UNIWERSYTET MIKOLAJA KOPERNIKAW TORUNIU
WYDZIAŁ NAUK BIOLOGICZNYCH I WETERYNARYJNYCH

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Promieniowce wyizolowane z gleb środowisk ekstremalnych i mało poznanych - różnorodność taksonomiczna i aktywność biologiczna

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Toruń 2023

Składam serdeczne podziękowania ***Pani dr hab. Patrycji Golińskiej, prof. UMK*** *za przekazaną wiedzę, nieocenioną pomoc w prowadzeniu badań i publikacji uzyskanych wyników, wsparcie w rozwoju naukowym oraz życzliwość.*

Pragnę wyrazić moją wdzięczność ***dr Magdalenie Wypij*** *za wsparcie, cenne rady, serdeczność i okazaną pomoc.*

Składam również podziękowania wszystkim koleżankom i kolegom oraz współpracownikom, którzy przyczynili się do powstania niniejszej pracy.

Szczególne wyrazy wdzięczności składam moim najbliższym za motywację i wsparcie w trudnych chwilach.

Spis treści

Wykaz skrótów

ANI – (ang. average nucleotide identity) średnie podobieństwo nukleotydów
ATCC – (ang. American Type Culture Collection) Amerykańska Kolekcja Szczepów Wzorcowych
BCAs – (ang. biocontrol agents) środki biokontrolujące rozwój patogenów roślin
BGCs – (ang. biosynthetic gene clusters) biosyntetyczne klastry genów
DDH – (ang. DNA-DNA hybridization) hybrydyzacja DNA:DNA
DNA – (ang. deoxyribonucleic acid) kwas deoksyrybonukleinowy
DSMZ – (niem. Deutsche Sammlung von Mikroorganismen und Zellkulturen) Niemiecka Kolekcja Mikroorganizmów i Kultur Komórkowych
HMDS – heksametylodisilazan
HNA – (ang. halophilic nutrient agar) halofilny agar odżywczy
HPLC – (ang. high-performance liquid chromatography) wysokosprawna chromatografia cieczowa
IOR – Instytut Ochrony Roślin
ISP – (ang. International *Streptomyces* Project) podłoże dedykowane promieniowcom opracowane w ramach międzynarodowego projektu badawczego nad promieniowcami
ISR – (ang. induced systemic resistance) indukowana odporność układowa
MLSA – (ang. multilocus sequence analysis) wielolokusowa analiza sekwencji nukleotydowej
NM – (ang. Nitrate medium) podłoże z azotanem
NRPS – (ang. non-ribosomal peptide synthetase) nierybosomalna syntetaza peptydowa
PCM – (ang. Polish Collection of Microorganisms) Polska Kolekcja Mikroorganizmów
PCR – (ang. polymerase chain reaction) reakcja łańcuchowej polimerazy
PKS1 – (ang. polyketide synthases type I) syntaza poliketydowa typu I
rRNA – (ang. ribosomal ribonucleic acid) rybosomalny kwas rybonukleinowy
SAR – (ang. systemic acquired resistance) nabyta odporność układowa
TLC 2D – (ang. two-dimensional thin-layer chromatography) dwukierunkowa chromatografia cienkowarstwowa
YCB – (ang. Yeast Carbon Base) podłoże do oznaczeń wykorzystywania źródeł azotu
YNB – (ang. Yeast Nitrogen Base) podłoże do oznaczeń wykorzystywania źródeł węgla

Streszczenie

Promieniowce są mikroorganizmami powszechnie występującymi w środowiskach naturalnych. Najbardziej znane są ze zdolności do syntezy różnych bioaktywnych metabolitów wtórnych, zwłaszcza antybiotyków. Poszukiwanie nowych, skutecznych związków antybiotycznych, w dobie narastającej oporności mikroorganizmów na te leki, ma szczególne znaczenie. Promieniowce poza antybiotykami wytwarzają szereg związków o działaniu przeciwnowotworowym, biokontrolującym rozwój patogenów roślin, immunosupresyjnym, a także o charakterze bionawozów i inne. Nowe, bioaktywne związki pochodzenia naturalnego są poszukiwane wśród mikroorganizmów środowisk ekstremalnych, w których trudne warunki selekcjonują szczepy o charakterystycznych cechach genetycznych i molekularnych, szczególnie takie o zdolności do wytwarzania wyspecjalizowanych metabolitów o dużym znaczeniu biotechnologicznym.

Celem niniejszej pracy było określenie rodzajowej różnorodności wśród promieniowców wyizolowanych z silnie wysuszonych i nasłonecznionych gleb pustyni Atakama, zasolonych gleb jeziora Lonar i gleb kwaśnych z dwóch stanowisk w lesie sosnowym, przynależności promieniowców do znanych lub potencjalnie nowych gatunków oraz ustanowienie kolekcji szczepów o dużym potencjale biotechnologicznym do wykorzystania w medycynie, przemyśle i rolnictwie.

Sto piętnaście reprezentatywnych szczepów, wyizolowanych z czterech środowisk, wybranych z poszczególnych grup kolorystycznych ustanowionych na postawie zabarwienia kolonii i pigmentów dyfundujących do podłoża, zidentyfikowano w oparciu o sekwencję nukleotydową genu 16S rRNA do rodzaju i określono ich przynależność do opisanych lub potencjalnie nowych gatunków promieniowców. Przeprowadzone badania wykazały, że większość zbadanych szczepów należała do rodzaju *Streptomyces*, niezależnie od źródła izolacji. Jednak wśród zbadanych izolatów wykryto również przedstawicieli rzadko występujących rodzajów między innymi *Modestobacter* (wśród izolatów z pustyni Atakama) czy *Actinacidiphila, Catenulispora* i *Streptacidiphilus* (wśród izolatów z gleby leśnej). Wśród zbadanych izolatów wykazano obecność wielu potencjalnie nowych taksonów. Wykorzystując podejście wielofazowe, oparte o badania filogenetyczne, analizy porównawcze sekwencji genomowych oraz badania fenotypowe, opisano po 2 nowe gatunki z rodzajów:

Catenulispora (*C. pinisilvae* i *C. pinistramenti*), *Modestobacter* (*M. excelsi* i *M. altitudinis*) i *Streptomyces* (*S. alkaliterrae* i *S. pinistramenti*).

W genomach izolatów stanowiących nowe gatunki promieniowców wykryto obecność licznych klastrów genów kodujących znane i nieznane metabolity wtórne m.in. o działaniu przeciwbakteryjnym, przeciwgrzybowym, przeciwwirusowym, czy przeciwnowotworowym. Genomy tych izolatów były również bogate w geny związane ze stresem, odpowiedzialne za przystosowanie się promieniowców do trudnych warunków środowiskowych oraz geny kodujące czynniki promujące wzrost roślin, takie jak siderofory.

Szczepy reprezentatywne w testach przeprowadzonych standardowymi metodami hodowlanymi wykazywały aktywność przeciwbakteryjną i przeciwdrożdżakową, a izolaty z gleby leśnej silną aktywność przeciwgrzybową wobec fitopatogenów. Ponadto u zbadanych promieniowców wykazano aktywność hydrolityczną (celulaz, chitynaz, lipaz, pektynaz, proteaz i ureaz), zdolność do syntetyzowania czynników promujących wzrost roślin, takich jak amoniak, auksyny, cyjanowodór i siderofory oraz zdolność do rozpuszczania fosforanów.

Podsumowując, uzyskane wyniki badań wykazały, że wśród promieniowców ze środowisk ekstremalnych i rzadko eksplorowanych takich jak silnie wysuszone gleby pustyni Atakama, zasolona i alkaliczna gleba krateru Lonar czy kwaśne gleby lasu sosnowego, oprócz powszechnie znanych, występują rzadkie taksony promieniowców, w tym nowe gatunki, a także izolaty o zróżnicowanej aktywności biologicznej i potencjale biotechnologicznym do wykorzystania w medycynie, przemyśle i produkcji roślin.

Streszczenie w języku angielskim (abstract)

Actinomycetes are microorganisms commonly found in natural environments. They are best known for their ability to synthesize various bioactive secondary metabolites, especially antibiotics. The search for new and effective antibiotics is of particular importance in the era of increasing resistance of microorganisms to these drugs. In addition to antibiotics, actinomycetes produce a number of compounds with anticancer, biocontrolling and immunosuppressive activities, as well as biofertilizers and others. New bioactive compounds of natural origin now being sought from extreme habitats as harsh environmental conditions therein select for novel strains with distinctive genetic and molecular features, notably an ability to produce specialized metabolites of biotechnological value.

The aim of this study was to determine the generic diversity among actinomycetes isolated from highly dried and insolated soils of the Atacama Desert, saline soils of Lonar Lake and acidic soils from two sites in a pine forest, the membership of actinomycetes to known or potentially novel species, and to establish a collection of strains with high biotechnological potential for use in medicine, industry and agriculture.

In the present study, one hundred and fifteen representative strains, isolated from four environments, selected from individual colour groups established on the basis of colony colour and pigments diffusing into the medium, were identified based on the nucleotide sequence of the 16S rRNA gene to the genera and their affiliation to the validly published or potentially new species of actinomycetes was determined.

The results of these studies showed that the majority of isolates belonged to the genus *Streptomyces*, regardless of the source of isolation. However, among the tested isolates, representatives of rare genera were also detected, including *Modestobacter* (among the Atacama Desert isolates) or *Actinacidiphila, Catenulispora* and *Streptacidiphilus* (among forest soil isolates). Among the examined isolates, the presence of several potentially new species has been demonstrated. Using a polyphasic approach, based on phylogenetic studies, comparative analyses of genome sequences and phenotypic studies, two new species of the following genera were described, namely *Catenulispora* (*C. pinisilvae* and *C. pinistramenti*), *Modestobacter* (*M. excelsi* and *M. altitudinis*) and *Streptomyces* (*S. alkaliterrae* and *S. pinistramenti*).

Numerous gene clusters that encode for known and unknown secondary metabolites with antibacterial, antifungal, antiviral and antitumor activity have been

recorded in the genomes of isolates belonging to new species. The genomes of the isolates were also rich in stress-related genes responsible for adaptation to harsh environmental conditions and genes encoding for plant growth promoting factors, such as siderophores.

The representative strains, in the standard culture tests, showed antibacterial and antifungal activity while isolates from forest soil strong antifungal activity against phytopathogens. In addition, isolates showed hydrolytic activity (cellulases, chitinases, lipases, pectinases, proteases and ureases), the ability to synthesize plant growth promoting factors (ammonia, auxins, hydrogen cyanide and siderophores) and solubilize phosphates.

In conclusion, the obtained results showed that among the actinomycetes isolated from extreme and rarely explored environments, such as hyper-arid, high-altitude Atacama Desert soils, saline and alkaline Lonar lake soil and acidic pine forest soils, both commonly known and rare taxa of actinomycetes, including new species, and isolates with diverse biological activity and biotechnological potential for use in medicine, industry and plant production were present.

I Wstęp

1. Charakterystyka promieniowców

Promieniowce to duża i zróżnicowana grupa polimorficznych bakterii Gram-dodatnich, o dużej zawartości guaniny i cytozyny w genomie (Ludwig i in., 2012). Ich komórki przybierają nieregularne kształty, u większości są wydłużone i nitkowate ze skłonnością do wytwarzania rozgałęzień - pseudomycelium (Barka i in., 2016). Z tego powodu promieniowce były w przeszłości mylnie klasyfikowane jako grzyby (Jakobson, 2012). Obecnie mikroorganizmy te zaliczane są do domeny *Bacteria* w obrębie *Prokariota*, ponieważ posiadają nukleoid i ścianę komórkową zbudowaną z peptydoglikanu oraz są wrażliwe na antybiotyki przeciwbakteryjne. Promieniowce rozmnażają się poprzez podział strzępek, fragmentację grzybni lub spory, co z kolei upodabnia je do grzybów strzępkowych (Barka i in., 2016).

Mikroorganizmy te są zazwyczaj tlenowe (Barka i in., 2016), do beztlenowych należą między innymi *Actinomyces dentalis* (Hall i in., 2005) czy *Propionibacterium acidifaciens* (Downes i Wade, 2009). W większości są organizmami heterotroficznymi, zdolnymi do wykorzystywania różnych związków organicznych oraz nieorganicznych jako źródła związków odżywczych, w tym polisacharydów. Przeważnie należą do chemoheterotrofów (Barka i in., 2016), jednak niektóre z promieniowców to chemoautotrofy tak jak *Acidimicrobium ferrooxidans* (Clark i Norris, 1996) czy *Carbonactinospora thermoautotrophica* (Gadkari i in., 1990; Volpiano i in., 2021). Promieniowce to w większości gatunki mezofilne, których temperatura optymalna do wzrostu mieści się w zakresie 25-30°C, chociaż gatunki termofilne, o optimum wzrostu około 50-60°C (Barka i in., 2016), jak na przykład *Thermoactinospora rubra* (Zhou i in., 2012), *Thermobifida cellulosilytica* (Kukolya i in., 2002) oraz psychrofilne, rosnące w zakresie temperatur 0-28°C, o optimum około 10°C (Kaari i in., 2022), takie jak *Subtercola boreus* i *Subtercola frigoramans* (Männistö i in., 2000) oraz *Salinibacterium xinjiangense* (Zhang i in., 2008) były również izolowane.

Promieniowce są szeroko rozpowszechnione w ekosystemach naturalnych, głównie w glebie, zwłaszcza bogatej w materię organiczną (Goodfellow i Williams, 1983). Chociaż wyizolowano je także z kompostu, zbiorników wodnych (Barka i in., 2016), roślin (Chankhamhaengdecha i in., 2013), gąbek (Liu i in., 2019) czy bezkręgowców takich jak wije (Shear, 2011), ślimaki morskie (Peraud i in., 2009) i mrówki (Qin i in., 2017) oraz kręgowców takich jak ryby (Jami i in., 2015).

Promieniowce występują głównie w powierzchniowej warstwie gleby (do około 20 cm głębokości), ale izolowano je również z próbek pobieranych na głębokości powyżej 2 metrów (Goodfellow i Williams, 1983; Barka i in., 2016). Większość poznanych dotychczas promieniowców wyizolowano z gleb o odczynie obojętnym (Barka i in., 2016), jednak mikroorganizmy te występują również w glebach kwaśnych (Teo i in., 2020) i alkalicznych (Wei i in., 2021), a także w środowiskach ekstremalnych takich jak zamarznięte obszary Arktyczne (Silva i in., 2020), gorące źródła (Bahamdain i in., 2020), silnie wysuszone piaski pustyni (Bull i Goodfellow, 2019), silnie alkaliczne jeziora (Wu i in., 2021), a także tereny zasolone (Sorokin i in., 2022). W takich środowiskach przyczyniają się do rozkładu materii organicznej i obiegu pierwiastków w przyrodzie (Goodfellow i Williams, 1983; Barka i in., 2016).

Zdolność do syntezy metabolitów o działaniu przeciwdrobnoustrojowym oraz enzymów degradujących ścianę komórkową innych mikroorganizmów sprawia, że promieniowce odgrywają ważną rolę w walce z fitopatogenami (Vurukonda i in., 2018). Ponadto, zdolność promieniowców do wywoływania nabytej odporności układowej (SAR) lub indukowanej odporności układowej (ISR) u roślin (Kämpfer, 2012; Hamedi i Mohammadipanah, 2015) wskazuje na możliwość zastosowania tych mikroorganizmów lub ich metabolitów jako czynników biokontrolujących rozwój patogenów roślin (ang. biocontrol agents, BCAs) (Guo i in., 2015). Promieniowce dzięki zdolności do solubilizacji fosforanów (Hastuti i in., 2012), produkcji cyjanowodoru zwiększającego dostęp fosforanów (Rijavec i Lapanje, 2016), amoniaku zwiększającego dostęp azotu, hormonów roślinnych (auksyny, gibereliny i cytokininy) i sideroforów wiążących jony żelaza, a tym samym ograniczających rozwój patogenów (Hamedi i Mohammadipanah, 2015; Barka i in., 2016), wpływają pozytywnie na wzrost oraz rozwój roślin i mogą być z powodzeniem stosowane jako alternatywa dla chemicznych nawozów i środków ochrony roślin (Hastuti i in., 2012; Vurukonda i in., 2018).

Promieniowce mają duże znaczenie w medycynie i przemyśle biotechnologicznym. Są źródłem enzymów stosowanych w detergentach oraz przemyśle farmaceutycznym, papierniczym, rafineryjnym, rolniczym, spożywczym i włókienniczym (Richa i Vivek, 2018). Wytwarzają bioaktywne metabolity wtórne takie jak immunomodulatory, związki przeciwnowotworowe oraz antybiotyki (Ouchari i in., 2019). Metabolity te nie są wymagane do wzrostu czy rozwoju promieniowców, ale dają im przewagę konkurencyjną nad innymi mikroorganizmami zasiedlającymi dane środowisko (Quinn i in., 2020). Promieniowce syntetyzują 2/3 znanych antybiotyków,

z których większość (około 70%) wytwarzają przedstawiciele rodzaju *Streptomyces* (Kämpfer, 2012; Barka i in., 2016). Analiza sekwencji całego genomu promieniowców, wykonana po raz pierwszy u *Streptomyces coelicolor,* wykazała znacznie większy potencjał tych mikroorganizmów do syntezy naturalnych metabolitów o aktywności przeciwdrobnoustrojowej niż wynikało to z badań przeprowadzonych w warunkach laboratoryjnych (Bentley i in., 2002). W genomie tego gatunku wykazano obecność „cichych" klastrów genów, które nie zawsze ulegają ekspresji (Chiang i in., 2011). Genomy promieniowców, szczególnie takie o wielkości powyżej 8 Mbp, charakteryzują się obecnością dużej liczby klastrów genów odpowiedzialnych za syntezę metabolitów wtórnych (ang. biosynthetic gene clusters, BGCs) (Baltz, 2017).

Od momentu odkrycia w latach 40-tych XX wieku pierwszych antybiotyków pochodzenia promieniowcowego (aktynomycyna, streptomycyna) do roku 1970 bardzo intensywnie opisywano gatunki rodzaju *Streptomyces* i wytwarzane przez nie antybiotyki, zaś po roku 1985 odkryto zaledwie 3 nowe klasy tych związków wytwarzanych przez promieniowce (Quinn i in., 2020). Odkrycie nowych związków biologicznie czynnych u promieniowców jest dużym wyzwaniem, ponieważ analiza dobrze poznanych dotychczas środowisk często prowadzi do reizolacji opisanych już szczepów (Rangseekaew i Pathom-aree, 2019) i wytwarzanych przez nie metabolitów wtórnych (Sivakala i in., 2021). Jednak przeprowadzone w ostatnich latach badania wykazały, że nietypowe środowiska, w tym ekstremalne, takie jak gleby kwaśne (Zhuang i in., 2020), alkaliczne (Králová i in., 2021), zasolone (Chen i in., 2018) czy pustynne (Castro i in., 2018) są źródłem nowych taksonów promieniowców wytwarzających nieznane dotąd połączenia chemiczne o interesujących właściwościach biologicznych (Bull, 2011; Bull i in., 2016; Goodfellow i in., 2018; Bull i Goodfellow, 2019). Uważa się, że stres abiotyczny (zasolenie, susza, mała dostępność związków odżywczych itp.) występujący w takich biomach wpływa na unikalną różnorodność mikroorganizmów (Bull, 2011; Mohammadipanah i Wink, 2016; Quinn i in., 2020) wynikającą z ich metabolicznej adaptacji do panujących warunków, a także stymuluje mikroorganizmy do ekspresji „cichych" klastrów genów prowadząc do wytwarzania antybiotyków (Quinn i in., 2020) i innych nieznanych związków chemicznych (Bull i Goodfellow, 2019). Obecnie analiza genomu i przesiewowe badania metabolomiczne promieniowców uważane są za najbardziej obiecujące strategie w celu odkrycia nieznanych związków biologicznie czynnych (Sivakala i in., 2021).

Z tego powodu analiza promieniowców nietypowych środowisk pozwala nie tylko na poznanie różnorodności tych mikroorganizmów, ale również ich potencjału do wytwarzania nowych metabolitów wtórnych o różnych właściwościach bioaktywnych (Golińska i in., 2023).

2. Promieniowce środowisk ekstremalnych

Środowiska ekstremalne, choć znane od dawna, przez długi czas ze względu na panujące w nich skrajne warunki (parametry fizyczne i/lub chemiczne) były uważane za nisze, w których nie rozwijają się żadne organizmy żywe (Horikoshi, 2011; Rampelotto, 2013). Takie przekonanie wynikało z interpretacji pojęcia „środowisko ekstremalne", w którym z perspektywy człowieka występują radykalne warunki, dalekie od znanych wartości fizjologicznych, uważane za ekstremalne. Jednak dla wielu mikroorganizmów są to warunki „normalne", w których mogą funkcjonować i prowadzić procesy metaboliczne (Zabłotni i Dziadosz, 2013; Merino i in., 2019). Na przestrzeni ostatniego stulecia graniczne wartości parametrów fizycznych i chemicznych środowisk, w których wykrywano organizmy żywe znacznie się przesunęły i obejmują szersze zakresy temperatury, pH, ciśnienia, promieniowania i zasolenia oraz większe ograniczenia dostępności składników odżywczych (Merino i in., 2019).

Mikroorganizmy zasiedlające środowiska ekstremalne, zwane ekstremofilami, nie tylko wykazują tolerancję, ale często wymagają takich warunków do wzrostu i rozwoju. Są one klasyfikowane według warunków w jakich rosną. Na tej podstawie wyróżnia się termofile i hipertermofile (organizmy rosnące odpowiednio w wysokich lub bardzo wysokich temperaturach), psychrofile (organizmy, które najlepiej rosną w niskich temperaturach), acidofile i alkalifile (organizmy optymalnie przystosowane do życia w odpowiednio kwaśnym lub zasadowym odczynie środowiska), barofile (organizmy, które rosną najlepiej pod zwiększonym ciśnieniem) oraz halofile (organizmy, które do wzrostu wymagają obecności soli, zwłaszcza chlorku sodu). Ekstremofile są często poliekstremofilne, czyli przystosowane do życia w warunkach, gdzie różne czynniki fizyko-chemiczne osiągają ekstremalne wartości (Horikoshi i Bull, 2011; Rampelotto, 2013).

Ekstremofile można podzielić na dwie grupy: organizmy ekstremofilne, które wymagają jednego lub więcej ekstremalnych warunków do wzrostu, oraz organizmy ekstremotolerancyjne, które mogą tolerować ekstremalne wartości jednego lub więcej

parametrów fizykochemicznych, chociaż optymalnie rosną w warunkach „normalnych" lub zbliżonych do „normalnych" (Horikoshi i Bull, 2011; Rampelotto, 2013).

Grupy promieniowców ekstremofilnych/ekstremotolerancyjnych zbadanych w niniejszej pracy scharakteryzowano poniżej.

2.1. Promieniowce środowisk alkalicznych

Promieniowce alkalifilne zostały po raz pierwszy opisane przez Mikami i współpracowników (1982). Można je podzielić na 3 grupy: alkalifile, wykazujące optimum wzrostu w pH > 10, umiarkowanie alkalifilne o wzroście w zakresie pH 7-10, ale słabym przy pH 7 oraz alkalitoleranty, rosnące w pH od 6 do 11 i wykazujące optymalny wzrost w pH około 7 (Shivlata i Satyanarayana, 2015). Najstabilniejszymi środowiskami alkalicznymi na Ziemi są pustynie i jeziora sodowe (Wani i in., 2006; Sharma i in., 2016), powszechnie zasiedlane również przez promieniowce alkalifilne i alkalitolerancyjne. Mikroorganizmy te są ważnym źródłem nowych antybiotyków, inhibitorów enzymów (Sharma i in., 2016) oraz enzymów alkalicznych, takich jak amylazy, ksylanazy i proteazy (Shivlata i Satyanarayana, 2015; Sharma i in., 2016).

Niepolarne pustynie stanowią około 1/3 powierzchni lądowej Ziemi, większość z nich ma położenie śródlądowe (Laity, 2009). Mikrobiom niepolarnych pustyń, takich jak pustynia Gobi, Sahara czy Thar (Wielka Pustynia Indyjska) był już wielokrotnie badany (Kurapova i in., 2012; Goodfellow i in., 2018; Ouchari i in., 2019). W ostatnich kilkunastu latach największą uwagę poświęcono badaniom nad mikroorganizmami, szczególnie promieniowcami, pustyni Atakama (Bull i in., 2016; Bull i Goodfellow 2019).

Pustynia Atakama w północnym Chile położona jest w strefie przybrzeżnej, zajmuje powierzchnię około 105 000 km^2, a z drugiej strony graniczy z Andami. Jest szczególnie interesująca, ponieważ jest najstarszą pustynią na Ziemi (Bull i in., 2016), na której skrajna susza utrzymuje się od 15 milionów lat, a susza od 150 milionów lat. Średnie opady są bardzo niskie i wynoszą poniżej 4 mm/m^2 (Azua-Bustos i in., 2018). Uważa się, że z powodu skrajnej suszy pustynia Atakama może odpowiadać warunkom środowiskowym panującym na Marsie (Navarro-González i in., 2003). Skrajna susza odpowiada za słabą erozję, przez co piaski pustyni są silnie zasolone, bogate w azotany, anhydryt, gips, siarczany i nadchlorany (Rech i in., 2003; Azua-Bustos i in., 2018) i niezwykle ubogie w materię organiczną (Azua-Bustos i in., 2018). Dodatkowo na terenie Atakamy odnotowano najwyższe na Ziemi promieniowanie UV (Carro i in.,

2019a). Skrajne warunki panujące na pustyni Atakama, sprawiają, że jest ona przykładem środowiska poliekstremalnego.

Środowiska suche, takie jak pustynia Atakama, są bogatym źródłem nowych gatunków promieniowców (Mohammadipanah i Wink, 2016; Goodfellow i in., 2018). W ostatnich latach z gleb pustyni Atakama wyizolowano i opisano stosunkowo dużo nowych taksonów promieniowców, które należą głównie do rodzajów *Actinocorallia*, *Actinomadura, Amycolatopsis*, *Blastococcus*, *Couchioplanes*, *Cryptosporangium*, *Geodermatophilus*, *Kineococcus*, *Kribbella*, *Lechevalieria*, *Lentzea*, *Microbispora*, *Microlunatus*, *Micromonospora*, *Modestobacter*, *Nocardia*, *Nocardiopsis*, *Nonomuraea*, *Prauserella*, *Pseudonocardia*, *Rhodococcus, Saccharothrix*, *Streptomyces* i *Williamsia* (Okoro i in., 2009; Idris i in., 2017a; Goodfellow i in., 2018; Carro i in., 2019a, b). Niezależne od hodowli analizy bioróżnorodności promieniowców w glebach pustyni Atakama wykazały obecność 245 rodzajów, z których 45% nie zidentyfikowano. Wśród znanych rodzajów dominującymi były *Arthrobacter, Blastococcus, Friedmanniella, Geodermatophilus, Jatrophibitans, Modestobacter, Mycobacterium, Nocardioides, Pseudonocardia* i *Terrabacter* (Idris, 2016).

Do szczególnie interesujących gatunków promieniowców wyizolowanych z pustyni Atakama, syntetyzujących nowe bioaktywne metabolity należą *Streptomyces leeuwenhoekii,* wytwarzający rzadkie 22-członowe poliketydy makrolaktonowe (chaxalactins A-C) o aktywności przeciw bakteriom Gram-dodatnim (Rateb i in., 2011a, b) oraz nowy lasso peptyd (chaxapeptin) działający hamująco na komórki raka płuc (Elsayed i in., 2015), *Streptomyces asenjonii* syntetyzujący nowy poliketyd należący do rzadkiej rodziny β-diketonu (asenjonamides A–C) o działaniu przeciw bakteriom Gram-dodatnim (Abdelkader i in., 2018), *Lentzea chajnantorensis* (Idris i in., 2017b) produkujący nowe glikozydy dienowe (lentzeosides A–F), hamujące rozwój wirusa HIV (Wichner i in., 2017) oraz *Micromonospora acroterricola* (Carro i in., 2019b), *Micromonospora arida*, *Micromonospora inaquosa* i *Micromonospora ureilytica* (Carro i in., 2019a) syntetyzujące przeciwgrzybowy lipopeptyd (fengyacin), którego nie wykryto u przedstawicieli rodzaju *Micromonospora* wyizolowanych z innych środowisk (Carro i in., 2018).

Przeprowadzone dotychczas badania wykazały, że genomy promieniowców wyizolowanych z pustyni Atakama są bogatym źródłem wielu biosyntetycznych klastrów genowych kodujących takie związki jak lantipeptydy, nierybosomalne syntetazy peptydowe, syntazy poliketydowe, kodujące między innymi nowe metabolity o działaniu

immunosupresyjnym, przeciwdrobnoustrojowym i przeciwnowotworowym oraz siderofory i terpeny. Powyższe doniesienia wskazują, że potencjał promieniowców pustyni Atakama, jako producentów związków biologicznie czynnych nie został jeszcze w pełni odkryty (Goodfellow i in., 2018; Carro i in., 2019a).

Kolejnym przykładem środowiska poliekstremalnego, zasiedlanego przez promieniowce, są jeziora sodowo-alkaliczne takie jak Lonar w Indiach czy Mono w Kalifornii (USA) (Antony i in., 2013). Na charakterystyczne właściwości takich jezior składa się kombinacja czynników geograficznych, geologicznych i klimatycznych. Jeziora sodowo-alkaliczne zawierają duże ilości węglanu wapnia lub sodu (Joshi i in., 2008), a w wyniku parowania dochodzi w nich do zwiększenia stężenia również innych soli takich jak chlorek sodu (Wani i in., 2006; Joshi i in., 2008).

Jezioro Lonar w dystrykcie Buldana, w stanie Maharasztra w Indiach powstało 50 tysięcy lat temu w wyniku uderzenia meteoru (Antony i in., 2013; Paul i in., 2016). Jezioro to jest unikatowym, trzecim co do wielkości jeziorem meteorytowym na Ziemi (Joshi i in., 2008), o kształcie zbliżonym do okręgu, którego średnica wynosi około 1,88 km i powstałym w powodziowych, wulkanicznych skałach bazaltowych (Antony i in., 2013; Paul i in., 2016), przez co jest odpowiednikiem kraterów występujących na Marsie (Paul i in., 2016). Bazaltowa gleba jeziora jest bogata w azot (zarówno w formie azotanów jak i azotu oznaczanego metodą Kjeldahla), fosforany, magnez, siarczany i siarczki oraz jony metali ciężkich takich jak kobalt, nikiel i żelazo (Wani i in., 2006; Antony i in., 2013). Wysokie pH (około 10) wody i osadów związane jest z obecnością węglanu sodu (Surve i in., 2021).

Istnieje kilka doniesień o różnorodności mikrobiologicznej jeziora Lonar, jednak dotychczas niewiele uwagi poświęcono różnorodności promieniowców w tym środowisku (Wani i in., 2006; Joshi i in., 2008; Sharma i in., 2016). Pierwszym nowym gatunkiem promieniowca z rodzaju *Streptomyces* wyizolowanym z jeziora Lonar był *Streptomyces alkalithermotolerans* (Sultanpuram i in., 2015).

Analiza osadów i próbek wody wykazała dominację bakterii typu *Firmicutes*, a następnie *Gammaproteobacteria*, *Actinobacteria*, *Alphaproteobacteria* i *Betaproteobacteria* (Joshi i in., 2008). Natomiast niezależne od hodowli badania różnorodności i liczebności mikroorganizmów przeprowadzone przez Paula i współpracowników (2016) wykazały obecność 28 różnych gromad bakterii, wśród których do najliczniejszych należały *Proteobacteria* (30%), a następnie *Actinobacteria* (24%), *Firmicutes* (11%) oraz *Cyanobacteria* i *Candidatus Saccharibacteria* TM7

(takson zidentyfikowany, ale niehodowalny; udział obu rodzajów wynosił poniżej 10%). Pozostałe wykryte w niewielkiej liczebności taksony stanowiły *Bacteroidetes*, *Candidatus Gracilibacteria* BD1-5 (takson zidentyfikowany, ale niehodowalny), *Nitrospirae*, *Verrucomicrobia* i *Spirochaetes* (Paul i in., 2016).

Mikroorganizmy środowisk zasadowych mają duży potencjał biotechnologiczny, ponieważ są źródłem enzymów alkalicznych (Antony i in., 2013), tak jak wyizolowany z jeziora Lonar *Streptomyces lonarensis,* syntetyzujący alkaliczną amylazę rozkładającą skrobię do mieszaniny maltozy, maltotriozy, maltotetraozy i maltooligosacharydów, mających zastosowanie w przemyśle spożywczym (Sharma i in., 2016) oraz *Streptomyces* sp. wytwarzający alkaliczną metaloproteazę, uczestniczącą w bioremediacji fenolu (Joshi i in., 2008). Wiele szczepów promieniowców przebadanych przez Joshiego i współpracowników (2008) wykazało zdolność do syntezy alkalicznej amylazy, celulazy i kazeinazy, a te wyizolowane z gleby pochodzącej z krateru Lonar przez Rauta i Bajekala (2009) zdolność do wiązania azotu atmosferycznego.

Promieniowce wyizolowane z jeziora Lonar wykazały również duży potencjał biomedyczny, hamując wzrost bakterii takich jak *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* i *Salmonella typhi* oraz działając przeciwnowotworowo wobec linii komórkowej raka płuc A549 (Kharat i in., 2009).

2.2. Promieniowce środowisk kwaśnych

Badania nad promieniowcami środowisk kwaśnych rozpoczęto w latach 60-tych XX wieku (Corke i Chase, 1964; Williams i in., 1971). Jednak do tej pory poświęcono im stosunkowo niewiele uwagi w porównaniu z promieniowcami środowisk obojętnych i alkalicznych (Golińska i in., 2023). Uważa się, że izolacja promieniowców acidofilnych jest trudniejsza niż neutrofili czy alkalifili, ponieważ ich optimum pH do wzrostu jest zbieżne z tym dla wzrostu grzybów. W związku z tym stosowanie zakwaszonych podłoży izolacyjnych wymaga dodania antybiotyków przeciwgrzybowych, z których duża część jest niestabilna w niskim pH (Williams i in., 1971; Poomthongdee i in., 2015).

Wśród promieniowców środowisk kwaśnych wyróżniamy acidofile rosnące w zakresie pH 3,5-6,5 i wykazujące optymalny wzrost w pH 4,5 oraz szczepy acidotolerancyjne (określane też neutrotolerancyjnymi acidofilami) rosnące w pH od 4,5 do 7,5, optymalnie w odczynie 5,0-5,5 (Eftekharivash i in., 2021; Golińska i in., 2023). Promieniowce kwasolubne występują powszechnie w glebach lasów szpilkowych (Corke

i Chase, 1964; Williams i in., 1971), izolowano je również z gleby leśnej bagien torfowych (Teo i in., 2020), wulkanicznej (Itoh i in., 2011), pól herbacianych, upraw ryżu (Eftekharivash i in., 2021) i drzew kauczukowych (Poomthongdee i in., 2015), z kopalni węgla (Oyuntsetseg i in., 2017) czy źródeł geotermalnych (Clark i Norris, 1996).

Lasy w Polsce w większości porasta sosna zwyczajna, która z powodu niskich wymagań glebowych tworzy drzewostany leśne na terenach ubogich, w klimacie umiarkowanym (Czerwiński, 1993). Drzewa iglaste charakteryzują się większym zakwaszaniem ściółki, niż drzewa liściaste, wpływając na jakość i pH gleby, która ma bezpośredni wpływ na skład mikroorganizmów (Lladó i in., 2017). Przyjmuje się, że wśród promieniowców zasiedlających gleby lasów iglastych 80-90% stanowią promieniowce kwasolubne (Zenova i in., 2011). Większość promieniowców wyizolowanych z kwaśnych gleb leśnych należy do rodzaju *Streptomyces*, a pozostałe między innymi do rodzajów *Actinospica, Catenulispora, Kitasatospora*, *Kribbella*, *Nocardia, Micromonospora, Streptacidiphilus i Streptosporangium* (Davies i Williams, 1970; Sharma i Thakur, 2020; Golińska i in., 2023). Promieniowce rozwijające się w glebie, szczególnie leśnej, powszechnie wytwarzają celulazy, dzięki którym rozkładają zdrewniałe łodygi i inne tkanki roślinne. Dodatkowo zdolność promieniowców kwaśnych gleb leśnych do rozkładu ligniny (Sharma i Thakur, 2020) wpływa na zakwaszenie tego środowiska (Morales i in., 2020).

Promieniowce acidofilne wykazują większą aktywność przeciwgrzybową niż promieniowce neutrotolerancyjne (Zakalyukina i Zenova, 2007). Związane jest to ze współzawodnictwem, do którego dochodzi pomiędzy promieniowcami kwaśnych gleb leśnych a grzybami zasiedlającymi to samo środowisko (Niyasom i in., 2015). Chitynazy wytwarzane przez promieniowce środowisk kwaśnych są jednym z mechanizmów kontrolujących rozwój grzybów zasiedlających tę niszę ekologiczną (Golińska i in., 2023). Badania przeprowadzone przez Poomthongdee i współpracowników (2015) wykazały, że acidofilne promieniowce ryzosferowe wyizolowane z ryżu i kauczukowca wykazywały zdolność do hamowania wzrostu grzybów *Fusarium moniliforme*, *Helminthosporium oryzae* oraz *Rhizoctonia solani.* Podobnie badania *in vitro* przeprowadzone przez Golińską i Dahm (2013) wykazały silną aktywność przeciwgrzybową promieniowców pochodzących z gleby lasu sosnowego wobec *Fusarium oxysporum, Fusarium culmorum* i *Rhizoctonia solani.* Poza tym, przeprowadzone przez autorki doświadczenia donicowe wykazały możliwość wykorzystania tych promieniowców jako czynników biokontrolujących rozwój patogena

siewek sosny - *R. solani*. Promieniowce pochodzące z gleby torfowej zbadane przez Niyasoma i współpracowników (2015) wykazywały zarówno działanie przeciwgrzybowe wobec *Colletotrichum gloeosporioides, Curvularia* sp. i *Fusarium* sp., jak i przeciwbakteryjne wobec *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* i *Staphylococcus aureus*. Podobnie, promieniowce z gleb leśnych wyizolowane przez Sharmę i Thakura (2020) wykazywały aktywność przeciwbakteryjną wobec *E. coli, Micrococcus luteus, P. aeruginosa, S. aureus* oraz przeciwdrożdżakową wobec *Candida albicans.*

W ostatnich latach wykazano, że promieniowce kwasolubne takie jak *Streptomyces abietis* (wyizolowany z gleby lasu sosnowego), *Streptomyces pini* (wyizolowany z igieł sosny zwyczajnej, *Pinus sylvestris* L.) i *Streptomyces piniterrae* (pozyskany z gleby ryzosferowej sosny junan, *Pinus yunnanensis*) są źródłem enzymów takich jak celulazy i ksylanazy (Fujii i in., 2013), sideroforów oraz związku immunosupresyjnego - heliquinomycyny (Su i in., 2019; Zhuang i in., 2020).

Chociaż promieniowce kwaśnych środowisk posiadają zdolność do syntetyzowania związków przeciwgrzybowych i enzymów stabilnych w odczynie kwasowym to stosunkowo niewiele uwagi poświęcono poszukiwaniu nowych metabolitów wytwarzanych przez takie mikroorganizmy, pomimo, że ich genomy wykazują obecność znacznie powyżej 20 klastrów genów kodujących szeroki zakres wyspecjalizowanych metabolitów, w szczególności lantipeptydów, nierybosomalnych syntetaz peptydowych (NRPS) i syntaz poliketydowych typu I (PKS1). Na tej podstawie uważa się, że promieniowce kwasolubne mają znacznie większy potencjał biosyntetyczny niż do tej pory sądzono (Golińska i in., 2023).

2.3. Adaptacja promieniowców alkalifilnych i acidofilnych do środowiska

Ekstremofile i ekstremotoleranty, wśród których dużą grupę stanowią promieniowce, prezentują szeroką i wszechstronną różnorodność metaboliczną w połączeniu z niezwykłymi zdolnościami fizjologicznymi do kolonizacji ekstremalnych środowisk (Bull, 2011; Rampelotto, 2013). Wykazują one kilka strategii adaptacyjnych pozwalających im na przystosowanie się do panujących warunków środowiskowych. Za jedną z najistotniejszych, dającą promieniowcom przewagę nad innymi mikroorganizmami, uważana jest antybioza, mająca szczególne znaczenie w środowiskach zawierających niewiele związków odżywczych (Shivlata i Satyanarayana, 2015).

Promieniowce acidofilne i alkalifilne posiadają w błonie komórkowej pompę protonową, regulującą wewnątrz- i zewnątrzkomórkowe stężenie jonów H^+, co pozwala na utrzymanie fizjologicznego pH (Shivlata i Satyanarayana, 2015). Mikroorganizmy acidofilne aktywnie usuwają kwaśne produkty metabolizmu i syntetyzują związki o charakterze alkalicznym, neutralizujące kwasy powstające podczas metabolizmu pozakomórkowego (Tamreihao i in., 2018). Alkalifile posiadają również ujemnie naładowane polimery stabilizujące błonę komórkową zmniejszając ładunek na powierzchni komórki. Strategia adaptacyjna haloalkalifili polega na syntetyzowaniu i akumulacji dużych ilości kompatybilnych substancji rozpuszczonych, które poprzez osmoregulację zapobiegają wysychaniu komórek. Posiadają również antyporter Na^+/H^+ przeciwdziałający nadmiernej akumulacji soli w komórce (Shivlata i Satyanarayana, 2015).

Chociaż molekularne mechanizmy wykorzystywane przez mikroorganizmy ekstremofilne/ekstremotolerancyjne do przetrwania w tak trudnych środowiskach wciąż nie są w pełni wyjaśnione, wiadomo, że organizmy te posiadają przystosowane do takich warunków biomolekuły i swoiste szlaki biochemiczne. Stabilność i aktywność takich cząsteczek, szczególnie enzymów, w warunkach ekstremalnych stanowi użyteczną alternatywę dla labilnych cząsteczek pozyskanych z organizmów zasiedlających środowiska neutralne, co jest bardzo interesujące dla celów biotechnologicznych. Co ciekawe, niektóre z tych enzymów wykazują poliekstremofilność, tj. stabilność i aktywność w więcej niż jednym ekstremalnym stanie (Rampelotto, 2013).

Obecność genów stresu w genomach promieniowców wyizolowanych ze środowisk ekstremalnych pozwala wyjaśnić w jaki sposób mikroorganizmy te przystosowują się do warunków panujących w tych trudnych środowiskach. U promieniowców zasiedlających środowiska alkaliczne takie jak pustynie wykazano obecność genów reakcji na szok osmotyczny, temperaturowy i wysychania oraz odpowiedzialnych za przetrwanie w środowisku ubogim w materię organiczną i w obecności silnego promieniowania UV (Busarakam i in., 2016). Obecność genów stresu u promieniowców zasolonych jezior, nie została jeszcze dobrze zbadana. Jednak w genomach promieniowców wyizolowanych z innych zasolonych środowisk, takich jak Wielkie Równiny Solne w USA wykazano obecność genów szoku temperaturowego i osmotycznego, kodujących odpowiednio białka stresu i białka odpowiedzialne za osmoregulację oraz genów syntezy choliny i aldehydu betainy glicynowej, będących prekursorem betainy glicynowej, której gromadzenie w komórkach chroni mikroorganizmy przed stresem osmotycznym

działając jak osmoprotektant. Ponadto, w genomach takich izolatów stwierdzono obecność genów biosyntezy ektoiny, będącej powszechnym osmolitem u promieniowców halofilnych i halotolerancyjnych (Cornell i in., 2018). W genomach promieniowców środowisk kwaśnych, takich jak gleby leśne, zidentyfikowano geny szoku cieplnego, stresu osmotycznego i oksydacyjnego oraz geny kodujące szlaki syntezy ektoiny i/lub trehalozy, pozwalające na przezwyciężenie stresu oksydacyjnego, a także geny odpowiedzialne za syntezę chitynaz pozwalających na adaptację do kwaśnych środowisk (Golińska i in., 2023).

3. Taksonomia promieniowców

Głównym wyzwaniem wszystkich systematyków bakterii jest ustalenie systemu hierarchicznego pozwalającego na odpowiednią klasyfikację grup, w szczególności wyższych rang (Salam i in., 2020). Gromada *Actinomycetota* (nazwa zaproponowana przez Goodfellowa, 2021) (Oren i Garrity, 2021, 2022), wcześniej klasyfikowana jako *Actinobacteria* (Goodfellow, 2012), to jedna z najliczniejszych gromad w domenie *Bacteria* (Ludwig i in., 2012; Salam i in., 2020). Członkowie tej gromady różnią się chemotaksonomicznie, morfologicznie, fizjologicznie i metabolicznie (Goodfellow i in., 2012; Barka i in., 2016; Oren i Garrity, 2021, 2022).

Według klasyfikacji zaproponowanej przez Salama i współpracowników (2020) gromada *Actinomycetota* (Oren i Garrity, 2021, 2022) obejmuje 6 klas: *Acidimicrobiia*, *Actinomycetia*, *Coriobacteriia*, *Nitriliruptoria*, *Rubrobacteria* i *Thermoleophilia*. Klasa *Actinomycetia* (wcześniej klasyfikowana jako *Actinobacteria*) dzieli się na 34 rzędy, 61 rodzin i 383 rodzaje. Jednak taksonomia bakterii, zwłaszcza promieniowców jest dynamiczna i wraz ze wzrostem wiedzy znacząco ewoluuje (Barka i in., 2016; Salam i in., 2020). Obecnie klasę *Actinomycetia* uznano za synonim, *Actinomycetes*, do której należy 20 poprawnie opublikowanych rzędów (w tym „*Streptomycetales*", rząd podlegający obecnie weryfikacji), 53 rodziny oraz 398 rodzajów (https://lpsn.dsmz.de/, ang. List of Prokaryotic names with Standing in Nomenclature, LPSN, data dostępu 11.10.2022; Parte i in., 2020).

W przeszłości wielokrotnie dochodziło do poprawiania klasyfikacji mikroorganizmów, w tym promieniowców (Goodfellow i in., 2012). Już w 1997 roku przy wprowadzaniu przez Stackebrandta i współpracowników wstępnego systemu hierarchicznej klasyfikacji klasy *Actinobacteria* było wiadomo, że będzie ona podlegać

modyfikacjom wraz z publikowaniem nowych sekwencji nukleotydowych genu 16S rRNA (Zhi i in., 2009). Jednak zmiany w hierarchicznej klasyfikacji filogenetycznej nie zmieniają opisu gatunków i rodzajów, ponieważ opisy te nie bazują wyłącznie na sekwencjach nukleotydowych genu 16S rRNA, ale również na właściwościach morfologicznych, chemotaksonomicznych i fizjologicznych (Stackebrandt i in., 1997; Zhi i in., 2009).

Każdy system klasyfikacji opierający się na niewielkiej liczbie porównywanych właściwości może prowadzić do uzyskania mylnych wyników (Rossello-Mora i Amann, 2001; Staley, 2006). Aby tego uniknąć stosuje się kompleksowe badania taksonomiczne opierające się na analizie wielu różnych cech (Alexander i in., 2015). Obecnie standardowe metody identyfikacji gatunków bakterii opierają się na zastosowaniu taksonomii wielofazowej bazującej na analizie porównawczej sekwencji nukleotydowej genu 16S rRNA (i/lub genów metabolizmu podstawowego – ang. housekeeping genes), hybrydyzacji DNA-DNA oraz analizach fenotypowych pomiędzy badanym izolatem a jego najbliższym filogenetycznym sąsiadem lub sąsiadami (Nakouti i Hobbs, 2012; Meier- Kolthoff i in., 2013b, Rodriguez i Konstantinidis, 2014). Obecnym trendem oprócz filogenetyki molekularnej (bazującej na sekwencji nukleotydowej genu 16S rRNA) (Alexander i in., 2015) coraz powszechniejsze jest stosowanie filogenomiki opartej na analizach porównawczych genomów. Jednak do momentu, aż genomy wszystkich poprawnie opublikowanych gatunków nie zostaną zsekwencjonowane, grupowanie filogenomowe będzie niekompletne (Salam i in., 2020).

Izolacja promieniowców ze środowiska naturalnego z reguły prowadzi do otrzymania dużej kolekcji tych mikroorganizmów. W celu zminimalizowania ryzyka przypadkowej selekcji szczepów, Williams i współpracownicy (1969) opracowali metodę przypisywania promieniowców wyhodowanych na agarze z wyciągiem z płatków owsianych (ISP3; Shirling i Gottlieb, 1966) do tzw. grup kolorystycznych (dereplikacja) na podstawie zdolności do wytwarzania barwników zabarwiających grzybnię substratową i powietrzną oraz tych dyfundujących do podłoża. Metoda dereplikacji pozwala na wybranie przedstawicieli grup kolorystycznych do dalszych badań przesiewowych zmniejszając liczbę analizowanych izolatów, jednocześnie eliminując całkowicie przypadkową selekcję szczepów (Williams i in., 1969).

Identyfikacja na poziomie gatunku i rodzaju opiera się na różnorodności morfologicznej, chemotaksonomicznej i molekularnej pomiędzy poszczególnymi taksonami (Laurent i in., 1999; Barka i in., 2016). Różnorodność morfologiczna odnosi

się przede wszystkim do morfologii grzybni podstawowej i powietrznej (jeśli jest wytwarzana) i ich zabarwienia, produkcji pigmentów melaninowych, a także kształtu strzępek sporonośnych i morfologii spor, która może być charakterystyczna dla rodzaju. Do najczęściej stosowanych markerów chemotaksonomicznych pozwalających na potwierdzenie przynależności rodzajowej promieniowców należą profile lipidów polarnych, menachinonów, kwasów tłuszczowych, cukrów i typ izoformy kwasu diaminopimelinowego w komórkach (Barka i in., 2016).

Filogenetyka molekularna pozwala na przypisanie badanych izolatów do rodzaju na podstawie analizy sekwencji nukleotydowej genu 16S rRNA i wskazanie procentowego podobieństwa do najbliżej spokrewnionego gatunku. Gen 16S rRNA jest silnie konserwowany w genomie bakterii, jednak posiada regiony, których zmienność jest charakterystyczna dla konkretnych rodzajów i gatunków bakterii pozwalając na ich identyfikację (Laurent i in., 1999). Obecnie wartość podobieństwa sekwencji nukleotydowej genu 16S rRNA pomiędzy analizowanym szczepem a jego najbliższym filogenetycznym sąsiadem wynosząca ≤98,7%, a nawet ≤99,0% uznawana jest za punkt odcięcia wskazując na przynależność izolatu do potencjalnie nowego gatunku (Meier-Kolthoff i in., 2013a; Kim i in., 2014). Chociaż wcześniej wartość ta wynosiła ≤97% (Stackebrandt i Goebels, 1994). Jednak, jak pokazują liczne badania, nawet prawie identyczna sekwencja nukleotydowa genu 16S rRNA badanych izolatów i ich najbliższych filogenetycznych sąsiadów nie zawsze oznacza, że analizowane szczepy reprezentują ten sam gatunek (Fox i in., 1992, Kim i in., 2014). Dlatego od kilku ostatnich dekad technika hybrydyzacji DNA:DNA (DDH) uważana jest za złoty standard pozwalający na porównanie podobieństwa genomowego analizowanych szczepów, powszechnie stosowany przy opisie i klasyfikacji nowych gatunków (Wayne i in., 1987). Wcześniej stosowane metody laboratoryjne hybrydyzacji DNA:DNA opierały się na zdolności do hybrydyzacji komplementarnych zasad azotowych jednoniciowych sekwencji nukleotydowych. Jednak postęp technologiczny w dziedzinie sekwencjonowania genomu wymagał zastosowania metod bioinformatycznych, które zastąpiły obarczoną błędem doświadczalnym laboratoryjną technikę DDH przez porównanie genomów *in silico*, polegające na obliczaniu dystansu genetycznego pomiędzy dwoma genomami i przeliczeniu uzyskanej wartości na procentowe podobieństwo sekwencji (Auch i in., 2010; Meier-Kolthoff i in., 2013b, 2014; Alexander i in., 2015; Lee i in., 2016). Wartość hybrydyzacji DNA:DNA pomiędzy dwoma blisko spokrewnionymi szczepami, na poziomie 70%, odpowiada co najmniej 97%

podobieństwa sekwencji nukleotydowej genu 16S rRNA i wskazuje, że badane szczepy stanowią jeden gatunek, natomiast wynik poniżej tej wartości potwierdza, że badany izolat jest nowym gatunkiem (Vandamme i in., 1996; Cho i Tiedje, 2001; Stackebrandt, 2011; Meier-Kolthoff i in., 2013a).

Kolejnym często stosowanym parametrem stosowanym do wyodrębniania nowego gatunku bakterii jest średnie podobieństwo nukleotydów (ang. average nucleotide identity, ANI). Jest to czuły parametr wyliczający średnie podobieństwo kolejności nukleotydów pomiędzy genomami porównywanymi *in silico*. Wartość ANI jest powiązana z wartością hybrydyzacji DNA:DNA, a wynik powyżej 95-96% podobieństwa potwierdza przynależność badanych mikroorganizmów do jednego gatunku (Goris i in., 2007; Lee i in., 2016).

II Cel pracy i hipotezy badawcze

Celem niniejszej pracy było określenie rodzajowej różnorodności wśród promieniowców wyizolowanych z silnie wysuszonych i nasłonecznionych gleb pustyni Atakama, zasolonych gleb jeziora Lonar i gleb kwaśnych z dwóch stanowisk w lesie sosnowym, przynależności promieniowców do znanych lub potencjalnie nowych gatunków oraz ustanowienie kolekcji szczepów o dużym potencjale biotechnologicznym do wykorzystania w medycynie, przemyśle i rolnictwie.

W trakcie planowania pracy postawiłam następujące hipotezy badawcze:

1. Gleby pustyni Atakama w Chile, jeziora Lonar w Indiach oraz lasu sosnowego z okolic Torunia stanowią źródło nowych i/lub rzadko występujących promieniowców.
2. Izolaty wykazują aktywność biologiczną, w tym przeciwdrobnoustrojową, wytwarzają enzymy hydrolityczne i czynniki promujące wzrost roślin, a tym samym duży potencjał biotechnologiczny.
3. Nowe gatunki promieniowców posiadają potencjał do syntetyzowania nieznanych dotąd metabolitów wtórnych.
4. Genomy promieniowców pozyskanych ze środowisk ekstremalnych, zawierają geny stresu mogące mieć związek z przystosowaniem do życia w danym środowisku.

III Materiały i metody

Materiał badawczy stanowiło 226 izolatów promieniowców pochodzących z trzech ekstremalnych lub słabo poznanych środowisk (silnie wysuszonych, alkalicznych gleb pustyni Atakama w Chile, gleby alkalicznego i zasolonego jeziora Lonar w Indiach oraz kwaśnej gleby północnego i południowego stoku wydmy śródlądowej lasu sosnowego w okolicy Torunia). Wszystkie szczepy w latach 2012-2014 wyizolowała dr hab. Patrycja Golińska, prof. UMK z Katedry Mikrobiologii Uniwersytetu Mikołaja Kopernika w Toruniu.

1. Analiza taksonomiczna i aktywność biologiczna izolatów

W tabeli 1 wymieniono materiały i metody zastosowane do analizy różnorodności taksonomicznej oraz aktywności biologicznej (potencjału biotechnologicznego) promieniowców wyizolowanych z gleb pustyni Atakama, jeziora Lonar i lasu sosnowego, które szczegółowo opisano w publikacjach stanowiących niniejsze osiągnięcie naukowe.

Tab. 1. Materiały i metody wykorzystane w badaniach promieniowców

Stosowane materiały i metody	**Publikacja**
Materiał badawczy:	I (Tabela S1)
Pochodzenie i sposoby przechowywania promieniowców z:	
- pustyni Atakama	I- III
- jeziora Lonar	I i IV
- gleby leśnej	I i V- VII
Mikroorganizmy wykorzystane w badaniach aktywności przeciwdrobnoustrojowej promieniowców:	
- szczepy dzikie bakterii oraz *Candida albicans* wyizolowane od pacjentów niewykazujących objawów chorobowych	V
- szczepy wzorcowe bakterii i *Candida albicans* oraz izolaty pochodzące z Stacji Sanitarno-Epidemiologicznej w Toruniu i kolekcji Collegium Medicum UMK	I i VII
- grzybowe i grzybopodobne patogeny roślin	I i VII
- dermatofity	VII
Grupowanie kolorystyczne promieniowców (Dereplikacja)	I
Molekularna identyfikacja promieniowców na podstawie sekwencji nukleotydowej genu 16S rRNA*	I
Aktywność przeciwdrobnoustrojowa:	
- metoda studzienkowa**	V
- metoda słupków agarowych*	I i VII

- metoda hodowli mieszanych*	I, V i VII
Produkcja enzymów hydrolitycznych: celulaz, chitynaz, lipaz, pektynaz, proteaz i ureaz*	I
Produkcja czynników promujących wzrost roślin: amoniaku, cyjanowodoru, kwasu indolilo-3-octowego, sideroforów oraz solubilizacja fosforanów*	I

*Badania przeprowadzono dla szczepów reprezentatywnych z każdej grupy kolorystycznej (115 szczepów) wybranych w grupowaniu kolorystycznym (publikacja I, tabela S1A-D).

** Badania przeprowadzono tylko dla izolatu $NH11^T$

2. Analiza taksonomiczna potencjalnie nowych gatunków

Na podstawie identyfikacji promieniowców w oparciu o sekwencje nukleotydowe genu 16S rRNA (publikacja I) do analiz taksonomicznych potencjalnie nowych gatunków wybrano po 6 izolatów z pustyni Atakama ($1G4^T$, $1G6^T$, 1G14, 1G50, 1G51, 1G52) i jeziora Lonar (IF11, IF17, IF19, $OF1^T$, OF3, OF8) oraz 5 izolatów z gleby leśnej (NF3, NF23, $NH11^T$, $NL8^T$, $SF28^T$). Szczepy te wykazywały stosunkowo małe podobieństwo sekwencji nukleotydowej genu 16S rRNA do najbliższych filogenetycznych sąsiadów lub należały do rzadko występujących rodzajów albo wyróżniały się interesującymi wynikami aktywności przeciwdrobnoustrojowej i enzymatycznej (publikacje I- VII).

Metody stosowane w celu ustalenia pozycji taksonomicznej wybranych potencjalnie nowych gatunków promieniowców, które szczegółowo opisano w publikacjach stanowiących osiągnięcie naukowe, podano w tabeli 2.

Tab. 2. Metody i programy stosowane w analizie taksonomicznej potencjalnie nowych gatunków promieniowców

Metody i programy	**Publikacja**
Analiza filogenetyczna izolatów w oparciu o sekwencje nukleotydowe genu 16S rRNA:	
- uzyskanych po sekwencjonowaniu metodą Sangera	I- V i VII
- wygenerowanych z genomu w programie SEED viewer na serwerze RAST	VI
Podobieństwo sekwencji (%) pomiędzy izolatami reprezentującymi ten sam gatunek oraz pomiędzy nimi i najbliżej spokrewnionymi sąsiadami filogenetycznymi określono z wykorzystaniem:	
- programu Phydit 1.0	III i IV
- serwera Single-Gene Phylogeny (DSMZ)	II, V- VII

Analiza filogenetyczna metodą największej wiarygodności i największej parsymonii na serwerze Single-Gene Phylogeny (DSMZ)	II- VII
Analiza filogenetyczna metodą najbliższego sąsiada w programie MEGA7	II- VII
Analiza MLSA (ang. Multilocus Sequence Analysis) przeprowadzona została dla izolatu SF28^T i jego najbliższych filogenetycznych sąsiadów, ponieważ analiza sekwencji nukleotydowej genu 16S rRNA oraz analiza filogenomowa nie dawały jednoznacznych wyników	VII
Analiza BOX-PCR przeprowadzona gdy potencjalnie nowy gatunek reprezentowany był przez więcej niż jeden izolat*	II- VI
Filogenomika i analizy porównawcze genomów:	
- analiza filogenomowa na serwerze TYGS (DSMZ)	III- VII
- hybrydyzacja DNA-DNA *in situ* na serwerze GGDC (DSMZ)	II-VII
- hybrydyzacja DNA-DNA metodą laboratoryjną	IV
- określenie wartości ANI z wykorzystaniem kalkulatora ANI na serwerze enve-omics (Konstantinidis group)	II, IV, V i VII
- określenie wartości ortoANI z wykorzystaniem kalkulatora ANI na serwerze EzBioCloud	III i VI
- oznaczanie właściwości genomów z wykorzystaniem serwera RAST	II, III, V-VII
- oznaczanie właściwości genomów z wykorzystaniem programu Prokka 1.11	IV, VI i VII
Właściwości fenotypowe:	
Analizy chemotaksonomiczne:*	
- typ izomeru kwasu diaminopimelinowego (DAP)	II- VII
- profil cukrów komórkowych	II- VII
- profil chinonów izoprenoidowych	II- VII
- profil lipidów polarnych	II- VII
- profil kwasów tłuszczowych	II - VII
Analizy morfologiczne*:	
- morfologia komórek za pomocą mikroskopu świetlnego	II i III
- morfologia zarodników i strzępek zarodnikonośnych za pomocą skaningowego mikroskopu elektronowego (SEM)	IV i VII
Właściwości hodowlane na podłożach agaryzowanych	II- VII
Testy tolerancji na pH, temperaturę i zawartość NaCl w pożywce	II-VII
Właściwości metaboliczne:	
- Biolog GEN III	II i III
- Zdolność do metabolizowania różnych źródeł węgla i azotu-klasyczna metoda hodowlana*	IV-VII
Właściwości biochemiczne i degradacyjne*	II-VII

Wytwarzanie enzymów - API-ZYM (bioMérieux)	II - VII
„Eksploracja genomu”	
-analiza biosyntetycznych klastrów genów (BGCs) z wykorzystaniem oprogramowania anti-SMASH	II-VII
-analiza genów funkcjonalnych i obecności genów stresu przeprowadzona w programie SEED viewer na serwerze RAST	II - VII

*Analizy, których szczegółowo nie przedstawiono w publikacjach stanowiących osiągniecie naukowe niniejszej dysertacji opisano poniżej.

2.1. Analiza sekwencji powtarzalnych BOX techniką PCR (BOX-PCR)

Analizę sekwencji powtarzalnych BOX w regionach pozagenowych wyizolowanego DNA techniką PCR przeprowadzono w celu sprawdzenia czy izolaty reprezentujące potencjalnie nowy gatunek nie są klonami (publikacje II-VI).

Skład mieszaniny reakcyjnej BOX-PCR (25 µl):

MyFi™ PCR master mix (Bioline)	12,5 µl
BOX-A1R (20 µM)	2,5 µl
ultra czysta woda	8,0 µl
DNA	2,0 µl

Sekwencja nukleotydowa startera BOX-A1R (Versalovic i in., 1994):
5’ CTACGGCAAGGCGACGCTGACG 3’

Warunki reakcji PCR:

faza 1	denaturacja wstępna	95°C	7 minut	1 cykl
faza 2	denaturacja	94°C	1 minuta	30 cykli
	hybrydyzacja	52°C	1 minuta	
	elongacja	72°C	3 minuty	
faza 3	końcowa elongacja	72°C	10 minut	1 cykl

Produkty reakcji PCR (5 µl) rozdzielono elektroforetycznie w 2% żelu agarozowym w buforze 1x TBE o pH 8,0 z barwnikiem Midori Green (Nippon Genetics, 5 µl/100 ml buforu). Rozdział prowadzono w obecności uniwersalnego markera wielkości DNA (Kapa Biosystems). Elektroforezę prowadzono przez 3 godziny przy napięciu 75 V (Trujillo i in., 2010). Rozdział produktów PCR zobrazowano wykorzystując Transiluminator (MultiDoc-It, UVP, Wielka Brytania).

2.2. Właściwości fenotypowe

2.2.1. Analizy chemotaksonomiczne

W celu potwierdzenia przynależności zbadanych izolatów do rodzaju analizowano ich chemiczne markery komórkowe, takie jak forma izomeru kwasu diaminopimelinowego (DAP), skład cukrów komórkowych, lipidów polarnych, menachinonów i kwasów tłuszczowych (publikacje II- VII).

Biomasę izolatów do analiz chemotaksonomicznych oraz najbliżej spokrewnionych gatunków pozyskano w wyniku hodowli w odpowiednim podłożu płynnym podanym w publikacjach II- VII.

2.2.1.1. Cukry komórkowe

Profil cukrów w hydrolizatach komórkowych izolatów zbadano stosując standardową chromatografię cienkowarstwową na płytkach celulozowych (Merck) o wielkości 10 x 10 cm.

W tym celu biomasę promieniowców (1 oczko ezy) zawieszono w 100 µl 0,25N HCl w krioprobówkach i ogrzewano przez 2 godziny w temperaturze 100°C.

Hydrolizaty komórkowe (3 µl) nanoszono w odległości 1 cm od dolnego brzegu płytki celulozowej oraz od standardów cukrów (1 µl). Chromatogram rozwijano dwukrotnie w fazie ruchomej (10 n-butanol : 6 woda : 6 pirydyna : 1 toluen). Po wysuszeniu płytkę spryskano roztworem ftalanu aniliny (Roth) i ogrzano w 100°C przez 4 minuty (Lechevalier i Lechevalier, 1980; Hasegawa i in., 1983). Standardy cukrów stanowiły arabinoza, galaktoza, glukoza, ksyloza, mannoza, ramnoza, ryboza (10mg/ ml pirydyny). Profil cukrów diagnostycznych w badanych próbkach ustalono w oparciu o zastosowane standardy.

2.2.1.2. Izomer kwasu diaminopimelinowego

Biomasę promieniowców zawieszono w 100 µl 6N HCl w krioprobówkach i ogrzano we wrzącej łaźni wodnej przez 8 godzin.

Na płytkę celulozową (Merck) w odległości 1 cm od dolnego brzegu płytki nanoszono po 1 µl standardu oraz 5 µl badanej próbki. Chromatogram rozwijano w fazie ruchomej (80 metanol : 26 woda : 4 6N HCl : 10 pirydyna) przez około 3 godziny. Po wysuszeniu płytki spryskano 0,2% ninhydryną w acetonie i ogrzano w 100°C przez 2 minuty (Staneck i Roberts, 1974; Lechevalier i Lechevalier, 1980).

Izoformę kwasu diaminopimelinowego określono względem standardu (mieszanina izoform *LL* i *mezo* kwasu diaminopimelinowego, Sigma-Aldrich).

2.2.1.3. Profil chinonów izoprenoidowych

Chinony izoprenoidowe wyekstrahowano z 50 mg zliofilizowanej biomasy promieniowców zawieszonej w 2 ml mieszaniny 0,3% chlorku sodu i metanolu (1 : 10 v/v) i ogrzewanej we wrzącej łaźni wodnej przez 5 minut. Następnie do schłodzonej próby dodano 2 ml eteru naftowego i całość mieszano na rotorze obrotowym przez 15 minut, a następnie odwirowano (Sorval Legend X1R, Thermo Fisher Scientific, USA) przy prędkości 2000 x *g* przez 5 minut. Górną warstwę zawierającą chinony przeniesiono do szklanych fiolek chromatograficznych. Procedurę ekstrakcji z eterem powtórzono. Próby odparowano pod dygestorium i przechowywano do dalszych analiz w temperaturze -20°C (Minnikin i in., 1984; Collins i in., 1985).

W celu analizy ilościowej i jakościowej chinonów próbki rozpuszczono w 200 µl izopropanolu i analizowano za pomocą wysokosprawnej chromatografii cieczowej (HPLC; In-Line Degasser AF, 515 HPLC Pump, Pump Control Module, 2487 Dual Absorbance Detector, 717 Plus Autosampler, Waters, USA), stosując mieszaninę metanol : izopropanol w stosunku 2 : 1 jako fazę ruchomą. Rozdział prowadzono na kolumnie C18 (Luna, 5 µm), przy długości fali 270 nm i szybkości przepływu 1 ml/min (Kroppenstedt, 1985).

2.2.1.4. Profil lipidów polarnych

Lipidy polarne ekstrahowano z biomasy pozostałej po izolacji chinonów izoprenoidowych. Do analizowanej próby dodano 2,3 ml mieszaniny chloroform : metanol : 0,3% chlorek sodu w stosunku 9 : 10 : 3 i całość mieszano na rotorze obrotowym przez 60 minut, a następnie odwirowano (Sorval Legend X1R, Thermo Fisher Scientific, USA) przy prędkości 2000 x *g* przez 5 minut. Supernatant przeniesiono do czystej probówki. Ekstrakcję lipidów polarnych powtórzono dwukrotnie dodając 0,75 ml mieszaniny chloroform : metanol : 0,3% chlorek sodu w stosunku 5 : 10 : 4, mieszając na rotorze obrotowym przez 30 minut, a następnie wirując przy prędkości 2000 x *g* przez 5 minut. Do zebranego supernatantu dodano 1,3 ml chloroformu i 1,3 ml 0,3% chlorku sodu, intensywnie wymieszano i odwirowano przy prędkości 2000 x *g* przez 5 minut. Dolną warstwę zawierającą lipidy polarne zebrano do szklanej

fiolki chromatograficznej i zostawiono do odparowania pod dygestorium (Minnikin i in., 1984; Kroppenstedt i Goodfellow, 2006).

W celu analizy wyizolowanych lipidów polarnych próby rozpuszczono w 80 µl mieszaniny chloroform : metanol w proporcji 2 : 1 i nanoszono na płytki aluminiowe z krzemionką (Merck) do chromatografii cienkowarstwowej o wielkości 10 x 10 cm. Do rozdziału lipidów polarnych zastosowano dwukierunkową chromatografie cienkowarstwową (TLC 2D). Chromatogramy najpierw rozwijano w mieszaninie chloroform : metanol : woda w stosunku 32,5 : 12,5 : 2, a po wysuszeniu płytkę TLC obrócono o 90° w prawą stronę i rozwijano w mieszaninie chloroform : kwas octowy : metanol : woda w stosunku 40 : 7,5 : 6 : 2.

Po wysuszeniu płytki spryskiwano:

a) 10% roztworem kwasu fosforomolibdenowego w etanolu (Sigma-Aldrich) i ogrzewano w 180°C przez minutę w celu wykrycia wszystkich wyizolowanych lipidów polarnych wybarwionych na ciemnoniebieski kolor na żółto-zielonym tle.
b) 0,2% roztworem ninhydryny w etanolu i ogrzewano w 110°C przez 5 minut w celu identyfikacji lipidów polarnych z wolnymi grupami aminowymi, które wybarwiały się na ciemno różowy kolor. Następnie płytkę spryskiwano roztworem błękitu molibdenowego (Sigma-Aldrich) wybarwiając fosfolipidy na ciemno-niebieski kolor.
c) odczynnikiem naftalenosiarkowym (10 ml 15% naftolu (Sigma-Aldrich) w etanolu, 4 ml wody destylowanej, 6,5 ml stężonego kwasu siarkowego, 40,5 ml etanolu) i ogrzewano przez 10 minut w 120°C, wybarwiając mannozydy fosfatydyloinozytolu (glikofosfolipidy) na brązowo na białym tle.

2.2.2. Analizy morfologiczne

2.2.2.1. Morfologia komórek

Morfologię komórek izolatów stanowiących nowe gatunki rodzaju *Modestobacter* (publikacje II i III), wyhodowanych przez 7 dni w 28°C na podłożu ISP2 (Shirling i Gottlieb, 1966) o pH 7,2 obserwowano pod mikroskopem świetlnym po wybarwieniu błękitem molibdenowym i fuksyną (Trujillo i in., 2015).

2.2.2.2. Morfologia zarodników i strzępek zarodnikonośnych

Kształt, wielkość i powierzchnię zarodników oraz morfologię strzępek zarodnikonośnych oznaczono u izolatów rodzaju *Streptomyces* wytwarzających grzybnię

powietrzną (szczepy OF1^T, OF3, OF8, IF11, IF17, IF19 i SF28^T), wykorzystując skaningową mikroskopię elektronową (publikacje IV i VII).

W tym celu izolaty OF1^T, OF3, OF8 hodowano na podłożu HNA (Atlas, 2010), a izolaty IF11, IF17, IF19 na agarze ISP5 (Shirling i Gottlieb, 1966) o pH 8,5 przez 14 dni w 28°C (publikacja IV). Szczep SF28^T hodowano na podłożu ISP2 (Shirling i Gottlieb, 1966) w 28°C przez 28 dni. Z hodowli promieniowców na szalkach Petriego wycinano aseptycznie za pomocą korkobora krążki o średnicy 1 cm i utrwalano w 2% glutaraldehydzie w buforze fosforanowym wg. Sorensona przez 24h w 4°C. Utrwalacz zlano, a próby przepłukano dwukrotnie wodą destylowaną i odwadniano inkubując przez 10 minut w wodnych roztworach etanolu (10, 20, 40, 60, 80, 100%). Następnie próby suszono chemicznie, umieszczając je na 10 minut w 25, 50 i 75% roztworach heksametylodisilazanu (HMDS; Sigma-Aldrich) w etanolu i w 100% HMDS aż do odparowania odczynnika. Tak przygotowane próby napylono złotem (wielkość cząstek 20 nm) i obserwowano za pomocą skaningowego mikroskopu elektronowego (Model 1430 VP, LEO Electron Microscopy Ltd, Wielka Brytania) (O'Donnell i in., 1993). Wykonano dokumentację fotograficzną i pomiary wielkości zarodników.

2.2.3. Badanie właściwości fizjologicznych z wykorzystaniem klasycznej metody hodowlanej

Zdolność do metabolizowania różnych źródeł węgla i azotu przez nowe gatunki promieniowców wyizolowane z gleby jeziora Lonar (publikacja IV) i kwaśnej gleby leśnej (publikacje V-VII) zbadano na podłożach podstawowych odpowiednio na YNB (ang. Yeast Nitrogen Base, Difco) z siarczanem amonu i bez aminokwasów oraz YCB (ang. Yeast Carbon Base, Difco) z glukozą i bez aminokwasów, o pH 8,0 dla promieniowców z gleby jeziora Lonar (publikacja IV) i 5,5 dla promieniowców z gleby leśnej (publikacje V-VII). 10-krotnie stężoną, wyjałowioną przez filtrację (Ø porów 0,2 μm; Nalgene) pożywkę mieszano ze sterylnym wodnym roztworem agaru (końcowe stężenie 1,5%) i wyjałowionym przez filtrację wodnym roztworem stanowiącym źródło węgla (końcowe stężenie 0,1 lub 1%, w/v; Tab. 3) lub azotu (końcowe stężenie 0,1%, w/v; Tab. 3) Podłoże po wymieszaniu wszystkich składników rozlewano do sterylnych 12-dołkowych płytek (Nest).

Po zestaleniu podłoży płytki zaszczepiono wodną zawiesiną zarodników promieniowców (0,5 w skali McFarlanda) za pomocą wielopunktowego, automatycznego inokulatora Mast Uri® Dot (Mast Group Ltd., Wielka Brytania) i hodowano przez

3 tygodnie w 28°C. Kontrolę negatywną w testach na wykorzystanie różnych źródeł węgla stanowiło podłoże YNB, a w testach na wykorzystanie różnych źródeł azotu YCB.

Tab. 3. Źródła węgla i azotu wykorzystane w badaniach nad nowymi gatunkami promieniowców.

Źródła węgla o stężeniu 1% (w/v)
adonitol, amigdalina, D-arabitol, L-arabitol, L-arabinoza, D-celobioza, dekstryna, *mezo*-erytrytol, D-fruktoza, , D-galaktoza, kwas D-glukuronowy, D-glukozamina, D-glukoza, glicerol, glikogen, *mezo*-inozytol, inulina, D-laktoza, D-maltoza, D-mannitol, D-melibioza, D-melezytoza, α-*metylo*-D-glukozyd, β-*metylo*-D-glukozyd, D-rafinoza, L-ramnoza, D-ryboza, salicyna, D-sacharoza, D-trehaloza, ksylitol, D-ksyloza.
Źródła węgla o stężeniu 0,1% (w/v)
kwas *para*-hydroksybenzoesowy, sole sodowe kwasu adypinowego, benzoesowego, bursztynowego, cytrynowego, fumarowego, hipurowego, masłowego, octowego, pirogronowego, propionowego, szczawiowego.
Źródła azotu o stężeniu 0,1% (w/v)
acetamid, L-alanina, L-asparagina, L-arginina, L-cysteina, etanoloamina, L-fenyloalanina, L-histydyna, L-hydroksyprolina, L-*izo*leucyna, kwas L-asparaginowy, kwas L-glutaminowy, L-metionina, L-seryna, L-treonina, L-walina.

2.2.4. Właściwości biochemiczne i degradacyjne

Zdolność do hydrolizy allantoiny, arbutyny, eskuliny i mocznika, redukcji azotanów i rozkładu różnych związków organicznych przez zbadane promieniowce oznaczono na podłożach podanych w tabeli 4 (publikacje II-VII).

Tab. 4. Substraty wykorzystane do badań właściwości biochemicznych i degradacyjnych promieniowców.

Związek	Stężenie związku w podłożu (%, w/v)	Podłoże
Hydroliza:		
Eskulina	0,1	wg. Kutznera (Kutzner i in. 1976)
Allantoina	0,1	wg. Gordona (1966)
Arbutyna	0,1	wg. Kutznera (Kutzner i in. 1976)
Mocznik	0,1	wg. Gordona (1966)

Testy degradacyjne:		
Adenina	0,5	ISP2 (Shirling i Gottlieb, 1966)
Kazeina	1,0	
Chityna	0,5	
Elastyna	0,3	
Guanina	0,5	
Hipoksantyna	0,4	
Skrobia	1,0	
L-tyrozyna	0,5	
Kwas moczowy	0,5	
Ksantyna	0,4	
Ksylan	0,4	
Żelatyna	0,4	
Tween 20	1,0	wg. Sierra (1957)
Tween 40	1,0	
Tween 60	1,0	
Tween 80	1,0	
Redukcja azotanów:		
KNO_3	0,1	NM podłoże z azotanem (ang. Nitrate medium; Williams i in., 1983)

Składniki pożywek, poza agarem rozpuszczono w wodzie destylowanej i ustalono pH za pomocą pH-metru (pH 211, Hanna instruments, Polska) i 1M roztworów HCl i NaOH. Następnie dodano agar i całość wysterylizowano w autoklawie w temperaturze 121°C przez 20 minut przy ciśnieniu 1 atm. Jałowe podłoża rozlano do płytek 12-dołkowych, a po zestaleniu zaszczepiono i hodowano jak opisano w punkcie 2.2.3.

Zdolność promieniowców do hydrolizy arbutyny i eskuliny charakteryzowała zmiana zabarwienia pożywki z żółtego do brązowego, natomiast allantoiny i mocznika z żółtego na różowo-czerwone. Rozkład Tween-ów ujawniał się w postaci kryształków soli wapniowych wytrąconych wokół kolonii promieniowców. Redukcję azotanów obserwowano poprzez pojawienie się różowego zabarwienia podłoża po dodaniu odczynnika Griessa (Avantor, Polska). Zdolność do rozkładu pozostałych związków organicznych obserwowano na podstawie pojawienia się stref hydrolizy wokół kolonii promieniowców.

IV Publikacje

I **Świecimska M**, Golińska P, Goodfellow M. Generation of a high quality library of bioactive filamentous actinomycetes from extreme biomes using a culture-based bioprospecting strategy. *Front Microbiol* 2022 (zaakceptowano 28.12.2022r.). Doi:10.3389/fmicb.2022.1054384.	IF- 6,064 MNiSW- 100
II Golińska P, Montero-Calasanz MDC, **Świecimska M**, Yaramis A, Igual JM, Bull AT, Goodfellow M. *Modestobacter excelsi* sp. nov., a novel actinobacterium isolated from a high altitude Atacama Desert soil. *Syst Appl Microbiol* 2020; 43:126051. Doi: 10.1016/j.syapm.2019.126051.	IF- 4,022 MNiSW- 100
III Golińska P, **Świecimska M**, Montero-Calasanz MDC, Yaramis A, Igual JM, Bull AT, Goodfellow M. *Modestobacter altitudinis* sp. nov., a novel actinobacterium isolated from Atacama Desert soil. *Int J Syst Evol Microbiol* 2020; 70:3513-3527. Doi: 10.1099/ijsem.0.004212.	IF- 2,747 MNiSW-70
IV **Świecimska M**, Golińska P, Nouioui I, Wypij M, Rai M, Sangal V, Goodfellow M. *Streptomyces alkaliterrae* sp. nov., isolated from an alkaline soil, and emended descriptions of *Streptomyces alkaliphilus*, *Streptomyces calidiresistens* and *Streptomyces durbertensis*. *Syst Appl Microbiol* 2020; 43:126153. Doi: 10.1016/j.syapm.2020.126153.	IF- 4,022 MNiSW- 100
V **Świecimska M**, Golińska P, Wypij M, Goodfellow M. Genomic-based classification of *Catenulispora pinisilvae* sp. nov., novel actinobacteria isolated from a pine forest soil in Poland and emended description of *Catenulispora rubra*. *Syst Appl Microbiol* 2021; 44:126164. Doi: 10.1016/j.syapm.2020.126164.	IF- 4,064 MNiSW- 100
VI **Świecimska M**, Golińska P, Wypij M, Goodfellow M. *Catenulispora pinistramenti* sp. nov., novel actinobacteria isolated from pine forest soil in Poland. *Int J Syst Evol Microbiol* 2021; 71:5063. Doi: 10.1099/ijsem.0.005063.	IF- 2,689 MNiSW- 70
VII **Świecimska M**, Golińska P, Goodfellow M. Genome-based classification of *Streptomyces pinistramenti* sp. nov., a novel actinomycete isolated from a pine forest soil in Poland with a focus on its biotechnological and ecological properties. *Antonie van Leeuwenhoek* 2022; 115:783-800. Doi: 10.1007/s10482-022-01734-8.	IF- 2,158 MNiSW-70
	Sumaryczny IF- 25,766 **Suma punktów MNiSW-** 610

Generation of a high quality library of bioactive filamentous actinomycetes from extreme biomes using a culture-based bioprospecting strategy

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Keywords: actinomycetes, systematics, antimicrobial activity, fungal phytopathogens, human pathogens, plant growth promoting bacteria, biocontrol agents.

Abstract

Introduction: Filamentous actinomycetes, notably members of the genus *Streptomyces*, remain a rich source of new specialized metabolites, especially antibiotics. In addition, they are also a valuable source of anticancer and biocontrol agents, biofertilizers, enzymes, immunosuppressive drugs and other biologically active compounds. The new natural products needed for such purposes are now being sought from extreme habitats where harsh environmental conditions select for novel strains with distinctive features, notably an ability to produce specialized metabolites of biotechnological value.
Methods: A culture-based bioprospecting strategy was used to isolate and screen filamentous actinomycetes from three poorly studied extreme biomes. Actinomycetes representing different colony types growing on selective media inoculated with environmental suspensions prepared from high-altitude, hyper-arid Atacama Desert soils, a saline soil from India and from a Polish pine forest soil were assigned to taxonomically predictive groups based on characteristic pigments formed on oatmeal agar. One hundred and fifteen representatives of the colour-groups were identified based on 16S rRNA gene sequences to determine whether they belonged to validly named or to putatively novel species. The antimicrobial activity of these isolates was determined using a standard plate assay. They were also tested for their capacity to produce hydrolytic enzymes and compounds known to promote plant growth while representative strains from the pine forest sites were examined to determine their ability to inhibit the growth of fungal and oomycete plant pathogens.
Results: Comparative 16S rRNA gene sequencing analyses on isolates representing the colour-groups and their immediate phylogenetic neighbours showed that most belonged to either rare or novel species that belong to twelve genera. Representative isolates from the three extreme biomes showed different patterns of taxonomic diversity and characteristic bioactivity profiles. Many of the isolates produced bioactive compounds that inhibited the growth of one or more strains from a panel of nine wild strains in standard antimicrobial assays and are known to promote plant growth. Actinomycetes from the litter and mineral horizons of the pine forest, including acidotolerant and acidophilic strains belonging to the genera *Actinacidiphila*,

genera *Actinacidiphila*, *Streptacidiphilus* and *Streptomyces*, showed a remarkable ability to inhibit the growth of diverse fungal and oomycete plant pathogens.
Discussion: It can be concluded that selective isolation and characterization of dereplicated filamentous actinomyctes from several extreme biomes is a practical way of generating high quality actinomycete strain libraries for agricultural, industrial and medical biotechnology.

1 Introduction

The phylum *Actinomycetota* (Oren and Garrity, 2021), formerly *Actinobacteria sensu* Goodfellow (2012), encompasses actinomycetes that are common in natural habitats where they have a major role in recycling organic matter (Bhatti et al., 2017) and in the transformation of environmental pollutants, such as pesticides (Alvarez et al., 2017). However, actinomycetes are known best for their unique ability to synthesize new specialized (secondary) metabolites, notably clinically significant antibiotics (Newman and Cragg, 2020; De Simeis and Serra, 2021). A new generation of antibiotics are urgently needed to control multi-drug resistant microbial pathogens which are causing a global health crisis (World Health Organisation, 2021).

Around 70% of known antibiotics are produced by filamentous actinomycetes, notably by members of the genus *Streptomyces* (Sánchez-Suárez et al., 2020; Donald et al., 2022), the type genus of the family *Streptomycetaceae* Waksman and Henrici 1943. Streptomycetes remain a rich source of new specialized metabolites, especially antibiotics (Sivalingam et al., 2019; Lacey and Rutledge, 2022) with the promise of more to come now that improved analytical procedures, such as genome mining and genetic engineering, are opening up new opportunities for drug discovery (Luo et al., 2014; Atanasov et al., 2021). However, the search for new natural products from streptomycetes using culture-dependent strategies needs to be tailored to meet developments in their ecology and systematics (Nouioui et al., 2018; Traxler and Rozen, 2022; Wang et al., 2022), as shown by the transfer of validly named acidotolerant and acidophilic *Streptomyces* species to the genera *Actinacidiphila, Streptantibioticus* and *Wenjunlia* while some *Streptacidiphilus* species have been moved to form the new genera *Peterkaempfera* and *Phaeacidiphilus* (Madhaiyan et al., 2022). Members of these poorly studied taxa are promising sources of new bioactive compounds (Golińska et al., 2023), as are *Kitasatospora* strains (Takahashi, 2017) which also belong to the family *Streptomycetaceae*.

In addition to therapeutic antibiotics, filamentous actinomycetes, including streptomycetes, are a valuable source of anticancer and biocontrol agents, biofertilizers, enzymes, immunosuppressive drugs and other biologically active compounds (Mukhtar et al., 2017; Sivalingam et al., 2019; Pacios-Michelena et al., 2021; Boukhatem et al., 2022). The new natural products needed for such purposes are now being sought from extreme habitats as harsh environmental conditions therein select for novel strains with distinctive genetic and molecular features, notably an ability to produce specialized metabolites of biotechnological value (Bull and Goodfellow, 2019; Sayed et al., 2020; Wilson and Brimble, 2021).

In practice, novel actinomycetes, especially streptomycetes, from extreme biomes are proving to be an especially rich source of new antibiotics, as shown by strains isolated from desert soils (Rateb et al., 2018; Djinni et al., 2019; Sun et al., 2023), deep-sea sediments (Nweze et al., 2020; Jagannathan et al., 2021) and marine organisms (Chen et al., 2021) whereas ones with growth promoting traits are being used to mitigate the effect of drought on crop plants (Chukwuneme et al., 2020). The extension of such studies to neglected extreme ecosystems can be expected to build upon these developments as biome type and geographical location are known to influence the composition of actinomycete communities (Charlop-Powers et al., 2015; Hernandez et al., 2021), not least with respect to streptomycetes (Andam et al., 2016; Arocha-Garza et al., 2017; Aallam et al., 2021). It is therefore timely to explore the taxonomic diversity and biotechnological potential of poorly studied actinomycetes from litter and mineral

horizons of coniferous forests (Golińska et al., 2023) and from saline soils (Zhao et al., 2016). Improved bioinformatic tools for recognizing prokaryotic species boundaries (Meier-Kolthoff et al., 2013; Sant'Anna et al., 2019) are bringing greater precision to bioprospecting campaigns, as are dereplication procedures designed to select representative isolates from extensive strain libraries for biotechnological purposes (Goodfellow et al., 2018).

Search and discovery campaigns intended to recover actinomycetes of potential biotechnological significance from extreme habitats are generally focused on the selective isolation, preliminary characterisation and antimicrobial screening of small numbers of isolates from individual biomes (Liu et al., 2016; Priyadarshini et al., 2016; Meklat et al., 2020). This partly reflects a tension between the need to screen representative isolates for new metabolites and the requirement to classify them using taxonomic methods that are difficult to apply to more than a few strains. This problem is being addressed by using dereplication procedures, such as MALDI-TOF mass spectrometry (Schumann and Maier, 2014), molecular fingerprinting (Carro et al., 2018) and genus specific primers (Castro et al., 2019), to distribute large numbers of isolates to taxonomically meaningful groups, representatives of which can be selected for further study. A practical and inexpensive way of dereplicating streptomycetes was introduced by Williams et al. (1969) who assigned large numbers of soil streptomycetes to colour-groups based on aerial spore mass, substrate mycelial and diffusible pigment colours formed on oatmeal agar and melanin pigments produced on peptone-yeast extract – iron agar. Subsequently, the number of colour-groups were used as an index of streptomycete diversity in diverse natural habitats (Atalan et al., 2000; Sembiring et al., 2000; Antony-Babu et al. 2010) following the discovery that representatives of such groups belonged to validly named or novel (previously unknown) *Streptomyces* species or species-groups based upon genotypic and phenotypic criteria (Manfio et al., 2003; Goodfellow et al., 2007), as exemplified by members of what became known as the *Streptomyces violaceusniger* clade (Sembiring et al., 2000; Goodfellow et al., 2007. Kusuma et al., 2021). Representatives of this taxon show a similar pattern of HPLC detected antibiotics (Ward and Goodfellow, 2004). Recently, principal component analyses of members of colour-groups composed of desert filamentous actinomycetes were shown to be positively correlated with corresponding levels of bioactivity recorded from antimicrobial assays (Goodfellow et al., 2018). The colour-group strategy has been extended to include representatives of other genera containing filamentous actinomycetes (Idris, 2016; Kusuma, 2020).

In the present study, filamentous actinomycetes isolated from hyper-arid, high altitude Atacama Desert soils, a saline soil and from two pine forest locations were assigned to colour-groups in order to gain an insight into the generic diversity at each of the sampling sites. 16S rRNA gene sequencing analyses were carried out on representatives of the colour-groups to determine whether they belonged to validly named species or to putatively new species and the resultant phylogenetic data used to establish the taxonomic identity of isolates assigned to the colour-groups. The antimicrobial activity of colour-group representatives was determined using a standard plate assay, as was their ability to produce hydrolytic enzymes and compounds known to promote plant growth. In addition, representative strains from the pine forest sites were examined to determine their ability to inhibit the growth of fungal and oomycete plant pathogens. The overall aim of the study was to compare and contrast the taxonomic and functional activities of representative isolates from the four sampling sites and to generate a high quality strain library for agricultural, medical and industrial biotechnology.

2 Materials and Methods

2.1 Sampling sites, selective isolation and maintenance of isolates

Filamentous actinomycetes were isolated from environmental samples taken from high altitude, hyper-arid, Atacama Desert soils on Cerro Chajnantor, Chile (Idris et al., 2017a, b; Bull et al., 2018), from litter and mineral layers of an acidic forest soil under *Pinus sylvestris*, near Toruń, Poland (Golińska et al., 2016; Świecimska et al., 2021a, b) and from a saline soil adjacent to Lake Lonar, India (Świecimska et al., 2020); these references include details on the sampling sites, their location and physico-chemical properties and on isolation procedures. Tenfold dilutions of the Atacama Desert soil samples were used to inoculate starch-casein agar (SCA; Küster and Williams, 1964), humic acid-vitamin agar (HA; Hayakawa and Nonomura, 1987), Gauze's no. 1 agar (G; Zakharova et al., 2003) and R2A agar (Becto-Dickinson, USA) plates which were incubated at 28ºC for 21 days. The pH of the Atacama soil samples ranged from 6.6 to 7.6, the organic matter content from 1.7 to 3.7%, the moisture content was zero %.
In total, the 226 representative actinomycetes consisted of 59 isolates from the hyper-arid Atacama Desert soils and 16 from the saline soil, including 3 classified as *Streptomyces alkaliterrae* by Świecimska et al. (2020). Similarly 151 isolates from the litter and mineral layers of the pine forest soils comprised 65 and 86 strains from the northern and southern slopes of the inland dune system supporting pine, including pairs of strains classified as *Catenulispora pinisilvae* (Świecimska et al., 2021a) and *Catenulispora pinistramenti* (Świecimska et al., 2021b). The isolates from the desert soils included 6 classified as either *Modestobacter altitudinis* (Golińska et al., 2020a) or *Modestobacter excelsi* (Golińska et al., 2020b) and the type strain of *Micromonosporra acroterricola* (Carro et al., 2019). Isolates were taken from the selective isolation plates based on characteristic colonial properties. Isolates from the Atacama Desert soils were maintained on slopes of modified Bennett's agar (Jones, 1949), ones from the saline soil on halophilic nutrient agar (Atlas, 2010) and those from the pine forest sites on SCA slopes, at room temperature and as suspensions of spores and mycelial fragments or as rods and cocci in 20%, v/v glycerol at −80ºC.

2.2 Assignment of isolates to colour-groups

The representative isolates were grown for 4 weeks at 28°C on oatmeal agar (International *Streptomyces* Project [ISP] medium 3) (Shirling and Gottlieb, 1966) at pH 5.5, 7.5 and 8.0 in the case of isolates from the pine forest, saline and Atacama Desert sampling sites; the ISP3 medium was adjusted to pH 5.5 using 1M HCl. The isolates were then assigned to four sets of colour-groups, representing the desert, saline and the two pine forests sites based on aerial spore mass, substrate mycelial and diffusible pigment colours using NBS/IBCC Colour Charts (Kelly, 1958). All of the isolates were grown on peptone-yeast extract-iron agar (ISP6, Shirling and Gottlieb, 1966) under the conditions described above and then examined to determine whether they produced melanin pigments.

2.3 Phylogenetic analyses

Most of the 99 isolates representing the colour-groups composed of 71 strains from the pine forest sites and 28 from the Atacama Desert soils were grown in ISP2 broth (Shirling and Gottlieb, 1966) at pH 5.5 and 7.5, respectively, the exceptions, desert isolates 1G4^T, 1G6^T, 1G14 and 1G50 to 1G52 were cultivated at pH 7.5 in modified Bennett's broth (Jones, 1949). The 16 isolates representing the colour-groups containing strains from the saline soil were grown at pH 8.0 in halophilic nutrient broth (Atlas, 2010) supplemented with 3% NaCl. All of the isolates were grown in shake flasks (150 rpm) at 28°C for 14 to 21 days, harvested by centrifugation, washed three times with sterile distilled water and stored at room temperature.

Genomic DNA was extracted from the biomass samples using a GenElute^{TM} Bacterial Genomic Kit (Sigma-Aldrich, Germany), according to the manufacturer's instructions. Amplification of 16S rRNA genes was performed using standard forward (27f:

AGAGTTTGATCCTGGCTCAG) and reverse (1525r: AAGGAGGTGATCCAGCC) primers (Lane, 1991) under the following conditions (25µl volume): 2x MyFi Mix (Bioline, United Kingdom) which contained DNA polymerase, dNTPs, $MgCl_2$ (at optimized concentrations), and 1 µl of each primer (20 µM), 1µl of 200 ng DNA and sterile distilled water; the negative control was sterile distilled water and the positive one DNA isolated from *Actinospica durhamensis* DSM 46820^T. The PCR reactions were carried out as follows: initial denaturation at 95°C for a minute, 30 cycles of 95°C for a minute, 55°C for a minute and 72°C for a minute, and finally 72°C for 5 minutes. The PCR products were purified using a purification kit (Qiagen, Germany), according to the instructions of the manufacturer. The concentration and quality of each of the purified PCR products were checked using a Nanodrop spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, USA) and by gel electrophoresis using a 1kb DNA ladder (Kapa Biosystems, United State). The resultant preparations were sequenced on an ABI 3730xl Genetic Instrument (Applied Biosystems, Thermo Fisher Scientific United State) at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw using the same pair of primers, as given above. Almost complete 16S rRNA gene sequences of the isolates were identified using a combination of two sequence similarity search engines (BLAST and MEGABLAST) (Altschul et al., 1997), followed by rigorous pairwise global sequence alignment (Myers and Miller, 1988), as previously described (Chun et al., 2007), as implemented through the EzBioCloud web server[1] (Yoon et al., 2017). Isolates showing 16S rRNA gene sequences equal to or <99.0% (maximum probability of error 1.0%) with their immediate phylogenetic neighbours were considered to belong to putatively new species (Meier-Kolthoff et al., 2013).

2.4 Antimicrobial activity

The 115 isolates representing the colour-groups composed of strains from the four sampling sites were examined for their ability to inhibit the growth of *Bacillus subtilis* PCM 2021, *Escherichia coli* PCM 2057, *Klebsiella pneumoniae* ATCC 700603, *Micrococcus luteus* ATCC 10240, *Proteus mirabilis* CM NCU (isolate from Collegium Medicum Nicolaus Copernicus University), *Pseudomonas aeruginosa* ATCC 10145, *Salmonella infantis* SES (isolate from the Sanitary-Epidemiological Station in Toruń, Poland), *Staphylococcus aureus* PCM 2054 and *Candida albicans* ATCC 10231 using a standard agar plug method (Fiedler, 2004), as described by Świecimska et al. (2022). Briefly, the isolates from the Atacama Desert and pine forest samples were grown on ISP2 and ISP3 agar (Shirling and Gottlieb, 1966) at pH 7.5 and 5.5, respectively, and those from the saline soil adjacent to Lake Lonar on HA agar (Atlas, 2010) supplemented with 3% NaCl and on ISP2 agar (Shirling and Gottlieb, 1966), pH 8.0, following incubation for 3 weeks at 28°C. Plugs (ø = 5 mm) of each of the isolates were cut aseptically using a sterile cork borer, and placed in square Petri dishes (120 x 120 mm). Overnight cultures of the reference bacteria and the yeast grown at 37°C in tryptic soy broth (TSB, Becton Dickinson, United State) and Sabouraud dextrose broth (SDB, Becton Dickinson, United State), respectively, were used to prepare inocula in Luria Bertani broth (LB, Becton Dickinson, United State) at an optical density (OD) of 0.6. These inocula were used to seed LB broths prior to diluting them with the same volume of nutrient agar (NA, Becton Dickinson, United State). In all cases, the final concentration of the reference microorganisms was $1.5–2 \times 10^6$ CFU per mL. Each of these media were poured into the plates containing the agar plugs and the resultant preparations incubated for 24h at 37°C when inhibition zones around the agar plugs were measured in mm. All of the tests were carried out in triplicate. The data acquired from the three experiments were presented as mean values ± standard deviations (SD).

2.5 Screening against fungal and oomycete plant pathogens

These experiments were restricted to 71 isolates representing colour-groups composed of strains from the acidic forest samples; the isolates from the desert and saline soils were not considered as they did not grow optimally, if at all, at pH 5.5-6.0, the required range for the antifungal assays. The pine forest isolates were tested for their ability to inhibit the growth of fungal and oomycete plant pathogens using the co-culture method, as described by Świecimska et al. (2021a; 2022). To this end, the isolates were streaked on the right side of Petri plates of potato dextrose agar (PDA, Becton Dickinson, USA) and incubated for 2 weeks at 28°C. These preparations were inoculated at the opposite side of the Petri plates with the agar plugs (ø = 8 mm). The pathogenic fungi and oomycetes, namely *Phytophthora cactorum, Phytophthora cryptogea*, *Phytophthora megasperma* and *Phytophthora plurivora*, were grown on PDA for 7-21 days at 28°C. The co-cultures were incubated for 7 days in the case of those involving *Alternaria alternata* IOR 1783 (isolated from kohlrabi), *Fusarium culmorum* IOR 2333 (isolated from a pine root), *Fusarium culmorum* D (isolated from wheat), *Phytophthora plurivora* IOR 2303 (isolated from oak rhizosphere), *Rhizoctonia solani* 13 (isolated from a pine root) and *Sclerotina sclerotiorum* IOR 2242 (isolated from broccoli); for 14 days with respect to those involving *Botritis cinerea* IOR 1873 (isolated from tomato), *Colletotrichum acutatum* IOR 2153 (isolated from blueberry), *Fusarium oxysporum* IOR 342 (isolated from pine), *Fusarium poae* A and *Fusarium tricinctum* A (isolated from wheat) and *Phytophthora cactorum* IOR 1925 (isolated from strawberry), and for 21 days in corresponding preparations involving *Fusarium graminearum* A and *Fusarium oxysporum* D (isolated from wheat), *Fusarium solani* IOR 825 (isolated from parsley), *Phytophthora cryptogea* IOR 2080 (isolated from Lawson cypress), *Phytophthora megasperma* IOR 404 (isolated from raspberry), and *Phoma lingam* IOR 2284 (isolated from rape). All of the tests were carried out in triplicate at 28°C. Activity against *Chalara fraxinea* (isolate from ash) was tested using the same procedure, but on malt extract agar (MEA; 20 g L^{-1}, malt extract, 15 g L^{-1} agar) (Kowalski, 2006) with incubation for 8 weeks at room temperature. The negative controls were cultures of the fungal and oomycete pathogens grown under the same incubation conditions. Inhibition (I) of pathogen growth was calculated using the formula: I (%) = (C-T/C) × 100, where C is the diameter of pathogen growth in the control sample and T the diameter of pathogen growth in each of the co-culture samples. Data obtained from *in vitro* experiments were reported as mean values ± standard deviations (SD).

2.6 Promotion of plant growth

The representative isolates were tested, in triplicate, for their ability to produce ammonia, indol-3-acetic acid (IAA) and hydrogen cyanide (HCN) using ISP2 medium (broth and agar, respectively) (Shirling and Gottlieb, 1966), at pH 5.5 and 7.5, as the basal media for the isolates representing the desert and pine forest colour-groups, respectively, whereas halophilic nutrient broth (Atlas, 2010), pH 8.0, supplemented with 3% sodium chloride was the basal medium used for the representatives of the colour-groups containing strains from the saline soil.

All of the isolates were examined for their ability to produce ammonia using the modified method described by Cappucino and Sherman (1992) and the basal media supplemented with L-tryptophan (5 mg mL^{-1}); flasks containing the inoculated media were shaken (150 rpm) for 14-21 days at 28°C then centrifuged (10,000 rpm for 10 min). One mL of each of the resultant supernatants was mixed with 0.5 mL of Nessler's reagent; the development of a yellow to brown colour indicated that ammonia had been formed. Similarly, the production of IAA was detected using the modified method described by Brick et al. (1991); the inoculated basal broth cultures supplemented with L-tryptophan (5 mg mL^{-1}) were shaken (150 rpm) for 14-21 days at 28°C, centrifuged at 10,000 rpm for 10 minutes and aliquots of the supernatants (50 µL) mixed with 100 µl Salkowski reagent (49 mL of 35% perchloric acid and 1 mL of 0.5M $FeCl_3$ solution) and the preparations incubated in the dark for 30 minutes. The development of a pink colour in

the resultant preparations indicated the presence of IAA; the negative control consisted of the corresponding growth media mixed with Salkowski reagent.

The isolates were also examined for their ability to solubilize phosphate using a medium containing 10 g glucose, 0.5 g NH_4SO_4, 0.8 g K_2HPO_4, 0.2 g KH_2PO_4, 0.2 g NaCl, 0.1 g KCl, 0.3 g $MgSO_4 \cdot 7H_2O$, 2 g yeast extract, 2.5 g $Ca_3(PO_4)_2$, 0.5 g arabic gum and 20 g agar; the arabic gum was used to keep the $Ca_3(PO_4)_2$ in suspension. The width of colonies and hydrolysis zones were measured in millimetres after 14 days of incubation at 28°C and activity indices calculated as follows: $Wact = Sh^2 (Sc \times t)$ where Sh indicates the diameter of the hydrolysis zone, Sc the colony diameter and t the time of incubation (Hrynkiewicz et al., 2010).

The production of HCN was determined after Lock (1948). The isolates were grown on slopes of the basal agar media supplemented with glycine (4.4 g L^{-1}) and the inoculated tubes incubated for 7 days at 28°C when Whatman filter paper (Merck) strips soaked with 2% sodium carbonate in 0.5% picric acid solution were inserted into the neck of the tubes; the latter were sealed with parafilm and incubated in the dark for 14 days at 28°C. A colour change on the paper strips from yellow to brown indicated that HCN had been produced. Similarly, the ability of the isolates to synthesis siderophores was established using the method of Alexander and Zuberer (1991); the isolates were inoculated onto Chrome Azurol S (CAS) medium and incubated at 28°C for 14 days. A colour change in the media from blue to orange under and around the colonies indicated the presence of siderophores; the resultant activity indices were estimated, as described above. Both of these tests were conducted three times.

2.7 Synthesis of hydrolytic enzymes

The isolates representing the various colour-groups were examined to establish whether they synthesized a range of hydrolytic enzymes of ecological and potential industrial importance. Strains isolated from the desert, pine forest and saline environmental samples were grown on dedicated media, given below, at pH 7.5, 6.5 and 7.5, respectively. In all cases the isolates were inoculated at the centre of the agar plates; the latter were incubated for 14 days at 28°C when the width of hydrolysis zones around colonies were measured and coefficients of activity determined, as described above. The ability of the isolates to produce cellulases and chitinases were determined according to Berg and Pettersson (1977) and Lingappa and Lockwood (1962), respectively. The production of cellulases was established by flooding incubated plates with a 0.1% solution of Congo red for 15 minutes and then with 1M NaCl for 15 minutes; hydrolysis zones appeared as orange haloes against a red background. Lipolytic and pectinolytic activities were determined using procedures described by Gibson and Gordon (1974) and Strzelczyk and Szpotański (1989), respectively. Hydrolysis of pectin was detected by flooding incubated plates with 10% Cetrimide solution for 15 minutes; zones of clearing around colonies indicated positive results. Proteolytic activity was tested on a medium containing 5 g powdered skimmed milk, 0.1 g $(NH_4)_2SO_4$, 0.1 g $FeSO_4 \cdot 7H_2O$, 0.1 g yeast extract and 15 g agar. Clear zones around colonies were recorded as activity indices. Finally, urease activity was detected after Tidwell et al. (1955); changes in the colour of the medium from yellow to orange-pink were recorded as positive results. All tests for synthesis of hydrolytic enzymes were performed in triplicate.

3 Results

3.1 Colour-group assignment and dereplication of isolates

Most of the representative strains from the selective isolation plates formed extensively branched substrate mycelia bearing either aerial hyphae or distinct masses of coloured spores characteristic of the genus *Streptomyces*. The 59 isolates representing the Atacama Desert

colour-groups were assigned to one single- and 12 multi-membered colour-groups with between 3 and 7 isolates, as shown in Table S1A. None of the isolates formed diffusible pigments or melanin pigments and those belonging to colour-groups 2 and 4 did not produce aerial hyphae. Colour-group 1, one of the two largest taxa, consisted of isolates that exhibited a medium grey aerial spore mass and a moderate yellow green substrate mycelium. The 16 isolates from the saline soil fell into 4 single- and 4 multi-membered colour-groups with between 2 and 5 strains, all but one of which included isolates which produced characteristic diffusible pigments (Table S1B); none of the isolates formed melanin pigments. The largest taxon, colour-group 1, included isolates which formed a distinctive moderate yellow substrate mycelium and a characteristic pale purple diffusible pigment, but not aerial hyphae. The most extensive taxonomic variation was found amongst the 65 strains isolated from the northern slope of the inland pine dune. These isolates were assigned to 7 single- and 14 multi-membered colour-groups with between 2 and 9 strains all of which formed aerial hyphae, apart from isolates belonging to the first colour-group; many of these isolates produced characteristic diffusible pigments, as shown Table S1C. Similarly, the 86 isolates from the southern inland pine dune fell into 6 single- and 12 multi-membered colour-groups with between 2 and 24 strains, all of which formed aerial hyphae (Table S1D). Colour-group 1, the largest taxon, consisted of 24 isolates with distinctive features, a moderate yellow green aerial spore mass, brilliant greenish yellow substrate mycelia and brilliant yellow green diffusible pigments. Only two strains produced melanin pigments on PYEA, namely isolates NL30 and SL26 from the northern and southern sampling sites in the pine forest.

3.2 Taxonomic diversity of representative isolates

The 115 isolates representing the four sets of colour-groups are shown in Table 1 together with their closest phylogenetic neighbours based on 16S rRNA gene sequence similarities. Twenty-eight of the isolates (24%) shared sequence similarities with their nearest neighbours at or below the 99.0% species threshold, as exemplified by isolates NL23, SA10, 2SCA1, SF9, IF12, SL55, NL15 and NH28; these isolates can be considered to be putatively novel species of *Actinacidiphila*, *Actinospica, Kribbella, Nocardia, Nocardiopsis, Pilimelia, Streptacidiphilus* and *Streptomyces*, respectively. In contrast, representatives of several genera showed identical or almost identical sequences with the type strains of validly named species, as illustrated by isolates NF3 and NF23 which shared 100 and 99.86 % gene sequence similarities with the type strains of *C. pinisilvae* and *C. pinistramenti*, respectively. This was also the case with isolates 1G51, 1G52, and 1G14 (100% sequence similarities) and 1G50 (99.86% sequence similarity) with the corresponding type strains of *M. altitudinis* and *M. excelsi*. Similarly, identical or almost identical sequence similarities were found between isolate 5R2A3 and *M. acroterricola* 5R2A7^{T}, isolate OF4 and *Nocardiopsis metallicus* KBS6^{T} and isolate NF10 and *Streptacidiphilus torunensis* NF37^{T}. The most numerous strains showing identical 16S rRNA gene sequences were between isolates and the type strains of *Streptomyces* species, as exemplified by isolates OF2, OF3, OF7 and OF8 and *S. alkaliterrae* OF1^{T}, isolate NH5 and *S. atratus* NRRL B-16927^{T}, and isolates SF4, SF8, SH11 and SL3 and *S. celluloflavus* NRRL B-2493^{T}. Isolates assigned to the genus *Streptomyces* predominated within each of the four sets of colour-groups, as shown in Table 1.

The 28 isolates from the hyper-arid desert soils included in the 16S rRNA gene sequencing analyses were assigned to the genera *Kribbella* (3 strains), *Micromonospora* (2 strains), *Modestobacter* (6 strains), *Pseudonocardia* (4 strains) and *Streptomyces* (13 strains); the codes for the Atacama Desert isolates are given in the footnote to Table 1A. Similarly, the 16 isolates from the saline soil were found to belong to the genera *Nocardiopsis* (5 isolates) and *Streptomyces* (11 isolates). Isolates from the northern slope of the inland pine dune were assigned to the genera *Actinacidiphila* (6 strains), *Catenulispora* (5 strains), *Kitasatospora* (3

strains), *Pilimelia* (1 strain), *Streptacidiphilus* (10 strains) and *Streptomyces* (11 strains), and those from the corresponding southern slope to the genera *Actinacidiphila* (8 strains), *Actinospica* (1 strain), *Nocardia* (3 strains), *Pilimelia* (9 strains), *Streptacidiphilus* (1 strain) and *Streptomyces* (13 strains). The codes for the pine forest isolates in Tables 1C and 1D show that they were isolated from litter, fermentation, humus and mineral horizons.

3.3 Identification of isolates assigned to colour-groups

In general, isolates belonging to colour-groups can be assigned to genera given the distribution of reference isolates included in the 16S rRNA gene sequencing analyses (Supplementary Table S2). In the case of the Atacama Desert isolates, for instance, isolates comprising colour-groups 2 and 5 can be considered to belong to the genera *Micromonospora* and *Kribbella*, respectively, as they include reference isolates found to belong to these taxa. In this context, it is encouraging that colour-group 4 is composed of *M. altitudinis* and *M. excelsi* strains and that colour-group 3 encompasses three of the four isolates shown to belong to the genus *Pseudonocardia*. However, most of the colour-groups encompass isolates that can be considered to be members of the genus *Streptomyces*. Several of the Atacama Desert isolates can be considered to represent putatively novel species, as shown by relationships between isolates 2SCA1, 3G5, 4SCA5 and 2G9 and the type strains of their close phylogenetic neighbours, namely *Kribbella turkmenica* (98.94% sequence similarity), *Pseudonocardia xinjiangensis* (97.81% sequence similarity), *Streptomyces camponoticapitis* (97.58% sequence similarity) and *Streptomyces paradoxus* (98.79% sequence similarity), respectively.

Using the approach outlined above isolates from the saline soil fell into two genera, *Nocadiopsis* (colour-groups 4 to 6 and 8) and *Streptomyces* (colour-groups 1 to 3 and 7). Isolates OF4 and OF6 (colour-group 4) can be provisionally identified as *N. metallicus* as they shared identical or almost identical 16S rRNA gene sequence similarities with the type strain of this species. In contrast, isolates IT2 and IF12, which represent single-membered colour-groups 6 and 5, are putatively novel *Nocardiopsis* species as they show sequence similarities of 97.45 and 98.72% with the type strains of *Nocardiopsis flavescens* and *Nocardiopsis halotolerans*, respectively. Similarly, isolate IF7 from colour-group 3 shared a 97.24% sequence similarity with *Streptomyces cahuitamycinicus* 13K301^{T}. The two largest taxa, colour-groups 1 and 2, were composed of isolates which were found to have identical or very high sequence similarities with type strains of *Streptomyces alkaliterrae* and *Streptomyces alkaliphilus,* respectively.

The most pronounced taxonomic variation was found amongst the isolates from the litter and mineral layers of the northern slope of the inland pine dune. Following the procedure described above, colour-groups 7, 8 and 11 contain *Actinacidiphila* strains, colour-groups 4, 5, 13, 18, 19 and 21 *Streptomyces* strains whereas those in colour-groups 2 and 10 belong to the genus *Kitasatospora*. Similarly, isolates comprising colour-groups 1, 6, 12, 14, 15, 16, 17 and 20 can be considered to be members of the genus *Streptacidiphilus* and those forming colour-group 9 members of the genus *Pilimelia*. It is especially interesting that colour-group 3 encompasses nine isolates assigned to the genus *Catenulispora*, including the type strains of *C. pinisilvae* and *C. pinistramenti*. Several isolates, namely NA4, NL15 and NA21, can be considered as putatively novel species given low sequence similarities with their immediate phylogenetic neighbours, namely *Streptacidiphilus albus* NBRC 100918^{T} (97.73%), *Streptacidiphilus carbonis* DSM 41754^{T} (98.44%) and *Streptacidiphilis hamsterleyensis* (92.01%). Similarly, isolates NH28a, NL16, NL23, NF27, and NH28 are prospective novel species that are most closely related to the type strains of *Actinacidiphila alni* (98.50%), *Actinacidiphila bryophytorum* (98.48%), *Actinacidiphila paucisporea* (98.62%), *Actinacidiphila yanglinensis* (98.14%) and *Streptomyces paludis* (98.13%), respectively. Isolate NH17 shows a very low sequence similarity (92.32%) with its nearest neighbour, *Streptomyces luteireticuli* NBRC 13422^{T}, suggesting that it may belong to a novel genus.

A different pattern of taxonomic diversity was found with strains from the litter and mineral horizons on the southern slope of the inland pine dune. Once again, many of the taxa contained isolates associated with the genus *Streptomyces*, as witnessed by colour-groups 1, 3, 4, 12, 13, 14, 15, 17 and 18 whereas those assigned to colour-groups 7, 8, 10 and 11 contained *Actinacidiphila* strains. Further, most, if not all, of the isolates comprising colour-groups 2 and 5 were closely related to the genus *Pilimelia* whereas those assigned to colour-groups 9 and 16 can be considered to be *Nocardia* and *Streptacidiphilus* strains, respectively. Several strains were found to belong to putative novel species, as exemplified by isolates SL52, SL13, SL5, SA10, SF9, SF13 and SL54, which shared low sequence similarities with the type strains of *A. bryophytorum* (98.61%), *Actinacidiphila rubida* (97.49%), *A. yanglinensis* (98.70%), *Actinospica acidiphila* (98.07%), *Nocardia nova* (98.85%), *Nocardia vinacea* (98.22%) and *Streptomyces ferralitis* (97.65%), respectively. Isolates related to the type strains of *A. bryophytorum, A. yanglinensis, P. columellifera* subspecies *pallida*, *Streptacidiphilus torunensis*, *Streptomyces atratus* and *S. celluloflavus* were also isolated from the northern inland pine dune.

3.4 Antimicrobial activity

Seventy-nine of the 115 isolates (69%) representing the colour-groups were active against at least one of the reference strains in the antimicrobial screening assay (Tables 2, 3). Those from the Atacama Desert soils were more active following growth on ISP3 than on ISP2 agar whereas the isolates from the saline soil showed more activity when grown on HA than on ISP3 agar. In contrast, the isolates from the pine forest soils tended to show similar patterns of activity irrespective of whether they were cultivated on ISP2 or ISP3 agar. Only strains from the pine forest soils inhibited the growth of the *K. pneumoniae* and *P. mirabilis* strains; they also showed more activity against *C. albicans* ATCC 10231 than those from the other sampling sites.

Table 2A shows that the Atacama Desert isolates were most active against the Gram-positive reference strains. In contrast, only isolate 1SCA19, which is most closely related to *Streptomyces purpurascens* NBRC 13077^T (sequence similarity 99.15%), and isolates 5R2A3 and 1SCA21 inhibited the growth of the *E. coli* and *C. albicans* strains following cultivation on ISP2 agar. The most pronounced activity was shown by isolates 2G9, 3HA10 and 1SCA19 which are, in turn, most closely related to *Streptomyces paradoxus* NBRC 14887^T (98.79% sequence similarity), *Streptomyces galbus* DSM 40089^T (99.37% sequence similarity) and the type strain of *Streptomyces purpurascens* (99.15% sequence similarity), respectively. Twelve of the isolates from the hyper-arid Atacama Desert soils, namely ones belonging to the genera *Kribbella* (isolates 2G7 and 2SCA1), *Modestobacter* (isolates 1G4^T, 1G51, 1G52 and 1G14), *Pseudonocardia* (isolates 1HA3, 2R2A4 and 3G5) and *Streptomyces* (isolates 1R2A7, 3G6 and 1R2A1) did not inhibit the growth of any of the reference strains.

The most active strains from the northern slope of the inland pine dune were found to inhibit the growth of the *C. albicans, E. coli*, *M. luteus* and *S. infantis* reference strains. Isolates NA10a, NA24, NH7, NH17, NH28 and NL21 were particularly active against *C. albicans* ATCC 10231 and *M. luteus* ATCC 10240 (inhibition zones 3.0-15.0 mm) (Table 2B); isolates NA10a and NH7, and NA24 and NL21 were most closely related to the type strains of *S. celluloflavus* and *S. xanthochormogenes*, respectively, whereas isolates NH17 and NH28 are members of putatively novel *Streptomyces* species (Table 1). Other strains showing activity against the *C. albicans* strain (inhibition zones 2.0-15.0 mm) were isolates NA4, NA21, prospective members of novel *Streptacidiphilus* species, isolate NH22, which shares a 99.78% sequence similarity with *Streptacidiphilus neutrinimicus* DSM 41755^T, and NL23, a prospective novel *Actinacidiphila* isolates most closely related to *A. paucisporea* CGMCC 4.2025^T. Similarly, *C. pinisilvae* NH11^T and isolate NH28a, which are also members of putatively novel *Actinacidiphila* and *Streptomyces* species, respectively, showed pronounced activity against the

M. luteus strain; isolate NH28a also inhibited the growth of the *S. infantis* strain following growth on ISP2 agar, as did isolate NA21, a representative of a prospective novel *Streptacidiphilus* species.

The most active isolates from the northern slope of the inland pine dune, isolates NA10a and NH7, close relatives of the type strain of *S. celluloflavus*, inhibited the growth of seven of the reference strains following cultivation on either ISP2 or ISP3 agar. Eleven isolates inhibited the growth of *E. coli* PCM 2057 following cultivation on one or both of the cultivation media, namely isolates NA10a, NA21, NH7 and NH17 mentioned above, *C. pinistramenti* NL8^T, and isolates NF22, NH22, NF39 and NH16, which are most closely related to the type strains of *S. hamsterleyensis*, *S. neutrinimicus*, *K. herbaricolor* and *S. atratus*, respectively, and isolates NL16 and NL23, prospective members of novel *Actinacidiphila* species (Table 1). Five strains inhibited the growth of *K. pneumoniae* ATCC 700603 following growth on ISP2 agar, namely isolate NA21 mentioned above, isolates NH21 and NL21, which share sequence similarities of 99.07 and 99.93% with *A. yanglinensis* 1307^T and *S. xanthochromogenes,* NRRL B-5410^T, respectively, and isolates NL15 and NH28, presumptive members of novel *Streptacidiphilus* and *Streptomyces* species, respectively. Nine isolates inhibited the growth of *B. subtilis* PCM 2021, as shown in Table 2B. In contrast, the eleven isolates which did not inhibit the growth of any of the reference strains were members of the genera *Actinacidiphila* (isolate NF27), *Catenulispora* (isolates NF23 and NL13), *Streptacidiphilus* (isolates NA19a, NF10, NF20 and NH14) and *Streptomyces* (isolates NH5, NH15, NL3 and NL35).

The isolates from the corresponding southern slope of the pine forest exhibited a different pattern of activity to their counterparts from the northern slope (Table 2). Thirteen isolates, for instance, inhibited the growth of the *S. aureus* PCM 2054, namely isolate SF17, a close relative of *A. yanglinensis* 1307^T, isolates SA4, SF15 and SL4, which showed their highest sequence similarities with *P. columellifera* subsp. *pallida* MB-SK8^T, isolates SA7, SF4, SF8 and SH11, close relatives of *S. celluloflavus* NRRL B-2493^T, isolates SL5, SL7, SL10 and SL22, which are prospective novel members of *Actinacidiphila* species*,* and isolate SL55, a prospective member of a novel *Pilimelia* species (Table 1D) following growth on ISP2 and ISP3 media. Eleven isolates inhibited the growth of the *M. luteus* reference strain, namely isolates SA7, SF8, SH11, SL3, SL5, SL7, SL10 and SL22 mentioned above, isolate SH15, a close relative of *S. celluloflavus* NRRL B-2493^T and isolates SH56 and SH57 which are close to *Streptomyces cocklensis* BK 168^T and *Streptomyces halstedii* NBRC 12783^T, as shown in Table 1. *Bacillus subtilis* PCM 2021 was inhibited by isolates SF15, SH15, SL5, SL7, SL22 and SL55 mentioned above whereas *S. infantis* SES was inhibited by isolates SA7, SF8 and SH56 mentioned earlier, and isolate SH24, a close relative of *P. columellifera* subspecies *pallida* (inhibition zones ranging from 2.0 to 8.0 mm) following growth on ISP2 and ISP3 agar.

Many of the isolates from the southern slope of the pine forest dune inhibited the growth of the Gram-negative reference strains. Fifteen of them were active against *K. pneumoniae* ATCC 700603 (43%), the corresponding numbers for *E. coli* PCM 2057, *S. infantis* SES, *P. mirabilis* CM NCU and *P. aeruginosa* ATCC 10145 were found to be 12 (34%), 11 (31%), 8 (23%), and 7 (20%), respectively. Four of the eight isolates shown to be closely related to *P. columellifera* subspecies *pallida* MB-SK8^T (isolates SA4, SF15, SH24 and SL24) inhibited the growth of the *E. coli* strain (inhibition zones 4.0-7.0 mm), as did *S. pinistramenti* SF28^T, isolate SL10, a prospective member of a new *Actinacidiphila* species most closely related to the type strain of *A. yanglinensis* and isolate SL55, a prospective member of a novel *Pilimelia* species. Similarly, four of the six isolates closely related to *S. celluloflavus* NRRL B-2493^T (isolates SA7, SF8, SH11 and SL3) suppressed the growth of the *E. coli* strain (inhibition zones 2.0-5.0 mm) following growth on both cultivation media while isolate SH15, one of the two remaining *S. celluloflavus* isolates, showed similar activity following growth on ISP3 agar.

Five of the fifteen isolates cultivated on ISP2 or ISP3 that were active against *K. pneumoniae* ATCC 700603 were close relatives of either *P. columellifera* subspecies *pallida* MB-SK8^T (isolates SA4 and SF15) or *S. celluloflavus* NRRL B-2493^T (isolates SA7, SF8 and SH11). The ten isolates which inhibited the growth of this reference strain following growth on either ISP2 or ISP3 agar were isolates SH24 and SL24, and SF4 and SH15, close relatives of the type strains of *P. columellifera* subspecies *pallida* and *S. celluloflavus*, respectively, *S. pinistramenti* SF28^T, isolates SF17 and SA16, which are most closely related to *A. yanglinensis* and *S. sanglieri*, and isolates SL7, SH20 and SL55, which were shown to be members of putatively novel *Actinacidiphila*, *Nocardia* and *Pilimelia* species, respectively, as shown in Table 1. Similarly, the eight strains that inhibited the growth of *P. mirabilis* CM NCU included five related to the type strains of *P. columellifera* subspecies *pallida* (isolates SA4 and SH24) and *S. celluloflavus* (isolates SH11, SH15 and SL3), isolate SF13, a presumptively novel *Nocardia* species, and isolates SF10 and SH56 which share 100 and 99.15% sequence similarity to *Streptacidiphilus torunensis* NF37^T and *Streptomyces cocklensis* BK168^T, respectively. The seven strains active against the *P. aeruginosa* strain (inhibition zones 2.0-3.0 mm) were *S. pinistramenti* SF28^T and ones closely related to either the type strains of *P. columellifera* subspecies *pallida* (isolates SA4, SF15 and SH24) or *S. celluloflavus* (isolates SA7, SF8 and SH11). Four of the strains mentioned above, isolates SA7, SF8, SH24 and SH56, inhibited the growth of the *S. infantis* strain (inhibition zones ranging from 2.0 to 8.0 mm). In turn, several isolates inhibited the growth of *C. albicans* ATCC 10231, notably isolates SF8, SH11, SH15, SL3 and SL7 which gave inhibition zones ranging from 5.0 to 15.0 mm. The nine isolates which did not show any activity against the reference strains belonged to the genera *Actinacidiphila* (isolates SA23, SL13 and SL52), *Nocardia* (isolate SF9), *Pilimelia* (isolates SF23 and SL16) and *Streptomyces* (isolates SA8, SA20 and SL54).

Table 3 shows that some of the isolates from the saline soil inhibited the growth of the *B. subtilis, C. albicans, M. luteus, P. aeruginosa* and *S. infantis* strains. In contrast, none of them were active against *E. coli* PCM 2057, *K. pneumoniae* ATCC 700603, *P. mirabilis* CM NCU or *S. aureus* PCM 2054. It is noteworthy that all of the *S. alkaliterrae* isolates, apart from OF3, inhibited the growth of *P. aeruginosa* ATCC 10145 and *M. luteus* ATCC 10240 following growth on HA agar. Isolate IT2 was the only *Nocardiopsis* strain to show any activity; it inhibited the growth of the *B. subtilis* strain and may represent a novel species as it was only loosely associated with its closest phylogenetic neighbour, *Nocardipsis flavescens* CGMCC 4.5723^T (97.45% sequence similarity).

3.5 Plant growth promoting features

The results of the triplicated analyses designed to determine the ability of representatives of the colour-groups to produce plant growth promoting compounds are shown in Figure 1 and Table S3. It is apparent from the Figure 1 that many of the isolates from the Atacama Desert and pine forest samples produced ammonia, IAA and siderophores, but relatively few solubilized phosphate. In contrast, none of the isolates from the saline soil produced IAA or were active in solubilizing phosphate while few produced ammonia or siderophores, *Catenulispora pinistramenti* NL8^T was the only isolate found to produce hydrogen cyanide (HCN), a volatile compound that has a role in biocontrol by sequestering iron at the expense of phytopathogens (Gu et al., 2020).

Seven isolates from the Atacama Desert soils (25%) showed an ability to release ammonia while the corresponding figures for those from the saline soil and from northern and southern inland pine slopes were 1 (6%), 16 (44%) and 21 (60%), respectively. The most active strains were *S. pinistramenti* SF28^T, isolates NA10a and SA16, near phylogenetic neighbours to the type strains of *S. celluloflavus* and *S. sanglieri*, respectively, and isolates NL15 and NH28, which were found to be members of prospective novel species of *Streptacidiphilus* and *Streptomyces,*

as they share low sequence similarities with their close phylogenetic relatives, *S. carbonis* DSM 41954^T (98.44% sequence similarity) and *S. paludis* GSSD-12^T (98.13% sequence similarity), respectively. Strains showing a less pronounced ability to form ammonia included isolates NL3 and SA7 which are close to *S. celluloflavus* NRRL B-2493^T, isolates NA24 and NL21, which have a similar relationship to *S. xanthochromogenes* NRLL B-5410^T, and isolates NH9, NA19a and NL35, which are close to *K. herbaricolor* (99.43% sequence similarity), *Streptacidiphillus durhamensis* (99.86% sequence similarity) and *Streptomyces yanii* (99.12% sequence similarity), respectively. Isolate OT1, the only strain from the saline soil to produce ammonia, was found to share a sequence similarity of 99.50% with its immediate phylogenetic neighbour, *Nocardiopsis valliformis* DSM 45023^T.

It is evident from Supplementary Table S2 that eleven isolates (39%) from the Atacama Desert soils synthesized IAA, the corresponding numbers from the northern and southern slopes of the pine forest were 42 and 26%, respectively. The most active strains were isolates 1HA1, 5R2A3 and SA8, which have identical or almost identical sequence similarities with *K. italica* BC637^T, *M. acroterricola* 5R2A7^T and *S. sanglieri* NBRC 100784^T, as shown in Table 1. Twelve strains were less active in synthesizing IAA, including *C. pinisilvae* NH11^T. *C. pinistramenti* NL8^T, isolates NF10 and NF20, which share identical or almost identical sequence similarities with *S. torunensis* NF37^T, and isolate 1SCA21 mentioned earlier. This group of isolates can be extended to include NL16 and NF27, members of prospective novel *Actinacidiphila* species that are most closely related to *A. bryophytorum* NEAU-HZ10^T (98.48% sequence similarity) and *A. yanglinensis* 1307^T (98.14% sequence similarity), respectively.

Fifty-eight isolates (50%) produced siderophores with activity indices ranging from 0.3 to 17.7. Fifteen of those from the Atacama Desert (54%) showed positive activity indices whereas only three of the isolates from the saline soil (19%) did so; the corresponding numbers for those from the litter and mineral horizons of the northern and southern slopes of the inland pine dunes were 17 (47%) and 23 (66%), respectively. The six Atacama Desert strains with the highest activity indices (range 9.3 to 17.7) were isolates 1G2 and 1R2A1, close relatives to *S. flaveolus* NBRC 3715^T (99.72% sequence similarities), isolate 1SCA21 mentioned above, isolates 5SCA7 and 5HA2, near relatives to *Streptomyces marokkonensis* Ap1^T (99.50% sequence similarity) and *S. mutabilis* NBRC 12800^T (99.79% sequence similarity), respectively, and isolate 2G9, which is close to *S. paradoxus* NBRC 14887^T. Two of the three active isolates from the saline soil, OF5 and IF17, were found to be phylogenetically close to *S. alkaliterrae* and *S. alkaliphilu*s, respectively; the remaining strain, isolate IF7, as mentioned previously, represent a presumptively novel *Streptomyces* species. The seven isolates from the pine forest sampling sites which gave the highest activity indices (range 8.6-14.2) were of *C. pinisilvae* NF3, *S. pinistramenti* SF28^T, isolates NH7, SA7 and SH11, and isolate SH57, close relatives of *S. celluloflavus* NRRL 2493^T, *S. halstedii* NBRC 12783^T, respectively, and isolate NH17, a member of a prospective novel *Streptomyces* species. Similarly, five of the remaining six strains close to the type strain of *S. celluloflavus*, that is, isolates NA10a, SF4, SF8, SH15 and SL3, were found to have activity indices within the range 1.3-6.6. Similarly, isolates NF24, NF39 and NH9, phylogenetic neighbours to *K. herbaricolor* NBRC 12876^T, have activity indices ranging from 2.8-4.1.

Few isolates from the Atacama Desert and pine forest sites solubilized phosphate (Supplementary Table S3). The three active isolates from the desert soils were 5R2A3, 1R2A7 and 1SCA21, near relatives of *M. acroterricola* 5R2A7^T, *S. aquilus* GGCR-6^T and *S. tendae* ATCC 19812^T, respectively. The isolates from the pine forest sites which solubilized phosphate were isolates NF24, NF39 and NH9, and SF8, SH15 and SL3, which are closely related to the type strains of *K. herbaricolor* and *S. celluloflavus*, respectively, and isolate NL21, a close relative to *S. xanthochromogenes* NRRL B-5410^T.

3.6 Enzyme activity

The ability of isolates representing the colour-groups to synthesize hydrolytic enzymes is shown in Figure 2 and Supplementary Table S3. It is evident from the Figure that many of the isolates from each of the sampling sites produce cellulases, lipases, proteases and ureases, and to a lesser extent chitinases. All of the isolates from the saline soil hydrolysed tributyrin and showed proteolytic activity, and most from the pine forest sites hydrolysed urea. In contrast, only isolates from the Atacama Desert and pine forest soils hydrolyzed pectin. Eighty-eight of the isolates (77%) produced zones of clearing against tributyrin; the corresponding results for the hydrolysis of cellulase, chitin, milk powder, pectin and urea were 42 (37%), 26 (23%), 71 (62%), 16 (14%) and 74 (64%), respectively. In contrast, pectinases were mainly produced by isolates from the Atacama Desert soils. The isolates from the saline soil metabolized tributyrin, but not pectin, while only isolate IF17, a close relative to *S. alkaliphilus* DSM 42118^T, hydrolyzed chitin. Four pine forest isolates degraded pectin, namely NA10a and SH15, SH56, and SH57, which are close relatives to *S. celluloflavus* NRRL B-2493^T *S. cocklensis* BK168^T and *S. halstedii* NBRC 12783^T, respectively. In contrast, 12 of the 28 Atacama Desert isolates (43%) hydrolyzed pectin. Five of these strains showed activity indices which ranged from 12.6 to 21.4, namely isolates 1R2A7, 3HA10 and 1SCA21, which are phylogenetically close to *S. aquilus*, *S. galbus* and *S. tendae*, respectively, and isolates 2G9 and 4SCA5, members of putative novel *Streptomyces* species (Table 1). Similarly, isolate 1G2, a close relative of *S. flaveolus* NBRC 3715^T, and isolate 2SCA1, a member of potentially novel *Kribbella* species were found to have activity indices of 9.4 and 8.3, respectively. The pine forest strain, isolate SH56, a close relative to *S. cocklensis* BK168^T, had an activity index of 12.8.

Fifteen out of 28 (54%) isolates from the Atacama Desert soils and 10 out of 16 (63%) isolates from saline soil produced cellulases. The corresponding numbers from the northern and southern pine dune slopes were low at 25 and 23%, respectively. In general, the highest activity indices were shown by isolates from the desert and saline soils, as exemplified by eight Atacama Desert strains which had activity indices that ranged from 13.4 to 27.3 (Supplementary Table S3A). These isolates included 1R2A1 and 1G2, close relatives to *S. flaveolus* NBRC 3715^T, 3HA10 and 2SCA1 mentioned earlier, and 5HA2, and 5SCA5 and 5SCA7, which are phylogenetically close to *S. mutabilis* NBRC 12800^T and *S. marokkonensis* Ap1^T, respectively while 5R2A3 is closely related to *M. acroterricola* 5R2A7^T.

Seven isolates from the saline soil had activity indices for cellulose degradation ranging from 9.4 to 17.1; three of them, IF11, IF15 and IF19, are close to *S. alkaliphius* DSM 42118^T, two, OF4 and OF6 to *N. metallicus* KBS6^T whereas IT2 and IF7 were shown to be members, of prospective novel species of *Nocardiopsis* and *Streptomyces* (Table 1). Seven of the pine forest isolates showed activity indices for cellulase hydrolysis at or above 8.3, including NH28a and NL23, members of prospective novel *Actinacidiphila* species; the remaining isolates, namely SL16, NL3, SH56, SH57 and NL35 are near neighbours of the type strains of *P. columellifera* subspecies *pallida*, *S. celluloflavus*, *S. cocklensis*, *S. halstedii* and *S. yanii*, respectively. In contrast, isolates 1HA3, 2R2A4 and 3G5, all of which were assigned to the genus *Pseudonocardia*, did not degrade cellulose nor did they hydrolyze chitin, pectin or milk protein.

Twenty-six (23%) of the isolates from the sampling locations degraded chitin albeit with low activity indices (Supplementary Table S3). In marked contrast, all of the isolates from the saline soil hydrolyzed powdered milk, as did most of those from the Atacama Desert soils (57%); the corresponding results for isolates from the northern and southern pine dune slopes were 42 and 69%. High levels of proteolytic activity (activity indices at or above 12.0) were recorded for eleven of the isolates (9.6%), including 2G7 and NH7, close relatives of *K. flavida* DSM 17836^T and *S. celluloflavus* NRRL B-2493^T, respectively, and isolate 2G9, a member of a prospective novel *Streptomyces* species. In turn, the eight strains from the pine forest sites showed pronounced proteolytic activity, namely isolates NF24, NF39 and NH9, NH15, NH16, SA16,

and SL19 and SL24 which are close to the type strains of *K. herbaricolor*, *S. yanii*, *S. atratus*, *S. sanglieri* and *P. columellifera* subspecies *pallida*, respectively. Similarly, isolates SF23, SF8 and SA8, which are close to the type strains of *P. columellifera*, *S. celluloflavus* and *S. sanglieri,* respectively, were found to have activity indices ranging from 10.9 to 11.9. In addition, high indices of proteolytic activity, 11.1 and 11.6, were recorded for isolate IF15, a close relative to the type strain of *S. alkaliphilus*, and isolate 2SCA1, a member of a prospective novel *Kribbella* species.

Few of the isolates from the saline and Atacama Desert soils hydrolyzed urea. In contrast, 30 (83%) and 32 (91%) of those from the northern and southern pine dune slopes did so. Urease production in many of the pine forest strains was high, as exemplified by strains closely related to the type strains of *A. yanglinensis* (isolates NH21, SL5, SL10, SL22), *P. columellifera* subspecies *pallida* (isolates SA4, SF23, SL4, SL16, SL19, SL55 and SL24), *S. atratus* (isolates NH5 and NH16) and *S. celluloflavus* (isolates NA10a, SA7, SH11, SH15 and SL3).

3.7 Activity against fungal and oomycete plant pathogens

Many of the representative isolates from the pine forest soils showed a remarkable ability to inhibit the growth of the fungal and oomycete plant pathogens, as shown in Figure 3 and Supplementary Table S4. In general, isolates from the southern slope of the inland pine dune showed more activity than those from the northern slope. Isolates from the southern inland dune were particularly active against *F. culmorum* IOR 2333*, F. culmorum* D*, F. graminearum* A, *F. oxysporum* IOR 342*, P, cactorum* IOR 1925, and *P. plurivora* IOR 2303. In contrast, a few isolates from the northern slope showed pronounced activity against *P. lignam* IOR 2284.

Sixty-two out of the 71 (87%) representative isolates from the pine forest sites showed inhibition indices over 50% against at least one of the 19 pathogens. The highest activity levels were recorded for *S. pinistramenti* SF28^T which inhibited the growth of seventeen of the plant pathogens, including eleven where inhibition values fell within the range 82.4-94.6% It is encouraging that results from this study are in good agreement with those reported by Świecimska et al. (2022). Isolate NA10a, which was found to have an identical 16S rRNA gene sequence similarity with *S. celluloflavus* NRRL B-2493^T, inhibited the growth of eleven of the pathogens, as did isolate NH17, a member of a putative novel *Streptomyces* species. The growth of eight of the pathogens was markedly reduced by isolate SH15, a close relative of the type strain of *S. celluloflavus*; similar results were recorded for isolate NH28, another putatively novel *Streptomyces* strain.

Representatives of other genera shown to have an inhibition value >50% against some of the plant pathogens included isolates NH9 and NF39, close relatives of *K. herbaricolor* NBRC 12876^T, and isolates NH22 and SF10, which share high or identical sequence similarities with *S. neutrinimicus* DSM 41755^T and *S. torunensis* NF37^T, respectively. Isolates NH7 and SF9 can be added to this group, the former is a close relative to *S. celluloflavus* NRRL B-2493^T*,* and the latter is a member of a putatively novel *Nocardia* species. Isolates showing high levels of activity against the plant pathogens were isolated from each of the horizons of the pine forest.

The *C. fraxinea*, *F. graminearum* A and *P. plurivora* IOR 2303 strains were particularly sensitive to some of the isolates, as shown in Supplementary Table S4. In contrast, few of the strains inhibited the growth of either *F. solani* IOR 825 or *F. oxysporum* D. The exceptions were *S. pinistramenti* SF28^T and isolate NA24, these strains inhibited the growth of the *F. solani* strain to varying degrees. Isolates NF24 and NF39, close associates to *K. herbaricolor* NBRC 12876^T, inhibited the growth of *F. oxysporum* D, as did isolate NH17, one of the putatively novel *Streptomyces* species, and isolates SA20 and SH56, which were found to share high sequence similarities with *S. atratus* NNRL B-16927^T (99.79%) and *S. cocklensis* BK168^T (99.15%), respectively.

4 Discussion

4.1 Colour-group assignment, dereplication of isolates and phylogeny

The effectiveness of culture-dependent strategies designed to detect novel specialized metabolites of biotechnological interest tend to reflect the taxonomic diversity of filamentous actinomycetes isolated from extreme habitats (Goodfellow et al., 2018; Benaud et al., 2022; Pathom-aree et al., 2023). It is encouraging that in the present study filamentous actinomycetes chosen to represent colony types growing on selective isolation plates were recovered in a broad range of single- and multi-membered colour-groups given their ability to form distinctive pigments on oatmeal agar. Colour-groups have been used extensively as an index of actinomycete diversity in natural habitats, including extreme biomes (Goodfellow and Fiedler, 2010; Idris, 2016, Kusuma, 2020). It is interesting that in this study the most extensive actinomycete diversity was found in the litter and mineral horizons of the pine forest.

Confidence can be placed in the 16S rRNA gene sequence data, not least because few significant incongruities were found between whole-genome and 16S rRNA gene trees in an extensive genome-based classification of actinomycetes (Nouioui et al., 2018). The phylogenetic data showed that isolates representing each set of colour-groups had distinctive taxonomic profiles thereby underpinning the pioneering work of Williams and his colleagues who found that the distribution of actinomycete populations in different habitats was a product of different environmental variables, such as organic matter content, pH, temperature and water availability (Williams and Mayfield, 1971; Williams et al., 1972). Indeed, it is now known that in Atacama Desert soils intensive solar irradiation acts synergistically with desiccation in limiting the survival and growth of microbial life (Gómez-Silva, 2018).

Representative isolates from the high altitude, hyper-arid Atacama Desert soils and saline soil were assigned to the genera *Kribbella, Micromonospora, Modestobacter* and *Pseudonocardia,* and *Nocardiopsis*, respectively, results in good agreement with those from previous surveys (Idris, 2016; Meklat et al., 2020; Tsetseg, 2023). Similarly, acidotolerant and acidophilic actinomycetes from the pine forest sites assigned to the genera *Actinospica, Catenulispora, Kitasatospora, Nocardia* and *Streptacidiphilus* tallies with those from earlier studies (Golińska et al., 2023). Members of all of these taxa are known to synthesize bioactive compounds, including novel antibiotics (Takahashi, 2017; Hifnawy et al., 2020; Riahi et al., 2022; Virués-Segovia et al., 2022; Golińska et al., 2023).

The taxonomic status of individual colour-groups can be determined from the distribution of the reference strains known to represent specific genera or species (Atalan et al., 2000; Sembiring et al., 2000; Manfio et al., 2003). In this study, multi-membered colour-groups containing isolates from the Atacama Desert soils were found to belong to the genus *Streptomyces* and the four genera mentioned above. In turn, saline isolates assigned to multi-membered groups corresponded to or were closely related to *Nocardiopsis metallicus* (Schippers et al., 2002) and *Streptomyces alkaliterrae* (Świecimska et al., 2020) while ones forming single-membered colour-groups were most closely related to *Nocardiopsis halotolerans* (Al-Zarban et al., 2002), *Nocardiopsis flavescens* (Fang et al., 2011) and *Nocardipsis valiformis* (Yang et al., 2008). Similarly, multi-membered colour-groups encompassing isolates from the northern slope of the pine forest were equated with the genera *Actinacidiphila, Catenulispora, Kitasatospora, Pilimelia, Streptacidiphilus* and *Streptomyces*. Corresponding multi-membered colour-groups composed of isolates from the southern slope of the pine forest were either equated with the genera *Actinacidiphila, Actinospica, Nocardia* and *Streptomyces* or were close to or *bona fide* members of *Pilimelia columellifera* subspecies *pallida* (Vobis et al., 1986) and *Streptomyces celluloflavus* (Nishimura et al., 1953) Madhaiyan et al., 2020. These data provide further evidence that 16S rRNA gene sequencing remains a practical way of determining the taxonomic status of filamentous actinomycetes isolated from

extreme habitats (Goodfellow et al., 2018; Singh and Dubey, 2018; Sharma and Thakur, 2020; Liu et al., 2021).

Over 40% of the isolates assigned to the colour-groups belonged to the genus *Streptomyces*. The highest number, 69%, were from the saline soil and the lowest, 27%, from the northern slope of the inland pine dune. These results are in line with those from previous studies where streptomycetes were shown to be the dominant component of extreme habitats (Bull and Asenjo, 2013; Jiang et al., 2018; Sharma and Thakur, 2020). In contrast, streptomycetes have not featured as major components of actinomycete communities in culture-independent studies, as exemplified by analyses of actinomycetes in Atacama Desert soils (Idris et al., 2017a; Bull et al., 2018) and diverse extreme biomes in Indonesia (Kusuma, 2020). These disparities can be attributed to biases in culture-independent methods, such as difficulties in extracting DNA from streptomycetes, choice of PCR primers, RNA copy numbers and PCR amplification (Kutchma et al., 1998; Klappenbach et al., 2000; Engelbrektson et al., 2010; Zielińska et al., 2017), to associated data handling issues (Claesson et al., 2010; Escobar-Zepeda et al., 2015), and to the use of isolation media that select for streptomycetes (Goodfellow and O'Donnell, 1989).

Several isolates assigned to the genus *Streptomyces* were found to belong to putatively novel species whereas others showed relatively low sequence similarities with the type strains of their closest phylogenetic neighbours or were close to streptomycetes that have rarely been isolated from natural habitats, such as *Streptomyces atratus* (Shibata et al., 1962), *Streptomyces cocklensis* (Kim et al., 2012), *Streptomyces mutabilis* (Preobrazhenskaya and Ryabova, 1957), *Streptomyces flaveolus* (Waksman, 1923; Waksman and Henrici 1948) and *Streptomyces sanglieri* (Manfio et al., 2003). These results are particularly interesting as similar isolates from extreme habitats have been found to produce novel specialized metabolites (Donald et al., 2022), including *Streptomyces leeuwenhoeki* strains from hyper-arid Atacama Desert soils(Busarakam et al., 2014) which synthesize ansamycin and macrolactone polyketides (Goodfellow et al., 2018).

Twenty-two (19.1%) of representative isolates found to belong to putatively novel species were assigned to genera other than *Streptomyces*. Nearly half of them were most closely related to the type strains of *Streptomyces* species that were transferred to the genus *Actinacidiphila*, namely *A. alni, A. bryophytorum, A, paucisporea, A. rubida* and *A. yanglinensis* (Madhaiyan et al., 2022) whereas others were most closely related to three validly named *Streptacidiphulus* species, *S. albus* (Kim et al., 2003), *S. carbonis* (Kim et al., 2003) and *S. hamsterleyensis* (Golińska et al., 2013). It seems likely that presumptively novel isolates shown to be most closely related to the type strains of *Streptomyces cocklensis* (Kim et al., 2012) and *Streptomyces ferralitis* (Saintpierre-Bonaccio et al., 2004) will be found to belong to the genus *Actinacidiphila* given the close phylogenetic relationships of these taxa with *Streptomyces* species recently transferred to this genus (Labeda et al., 2012; 2017). It is also interesting that isolate SA10 is most closely related to the type strain of *Actinospica* acidiphila (Cavaletti et al., 2006), an actinomycete found to merit generic status (Golińska et al., 2023). Similarly, presumptively novel isolates from the other sampling sites were found be most closely related to *Kribbella turkmenica* (Saygin et al., 2019), *Nocardiopsis flavescens* (Fang et al., 2011), *Nocardiopsis halotolerans* (Al-Zarban et al., 2002) and *Pseudonocardia xinjiangensis* (Xu et al., 1999; Huang et al., 2002). It is likely that some of the isolates found to share sequence similarities above the 99.0% threshold with the type strains of their immediate phylogenetic neighbours will be members of putatively novel species. Indeed, it has been shown that actinomycetes sharing very high and almost identical 16S rRNA gene sequence similarities can be classified into different species based on extensive polyphasic taxonomic studies, as shown in the case of closely related members of the genera *Gordonia*, *Micromonospora* and *Streptomyces* strains (Riesco et al., 2018, 2022; Kusuma et al., 2021).

The taxonomic data acquired in this study show that selective isolation, dereplication and initial characterization of representative isolates from diverse extreme biomes is a simple and practical way of selecting putatively novel and rare filamentous actinomycetes for exploitative biotechnology. In addition, these data underpin the rational of ecologically guided bioprospecting campaigns featuring actinomycetes (Mitra et al., 2011; Nalini and Prakash, 2017; Liu et al., 2021; Wang et al., 2022). Many of the isolates found to belong to rare and putatively novel taxa were from the litter and mineral horizons of the pine forest indicating that acidophilic and acidotolerant filamentous actinomycetes should feature more prominently in the search for novel bioactive compounds, especially given evidence that such strains are a source of novel antibiotics and acid-stable enzymes (Golińska et al., 2023). Further studies are also needed to determine whether litter and mineral horizons in coniferous woodlands contain characteristic actinomycete communities, as implied by Goodfellow and Dawson (1978).

4.2 Antimicrobial activity

There is a urgent need to find a new generation of antibiotics that are effective against multidrug-resistant microbial pathogens (Tacconelli et al., 2018), especially Gram-negative ones that cause high mortality rates in hospital acquired infections (Mehrad et al., 2015). It is encouraging that nearly 70% of the dereplicated isolates included in the antimicrobial screens showed activity against at least one of a panel of reference strains, as was the case in corresponding studies on isolates from extreme hyper-arid Atacama Desert soils where hit rates of 68% were recorded (Busarakam, 2014; Idris, 2016). These figures are much higher than those recorded in comparable studies on non-dereplicated isolates (Sengupta et al., 2015; Priyadarshini et al., 2016; Prieto-Davó et al., 2016). Further, the importance of growing dereplicated isolates on more than one production medium (Goodfellow and Fiedler, 2010) was underlined by instances where positive results were only reported for isolates grown on only one of the cultivation media.

The isolates from the pine forest sites, notably those from the southern inland pine dune, were especially effective in inhibiting the growth of the *E. coli* (34%), *K. pneumoniae* (28%), *P. aeruginosa* (10%), *P. mirabilis* (15%) and *S. infantis* (30%) strains following growth on one or both of the cultivation media. The most active isolates were *S. pinistramenti* SF28^{T} and those closely related to *P. columellifera* subspecies *pallida* and *S. celluloflavus*, as they inhibited the growth of all but one of the reference strains. In contrast, few, if any, of the isolates from the Atacama Desert and saline sites inhibited the growth of the *E. coli, K. pneumoniae* and *P. mirabilis* strains. In contrast, the five *S. alkaliterrae* isolates from the saline soil inhibited the growth of the *P. aeruginosa* strain following growth on HA agar; isolate 1G2, a near relative of *S. flaveolus*, from the Atacama Desert soil also inhibited the growth of this reference strain when cultivated on ISP3 agar.

The ability of the isolates to inhibit the growth of the Gram-positive reference strains was evenly distributed across all of the sampling sites though none of the isolates from the saline soil were active against *S. aureus* PCM 2054 and only a putatively novel *Nocardiopsis* strain inhibited the growth of *B. subtilis* PCM 2021. Thirty-five of the isolates (30%) showed activity against the latter, the corresponding figures for the *M. luteus* and *S. aureus* reference strains were 53 (46%) and 38 (33%), respectively, following growth on at least one of the production media. Comparable results were recorded against the *B. subtilis* and *S. aureus* reference strains for dereplicated filamentous actinomycetes from high altitude Atacama Desert soils (Idris, 2016).

Most of the twenty-five isolates (22%) active against all of the Gram-positive reference strains were from the pine forest sites. These isolates included *S. pinistramenti* SF28^{T} and ones found to be closely related to *S. celluloflavus*, *P. columellifera* subspecies *pallida*, and *S. xanthochromogenes*; four of the remaining strains were assigned to putatively novel

Actinacidiphila species and another two were members of prospective novel *Streptomyces* species. Similarly, two of the four corresponding isolates from the Atacama Desert soils were found to be putatively novel *Streptomyces* species; the remaining ones, isolates 3HA10 and 1SCA19, were most closely related to *Streptomyces galbus* (Frommer, 1959) and *Streptomyces purpurascens* (Lindenbein, 1952), respectively.

Most of the thirty-three isolates (29%) shown to be active against *C. albicans* ATCC 10231 were from the pine forest soils though the five *S. alkaliterrae* isolates from the saline soil also gave positive results. The fifteen isolates from the pine forest which gave positive results following growth on ISP2 and ISP3 agar included five that were close to *S. celluloflavus*, two to *S. xanthochromogenes,* one to *P. columellifera* subspecies *pallida* (isolate NL28), and six belonging to presumptively novel *Actinacidiphila*, *Streptacidiphilus* and *Streptomyces* species. The final strain, isolate NH22, is phylogenetically close to the type strain of *Streptacidiphilus neutrinimicus*.

Little, if anything, is known about the antimicrobial activities of the taxa cited above though a strain from an acid mangrove soil identified as *S. celluloflavus* produced diverse specialized metabolites (Nguyan and Cao, 2022). Similarly, a soil isolate identified as *S. purpurascens* synthesized several bioactive compounds, notably rhodomycin C, which is particularly active against *B. subtilis* (Holkar et al., 2013). Further, a strain identified as *S. xanthochromogenes* showed antifungal activity (Singh et al., 2016), as did a *P. columellifera* subspecies *pallida* isolate which was active against fungi-causing superficial mycoses (Wypij et al., 2017).

4.3 Plant growth promoting features

Microbial inoculants are needed to promote plant growth and control plant diseases given challenges posed by climate change and sustainable agriculture (Debasis et al., 2019; Pacios-Michelena et al., 2021). Actinomycetes from extreme habitats are increasingly being seen as relevant in this respect given their role in nutrient recycling and promoting plant growth, as witnessed by their capacity to solubilize phosphate, synthesize phytohormones, notably IAA, lytic enzymes and siderophores (Boukhatem et al., 2022; Pathom-aree et al., 2023). The use of *Streptomyces venezuelae* as a biofertilizer, for example, increased maize production under drought conditions due to its ability to secrete high levels of IAA and L-aminocyclopropane (ACC) (Chukwuneme et al., 2020); ACC is an immediate precursor of the gaseous hormone, ethylene. Plant growth promoting bacteria (PGPB) play an important role lowering plant stress by reducing ethylene levels by hydrolyzing ACC to ammonia, α-ketobutyrate and methionine thereby regulating ethylene production (Mulani et al., 2021); plants inoculated with ACC-deaminase producing bacteria are more resistant to abiotic and biotic stress (Gupta and Pandey, 2019). In the present study, representative isolates from the Atacama Desert and pine forest sampling sites produced ammonia and IAA, solubilized phosphate and synthesized siderophores. In contrast, none of those from the saline soil produced IAA or solubilized phosphate and only one, isolate OT1, a close relative to *Nocardiopsis valliformis*, formed ammonia. However, actinomycetes are known to promote the growth of plants adapted to saline conditions, as exemplified by an endophytic *Micromonospora chalcea* strain which enhanced the growth of *Salicornia bigelovii* (El-Tarabily et al., 2019).

In general, actinomycetes isolated from the pine forest were the most proficient in synthesizing PGP-metabolites, as shown by five isolates that were most active in producing ammonia, namely *S. pinistramenti* SF28^{T}, strains close to the type strains of *S celluloflavus* and *S. sanglieri* and ones considered to be members of presumptive novel species of *Streptacidiphilus* and *Streptomyces*. Isolates 1HA1 and 5R2A3, near relatives of *Kribbella italica* (Everest et al., 2015) and *Micromonospora acroterricola* (Carro et al., 2019), respectively, were particularly gifted in their ability to produce IAA, as was isolate SA8, another close relative of *S. sanglieri*.

Thirteen isolates were shown to be especially active in synthesizing siderophores (activity indices 8.6-17.7). They included six from the Atacama Desert, namely ones phylogenetically close to *Streptomyces flaveolus*, *Streptomyces marokkonensis* (Bouizgarne et al., 2009), *Streptomyces mutabilis* (Preobrazhenskaya and Ryabova, 1957) and *Streptomyces tendae* (Ettlinger et al., 1958), the remaining strain, isolate 2G9, belongs to a putatively novel *Streptomyces* species most closely related to *Streptomyces paradoxus* (Krasil'nikov and Yuan, 1961; Goodfellow et al., 1986). The corresponding organisms from the pine forest sites were *C. pinisilvae* NF3, *S. pinistramenti* SF28^{T}, isolates close to *S. celluloflavus* and *S. halstedii,* and isolate NH17, a presumptively novel *Streptomyces* species. The remaining isolates close to *S. celluloflavus* also produced siderophores, notably NA10a, SH15, SF8 and SL3, which showed activity indices ranging from 4.7-6.6. These results provide additional evidence that taxonomically diverse filamentous actinomycetes are a valuable source of iron-binding compounds (Franco-Correa and Chavarro-Anzola, 2016), many of which promote plant growth (Boukhatem et al., 2022).

4.4 Enzyme activity

Actinomycetes also promote plant growth by secreting hydrolytic enzymes, notably cellulases and chitinases, which convert insoluble polymers into nutrients which act as natural fertilizers (Jog et al., 2016; Nouioui et al., 2019). Novel hydrolytic enzymes are also a valuable resource for industrial processes (Mukhtar et al., 2017; Jin et al., 2019). Isolates from all of the sampling sites produced cellulases, chitinases, lipases and proteinases though pectinase activity was restricted mainly to ones from the Atacama Desert soils, notably isolates 1R2A7, 3HA10 and 1SCA21, which were found to be close relatives to the type strains *Streptomyces aquilus*, *S. galbus* and *S. tandae*, respectively, and 2G9 and 4SCA5, members of two prospective novel *Streptomyces* species. Several isolates showed a marked ability to produce cellulases (activity indices ≥15.0), mainly ones related to *M. acroterricola* , *N. metallicus, S. cocklensis* , *S. flaveolus, S. galbus*, *S. marokkonensis* and isolate IF7, which was assigned to a putatively novel *Streptomyces* species. Atacama Desert isolates also showed an ability to produce lipases (activity indices >10), as shown by strains closely related to *K. flavida*, *M. acroterricola*, *Pseudonocardia khuvsgulensis* (Ara et al., 2011), *Pseudonocardia rhizophila* (Li et al., 2010) and *S. mutabilis*; two additional strains, isolates 2SCA1 and 3G5, were shown to be putatively novel *Kribbella* and *Pseudonocardia* species, respectively.

The seventy-one isolates (62%) which secreted proteases included all of those from the saline soil. Isolates showing high or very high activity indices (>10) were recovered from all of the sampling sites. Most of the isolates were streptomycetes that either belonged or were closely related to *S. alkaliphilus*, *S. alkaliterrae*, *S. atratus*, *S. celluloflavus*, *S. sanglieri*, *S. yanii* and *S. xanthochromogenes* or, as in the case of isolate 2G9, belonged to a putatively novel *Streptomyces* species. The most highly active non-streptomycetes isolates were either closely related to the type strains of *K. herbaricolor* and *K. flavida* or represented a prospective novel *Kribbella* species. These results are in sync with those reported for many strains isolated from extreme habitats, as exemplified by a strain found to be closely related to the type strain of *Kribbella gitaiheensis* (Tsetseg and Badamgavar, 2021), a soil isolate (Guo L. et al., 2015).
It is not surprising that isolates from each of the sampling sites hydrolyzed urea, as this is common feature of streptomycetes. However, all of the most highly active strains were from the pine forest soils, including *S. pinistramenti* SF28^{T}, and ones closely related to *S. atratus* and *S. yanii*. In addition, seven out of the nine isolates closely related to the type strain of *S. celluloflavus* gave strong reactions. Similarly, all but one of the isolates closely related to *P. columellifera* subspecies *pallida* gave highly positive results, the exception, isolate SF15, was negative. Most of the putatively novel *Actinacidiphila*, *Streptacidiphilus* and *Streptomyces* isolates gave markedly positive results.

4.5 Activity against fungal and oomycete plant pathogens

New effective eco-friendly methods are needed to control phytopathogenic fungi, especially ones that reduce yields of staple crops (Law et al., 2017; Debasis et al., 2019; Boukhatem et al., 2022; Ebrahimi-Zarandi et al., 2022). Actinomycetes are to the fore amongst microbial control agents given their ability to produce antifungal compounds and siderophores, secrete enzymes that degrade fungal cell walls and compete for nutrients (Guo X. et al., 2015; Alblooshi et al., 2021). Taxonomically diverse actinomycetes, notably streptomycetes, inhibit the growth of phytopathogens, as exemplified by Abdelrahman et al. (2022) who found that streptomycetes from rhizosphere and soil samples in the Sudan inhibited the growth of the devastating oomycete pathogen *Phytophthora infestans*. Further, an actinomycete closely related to *Streptomyces spectabilis* inhibited the growth of eleven plant pathogens (Chen et al., 2018) whereas *Nocardiopsis* and *Streptomyces* were found to show *in vitro* and *in planta* activity against bacterial and fungal pathogens of carrots and tomatoes (Djebaili et al., 2021). In this context, it is remarkable that so many of the isolates from the litter and mineral horizons of the pine forest inhibited the growth of the fungal pathogens. Little is known about the functional roles of actinomycetes in acidic forest soils though it seems likely that they will compete with fungal populations for nutrients (Matthies et al., 1997; Rousk et al., 2009).

Taxonomically diverse isolates, albeit mainly streptomycetes, showed pronounced broad spectrum activity (>70% inhibition) against many of the fungal and oomycete pathogens, notably isolates showing high or identical sequence similarities with the type strains of *S. celluloflavus* and *S. xanthochromogenes*. The most active isolate in this respect was *S. pinistramenti* SF28^{T} which inhibited the growth of 17 of the 19 plant pathogens, a result that built upon the earlier work of Świecimska et al. (2022). These results provide further evidence, that streptomycetes from poorly studied extreme habitats have the ability to control the growth of plant pathogens (Suksaard et al., 2017; Pathom-aree et al., 2019; Qi et al., 2019).

Non-streptomycete isolates which showed either pronounced or notable broad spectrum activity against some of the fungal pathogens included ones closely related to *Catenulispora pinisilvae*, *Kitasatospora herbaricolor*, *Streptacidiphilus neutrinimicus* and *Streptacidiphilus torunensis,* and putatively novel isolates that were most closely related to the type strains of *Nocardia nova* and *Streptacidiphilus hamsterleyensis*. These results provide further evidence that isolates assigned to several actinomycete genera have the ability to inhibit the growth of fungal pathogens (Martínez-Hidalgo et al., 2015; Ebrahimi-Zarandi et al., 2022). Few of the isolates showed pronounced activity against the oomycetes, a notable exception was *S. pinistramenti* SF28^{T}, which inhibited the growth of *P. cactorum* and *P. plurivora*; several isolates which shared high or identical sequence similarities with the type strains of *P. columellifera* subspecies *pallida* and *S. celluloflavus* also inhibited the latter.

5 Conclusions

Initial steps in natural product pipelines designed to discover new bioactive compounds from actinomycetes are often taken for granted given an understandable focus on outcomes, that is, the commercial exploitation of novel chemical compounds. Nevertheless, the selective isolation, characterization and screening of actinomycetes from unexplored or poorly studied extreme biomes highlights actinomycetes that can be prioritized in the search for new bioactive compounds using state-of-the-art technologies, such as genome mining, genetic engineering and procedures that allow rapid dereplication of chemical entities from complex biological extracts.

In the present study the importance of the early steps in the natural product pipeline was underpinned by the isolation of taxonomically diverse actinomycetes from three extreme ecosystems which produced a broad range of bioactive compounds. It was particularly

significant that dereplicated isolates from the litter and mineral horizons of the pine forest included members of rare and novel genera which not only showed an extraordinary ability to inhibit the growth of diverse fungal and oomycete phytopathogens, but also inhibited the growth of members of Gram-negative taxa that are on the list of multidrug-resistant taxa highlighted by the World Health Organization. In addition, representatives of several genera, notably *Actinacidiphila*, *Pilimelia* and *Streptomyces,* not only inhibited the growth of a panel of microorganisms in the antimicrobial assays, but also produced compounds that promote plant growth. Another notable outcome of this study was the isolation of so many putatively novel species, especially ones belonging to the genera *Streptomyces* and the association of other isolates with rare validly named species belonging to poorly studied genera, such as *Actinacidiphila, Kribbella, Pilimelia* and *Streptacidiphilus*. Further work is now in the progress to build upon these developments.

Consequently, all-embracing culture-dependent studies such as the present one should be seen as an integral part of platforms designed to foster research collaboration on actinomycetes designed to address some of the critical challenges facing humankind, not least the need to find new antibiotics for multiple purposes and effective biofertilizers and bioinoculants for sustainable agricultural practices. It is also worth noting that enforceable measures are needed to safeguard actinomycete communities in fragile biomes given problems associated with habitat destruction and the effects of climate breakdown.

6 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7 Author Contributions

PG and MG: conceptualization, writing and editing the manuscript. PG and MŚ: methodology and validation. MŚ formal analysis, investigation, resources, data curation, writing—original draft preparation, visualization, project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

8 Funding

This research was funded by Nicolaus Copernicus University, grant Nos. 90-SIDUB.6102.40.2021.G4NCUS1 and 1207-B and the APC was funded by IDUB NCU.

9 Acknowledgments

Not applicable

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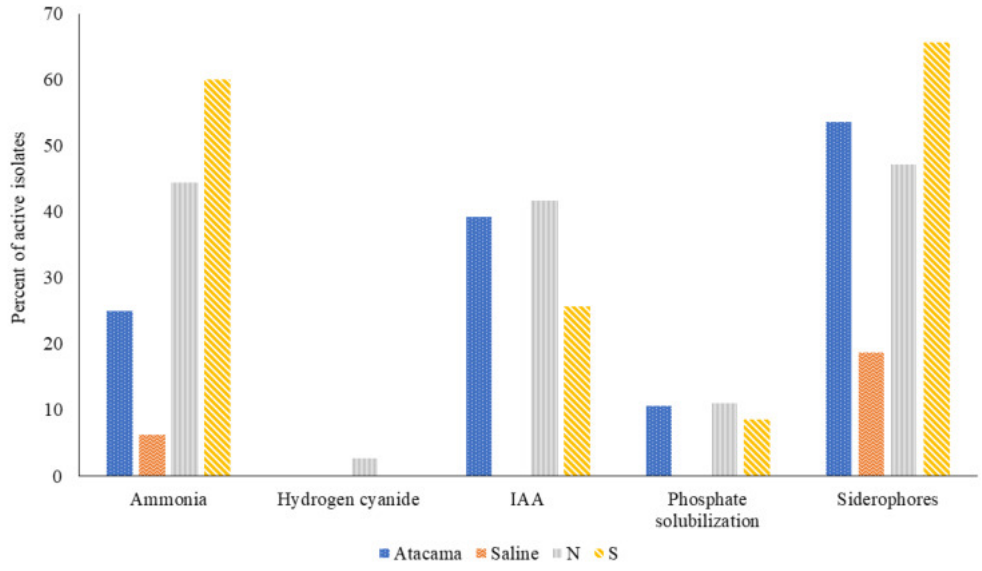

Figure 1. Representative isolates [%] from the Atacama Desert, saline and acid forest soil sampling sites which were found to produce compounds that promote plant growth. N and S, isolates from the northern and southern slopes of the inland dunes of the pine forest.

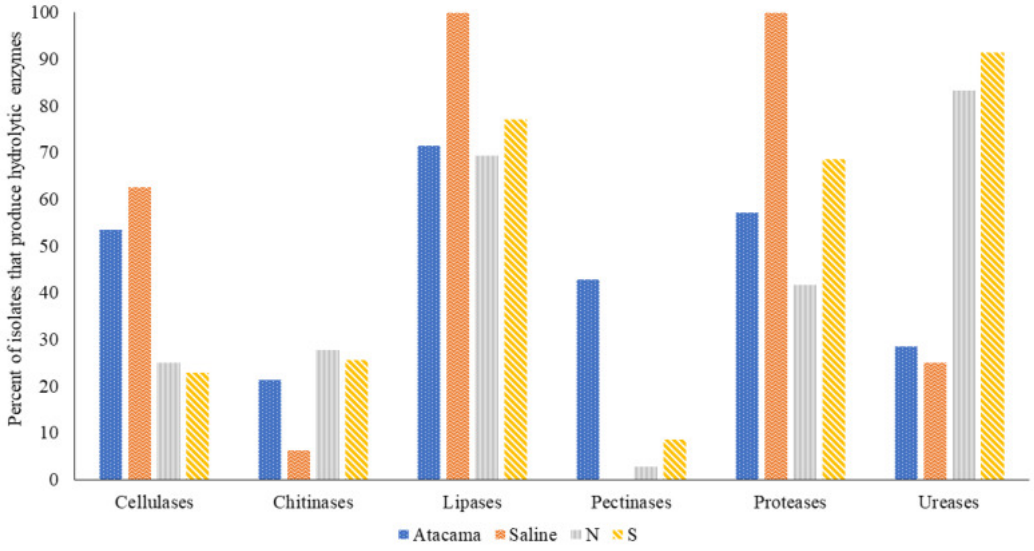

Figure 2. Representative isolates [%] from the Atacama Desert, saline and acidic pine forest soil which were found to produce hydrolytic enzymes. N and S, isolates from the northern and southern slopes of the inland dunes of pine forest.

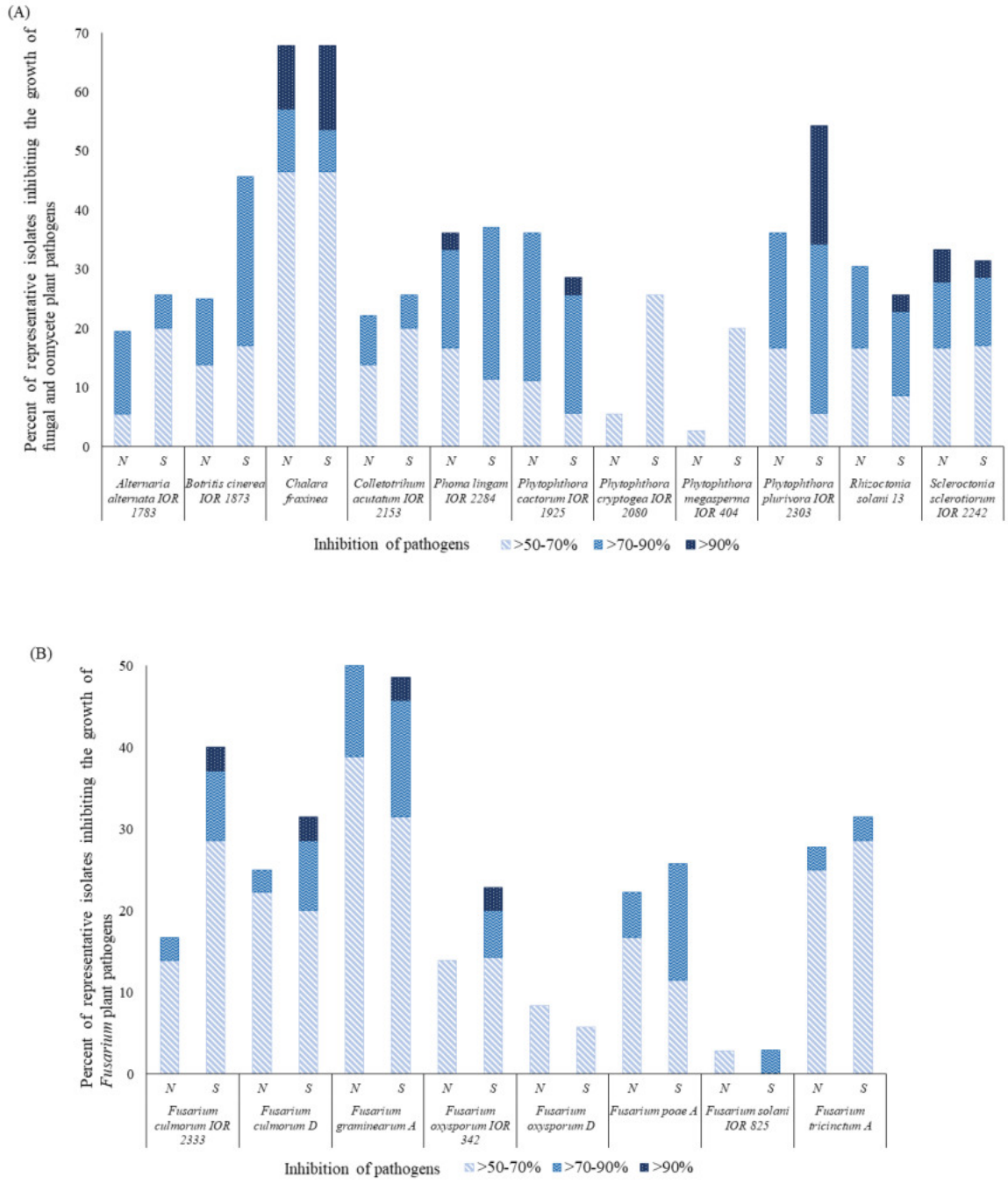

Figure 3. Representative isolates [%] inhibiting fungi and fungi-like organisms tested using a co-culture method. N and S, isolates from the northern and southern slopes of the inland dunes of the pine forest. (A) Fungal and fungal-like pathogens; (B) *Fusarium* species that are agents of a plant diseases

1628 **Table 1.** Nearest neighbours of isolates based on 16S rRNA gene sequence similarities using
1629 the EzBioCloud server (Yoon et al., 2017).
1630

Isolates	Nearest neighbours	Codes of type strains	Sequence similarity	Nucleotide differences/ total number of nucleotides	Length of 16S rRNA genes
(A) Representative isolates from the hyper-arid Atacama Desert soils					
2G7	*Kribbella flavida*	DSM 17836^T	99.43	8/1411	1416
1HA1	*Kribbella italica*	BC637^T	99.36	9/1411	1416
2SCA1	***Kribbella turkmenica***	**16K104^T**	**98.94**	**15/1413**	**1417**
5R2A3	*Micromonospora acroterricola*	5R2A7^T	99.72	4/1407	1411
5R2A7^T*	*M. acroterricola*	5R2A7^T	100	0/1413	1413
1G4^T*	*Modestobacter altitudinis*	1G4^T	100	0/1526	1526
1G51*	*M. altitudinis*	1G4^T	100	0/1397	1398
1G52*	*M. altitudinis*	1G4^T	100	0/1391	1394
1G6^T*	*Modestobacter excelsi*	1G6^T	100	0/1526	1526
1G14*	*M. excelsi*	1G6^T	100	0/1408	1416
1G50*	*M. excelsi*	1G6^T	99.86	2/1410	1417
1HA3	*Pseudonocardia khuvsgulensis*	MN08-A0297^T	99.36	9/1399	1399
2R2A4	*Pseudonocardia rhizophila*	YIM 67013^T	99.72	4/1411	1416
4R2A1	*P.rhizophila*	YIM 67013^T	99.64	5/1403	1403
3G5	***Pseudonocardia xinjiangensis***	**AS 4.1538^T**	**97.81**	**29/1325**	**1408**
5SCA4	*Streptomyces albogriseolus*	NRRL B-1305^T	99.43	8/1412	1412
1R2A7	*Streptomyces aquilus*	GGCR-6^T	99.28	10/1393	1405
3G6	*Streptomyces bungoensis*	DSM 41781^T	99.51	7/1419	1423
4SCA5	***Streptomyces camponoticapitis***	**2H-TWYE14^T**	**97.58**	**34/1407**	**1412**
1G2	*Streptomyces flaveolus*	NBRC 3715^T	99.72	4/1419	1424
1R2A1	*S. flaveolus*	NBRC 3715^T	99.72	4/1411	1411
3HA10	*Streptomyces galbus*	DSM 40089^T	99.37	9/1419	1424
5SCA5	*Streptomyces marokkonensis*	Ap1^T	99.50	7/1412	1412
5SCA7	*S. marokkonensis*	Ap1^T	99.50	7/1412	1412
5HA2	*Streptomyces mutabilis*	NBRC 12800^T	99.79	3/1422	1426
2G9	***Streptomyces paradoxus***	**NBRC 14887^T**	**98.79**	**17/1405**	**1411**
1SCA19	*Streptomyces purpurascens*	NBRC 13077^T	99.15	12/1411	1415
1SCA21	*Streptomyces tendae*	ATCC 19812^T	99.15	12/1411	1411
(B) Representative isolates from saline soil adjacent to Lake Lonar					
IT2	***Nocardiopsis flavescens***	**CGMCC 4.5723^T**	**97.45**	**36/1412**	**1415**
IF12	***Nocardiopsis halotolerans***	**DSM 44410^T**	**98.72**	**18/1410**	**1411**
OF4	*Nocardiopsis metallicus*	KBS6^T	100	0/1419	1417
OF6	*N. metallicus*	KBS6^T	99.86	2/1417	1417
OT1	*Nocardiopsis valliformis*	DSM 45023^T	99.50	7/1413	1421
IF11*	*Streptomyces alkaliphilus*	DSM 42118^T	99.42	8/1387	1389
IF15	*S. alkaliphilus*	DSM 42118^T	99.36	9/1414	1414
IF17*	*S. alkaliphilus*	DSM 42118^T	99.52	7/1451	1535
IF19*	*S. alkaliphilus*	DSM 42118^T	99.50	7/1402	1402
OF1^T*	*Streptomyces alkaliterrae*	OF1^T	100	0/1534	1534
OF2	*S. alkaliterrae*	OF1^T	100	0/1406	1406
OF3*	*S. alkaliterrae*	OF1^T	100	0/1450	1534
OF5	*S. alkaliterrae*	OF1^T	99.79	3/1413	1413
OF7	*S. alkaliterrae*	OF1^T	100	0/1401	1401
OF8*	*S. alkaliterrae*	OF1^T	100	0/1450	1534
IF7	***Streptomyces cahuitamycinicus***	**13K301^T**	**97.24**	**39/1411**	**1411**
(C) Representative isolates from the litter and mineral horizons of the northern slope of inland dune of the pine forest					
NH28a	***Actinacidiphila alni***	**D65^T**	**98.50**	**21/1403**	**1403**
NL16	***Actinacidiphila bryophytorum***	**NEAU-HZ10^T**	**98.48**	**21/1386**	**1388**
NL23	***Actinacidiphila paucisporea***	**CGMCC 4.2025^T**	**98.62**	**19/1377**	**1385**

NF27	***Actinacidiphila yanglinensis***	**1307^{T}**	**98.14**	**26/1395**	**1406**
NH21	*A. yanglinensis*	1307^{T}	99.07	13/1401	1406
NH27	*A. yanglinensis*	1307^{T}	99.22	11/1408	1415
NH11^{T}*	*Catenulispora pinisilvae*	NH11^{T}	100	0/1441	1525
NF3*	*C. pinisilvae*	NH11^{T}	100	0/1525	1525
NL13	*C. pinisilvae*	NH11^{T}	99.79	3/1401	1402
NF23*	*Catenulispora pinistramenti*	NL8^{T}	99.86	2/1441	1519
NL8 ^{T}*	*C. pinistramenti*	NL8^{T}	100	0/1398	1398
NF24	*Kitasatospora herbaricolor*	NBRC 12876^{T}	99.71	4/1403	1403
NF39	*K. herbaricolor*	NBRC 12876^{T}	99.64	5/1402	1402
NH9	*K. herbaricolor*	NBRC 12876^{T}	99.43	8/1404	1408
NL28	*Pilimelia columellifera subsp. pallida*	MB-SK 8^{T}	99.64	5/1408	1420
NA4	***Streptacidiphilus albus***	**NBRC 100918^{T}**	**97.73**	**31/1364**	**1371**
NL15	***Streptacidiphilus carbonis***	**DSM 41754^{T}**	**98.44**	**22/1407**	**1422**
NA13	*Streptacidiphilus durhamensis*	FSCA67^{T}	99.64	5/1385	1417
NA19a	*S. durhamensis*	FSCA67^{T}	99.86	2/1382	1383
NA21	***Streptacidiphilus hamsterleyensis***	**HSCA 14^{T}**	**92.01**	**109/1365**	**1406**
NF22	*S. hamsterleyensis*	HSCA 14^{T}	99.56	6/1361	1403
NH22	*Streptacidiphilus neutrinimicus*	DSM 41755^{T}	99.78	3/1377	1383
NF10	*Streptacidiphilus torunensis*	NF37^{T}	100.00	0/1381	1412
NF20	*S. torunensis*	NF37^{T}	99.86	2/1380	1401
NH14	*S. torunensis*	NF37^{T}	100.00	0/1381	1422
NH5	*Streptomyces atratus*	NRRL B-16927^{T}	100.00	0/1425	1435
NH16	*S. atratus*	NRRL B-16927^{T}	99.79	3/1419	1424
NA10a	*Streptomyces celluloflavus*	NRRL B-2493^{T}	100.00	0/1408	1408
NH7	*S. celluloflavus*	NRRL B-2493^{T}	99.86	2/1398	1398
NL3	*S. celluloflavus*	NRRL B-2493^{T}	100.00	0/1418	1425
NH17	***Streptomyces luteireticuli***	**NBRC 13422^{T}**	**92.32**	**107/1393**	**1431**
NH28	***Streptomyces paludis***	**GSSD-12^{T}**	**98.13**	**26/1390**	**1390**
NA24	*Streptomyces xanthochromogenes*	NRRL B-5410 ^{T}	100.00	0/1404	1404
NL21	*S. xanthochromogenes*	NRRL B-5410 ^{T}	99.93	1/1412	1412
NH15	*Streptomyces yanii*	NBRC 14669^{T}	99.20	11/1377	1397
NL35	*S. yanii*	NBRC 14669^{T}	99.12	12/1366	1409
(D) Representative isolates from the litter and mineral horizons of the southern slope of inland dune of the pine forest					
SL7	***Actinacidiphila bryophytorum***	**NEAU-HZ10^{T}**	**98.33**	**23/1381**	**1390**
SL52	***A. bryophytorum***	**NEAU-HZ10^{T}**	**98.61**	**19/1363**	**1368**
SL13	***Actinacidiphila rubida***	**13C15^{T}**	**97.49**	**35/1394**	**1408**
SA23	*Actinacidiphila yanglinensis*	1307^{T}	99.36	9/1416	1425
SF17	*A. yanglinensis*	1307^{T}	99.50	7/1400	1400
SL5	***A. yanglinensis***	**1307^{T}**	**98.70**	**18/1380**	**1387**
SL10	***A. yanglinensis***	**1307^{T}**	**98.84**	**16/1375**	**1381**
SL22	***A. yanglinensis***	**1307^{T}**	**98.78**	**17/1392**	**1399**
SA10	***Actinospica acidiphila***	**GE134766^{T}**	**98.07**	**27/1400**	**1406**
SF9	***Nocardia nova***	**NBRC 15556^{T}**	**98.85**	**16/1397**	**1399**
SF13	***Nocardia vinacea***	**NBRC 16497^{T}**	**98.22**	**25/1407**	**1413**
SH20	***N. vinacea***	**NBRC 16497^{T}**	**98.26**	**24/1378**	**1384**
SA4	*Pilimelia columellifera* subsp. *pallida*	MB-SK 8^{T}	99.71	4/1398	1403
SF15	*P. columellifera* subsp. *pallida*	MB-SK 8^{T}	99.71	4/1398	1403
SF23	*P. columellifera* subsp. *pallida*	MB-SK 8^{T}	99.71	4/1398	1401
SH24	*P. columellifera* subsp. *pallida*	MB-SK 8^{T}	99.71	4/1396	1412
SL4	*P. columellifera* subsp. *pallida*	MB-SK 8^{T}	99.72	4/1408	1410
SL16	*P. columellifera* subsp. *pallida*	MB-SK 8^{T}	99.71	4/1398	1414
SL19	*P. columellifera* subsp. *pallida*	MB-SK 8^{T}	99.71	4/1398	1414
SL24	*P. columellifera* subsp. *pallida*	MB-SK 8^{T}	99.71	4/1398	1414
SL55	***P. columellifera* subsp. *pallida***	**MB-SK 8^{T}**	**99.00**	**14/1406**	**1417**
SF10	*Streptacidiphilus torunensis*	NF37^{T}	100.00	0/1381	1418
SA20	*Streptomyces atratus*	NRRL B-16927^{T}	99.79	3/1419	1425

SA7	*Streptomyces celluloflavus*	NRRL B-2493^{T}	99.79	3/1422	1430
SF4	*S. celluloflavus*	NRRL B-2493^{T}	100.00	0/1404	1404
SF8	*S. celluloflavus*	NRRL B-2493^{T}	100.00	0/1404	1404
SH11	*S. celluloflavus*	NRRL B-2493^{T}	100.00	0/1419	1425
SH15	*S. celluloflavus*	NRRL B-2493^{T}	99.79	3/1401	1402
SL3	*S. celluloflavus*	NRRL B-2493^{T}	100.00	0/1423	1430
SH56	*Streptomyces cocklensis*	BK168^{T}	99.15	12/1404	1409
SL54	***Streptomyces ferralitis***	**SFOp68^{T}**	**97.65**	**33/1406**	**1411**
SH57	*Streptomyces halstedii*	NBRC 12783^{T}	99.79	3/1419	1427
SF28^{T}*	*Streptomyces pinistramenti*	SF28^{T}	100	0/1404	1404
SA8	*Streptomyces sanglieri*	NBRC 100784^{T}	100.00	0/1401	1401
SA16	*S. sanglieri*	NBRC 100784^{T}	99.79	3/1419	1426

Codes and names in bold indicate isolates with 16S rRNA sequence similarity at or below the 99.0% threshold for delineating actinomycete species.

*Isolates recently classified as new species: 1G4^{T}, 1G51 and 1G52 (Golińska et al. 2020b); 1G6^{T}, 1G14 and 1G50 (Golińska et al., 2020a); IF11, IF17, IF19, OF1^{T}, OF3 and OF8 (Świecimska et al., 2020); NF3 and NH11^{T} (Świecimska et al., 2021a); NF23 and NL8^{T} (Świecimska et al., 2021b); SF28^{T} (Świecimska et al., 2022) and 5R2A7^{T} (Carro et al., 2019).

Codes for the isolates from the Atacama Desert soils: numbers 1-5 at the beginning of the code indicate the sample sites, G, HA, R2A, SCA the isolation medium and then the isolate number. Codes: NA, NL, NF and NH/SA, SL, SF and SH refer to isolates from the mineral, litter, fermentation and humus layers of the northern and southern slopes of the inland dune of the pine forest. ^{T}, type strain.

Table 2. Antimicrobial activity of representative actinomycetes isolated from the Atacama Desert and pine forest soils using a standard plug assay.

Strains	*B. subtilis* PCM 2021		*E. coli* PCM 2057		*K. pneumoniae* ATCC 700603		*M. luteus* ATCC 10240		*P. mirabilis* (CM NCU)		*P. aeruginosa* ATCC 10145		*S. infantis* (SES)		*S. aureus* PCM 2054		*C. albicans* ATCC 10231	
Basal medium	**ISP2**	**ISP3**	**ISP2**	**ISP3**	**ISP2**	**ISP3**	**ISP2**	**ISP3**	**ISP2**	**ISP3**	**ISP2**	**ISP3**	**ISP2**	**ISP3**	**ISP2**	**ISP3**	**ISP2**	**ISP3**
(A) Representative isolates from the hyper-arid Atacama Desert soils																		
1G2	-	-	-	-	-	-	-	-	-	-	-	5.0* ±0.0	-	-	-	-	-	-
1G6^T	-	-	-	-	-	-	2.0 ±0.0	-	-	-	-	-	-	-	-	-	-	-
1G50	-	-	-	-	-	-	4.0* ±0.0	-	-	-	-	-	-	-	-	-	-	-
1HA1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.0* ±0.0	-	-	-
1SCA19	-	3.0 ±0.0	2.0 ±0.0	-	-	-	7.0 ±0.0	9.0* ±0.0	-	-	-	-	-	2.0 ±0.0	2.0 ±0.0	5.0 ±0.0	-	-
1SCA21	-	-	-	-	-	-	5.7 ±0.6	9.0 ±0.0	-	-	-	-	-	3.0 ±0.0	-	4.0* ±0.0	2.0 ±0.0	-
2G9	2.0 ±0.0	5.0 ±0.0	-	-	-	-	10.0 ±0.0	13.0 ±0.0	-	-	-	-	-	-	4.0 ±0.0	4.0 ±0.0	-	-
3HA10	-	9.0 ±0.0	-	-	-	-	-	6.0 ±0.0	-	-	-	-	-	-	2.0* ±0.0	4.0 ±0.0	-	15.0 ±0.0
4R2A1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.0 ±0.0	-	-	-
4SCA5	-	2.3 ±0.6	-	-	-	-	7.0 ±0.0	10.0 ±0.0	-	-	-	-	-	-	-	3.3 ±0.6	-	-
5HA2	2.0* ±0.0	4.0 ±0.0	-	-	-	-	5.0 ±0.0	4.3 ± 0.6	-	-	-	-	-	-	-	-	-	-
5R2A3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.0 ±0.0	-
5R2A7^T	-	-	-	-	-	-	-	2.0 ±0.0	-	-	-	-	-	-	-	-	-	-
5SCA4	-	5.0 ±0.0	-	-	-	-	-	2.0 ±0.0	-	-	-	-	-	-	-	-	-	-
5SCA5	4.0 ±0.0	3.0 ±0.0	-	-	-	-	-	-	-	-	-	-	-	-	6.0 ±0.0	-	-	-
5SCA7	3.0 ±0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	6.0 ±0.0	-	-	-

(B) Representative isolates from the litter and mineral horizons of the northern slope of the inland dune of the pine forest																		
NA4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.0 ±0.0	11.0 ±0.0
NA10a	4.0 ±0.0	7.0* ±0.0	2.0 ±0.0	3.0 ±0.0 4.0* ±0.0	-	-	5.0 ±0.0	13.0 ± 0.0	-	2.0 3.0* ±0.0	-	-	-	2.0 ±0.0	2.0 ±0.0	4.0 ±0.0	12.0 ±0.0	14.0 ±0.0
NA13	8.0 ±0.0	-	-	-	-	-	3.0 ±0.0	-	-	-	-	-	-	-	-	-	-	-
NA21	-	-	5.0 ±0.0	-	2.0 ±0.0	-	-	-	-	-	-	-	12.0 ± 3.6	-	-	-	9.3 ±0.6	10.0 ±0.0
NA24	-	8.0 ±0.0	-	-	-	-	14.3 ±0.6	10.0 ±0.0	-	-	-	-	-	-	10.0 ±2.0	8.0 ± 0.0	7.7 ±0.6	10.0* ±0.0
NF3	-	-	-	-	-	-	-	2.0* ±0.0	-	-	-	-	-	2.0 ±0.0	-	-	-	4.0* ±0.0
NF22	-	-	-	4.0 ±0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	13.0 ±0.0
NF24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.0* ±0.0
NF39	-	-	-	3.0 ±0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NH7	2.0 ±0.0	9.0* ±0.0	-	4.0 2.0* ±0.0	-	-	4.0 ±0.0	14.0 ±0.0	-	6.0 ±0.0	-	-	-	3.0 ±0.0	-	5.0 ±0.0	-	15.0 ±0.0
NH9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.0* ±0.0
NH11^{T}	5.0 ±0.0	-	-	-	-	-	8.7 ±1.6	2.0 ±0.0	-	-	-	-	-	-	-	-	-	3.0* ±0.0
NH16	-	-	-	2.0* ±0.0	-	-	-	5.0 ±0.0	-	-	-	-	-	-	-	-	-	-
NH17	-	4.0 ±0.0	5.7 ±0.6	-	-	-	8.7 ±0.6	11.0 ±0.0	-	-	-	-	-	3.0 ±0.0	-	6.0 ±0.0	9.3 ±0.6	12.0 ±0.0
NH21	-	-	-	-	2.0 ±0.0	-	-	-	-	-	-	-	-	-	-	-	-	-
NH22	-	-	-	2.0* ±0.0	-	-	-	-	-	-	-	-	-	-	-	-	15.0 ±0.0	8.0 ±0.0
NH27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NH28	1.3 ±0.6	-	-	-	2.0 ±0.0	-	6.7 ±0.6	10.0 ±0.0	-	-	-	-	-	2.0 ±0.0	-	4.0* ±0.0	7.3 ±0.6	9.0 ±0.0
NH28a	9.0 ±0.0	9.0 ±0.0	-	-	-	-	11.7 ±0.6	8.0 ±0.0	-	-	-	-	15.3 ±5.0	-	9.0 ±0.0	7.0 ±0.0	-	-

NL8^{T}	-	-	-	2.0* ±0.0	-	-	5.0 ±0.0	-	-	-	-	-	-	-	-	-	-	-
NL15	-	-	-	-	2.0 ±0.0	-	3.3 ±1.2	-	-	-	-	-	2.0* ±0.0	-	-	-	-	-
NL16	-	-	-	2.0* ±0.0	-	-	-	5.0 ±0.0	-	-	-	-	-	-	-	-	-	-
NL21	12.0 ±0.0	8.0 ±0.0	-	-	7.7 ±0.6	-	13.7 ±0.6	3.0 ±0.0	-	-	-	-	2.0* ±0.0	3.0 ±0.0	12.0 ±1.0	8.0 ±0.0	9.7 ±0.6	9.0* ±0.0
NL23	-	-	-	2.0* ±0.0	-	-	-	-	-	-	-	-	-	-	-	-	2.0 ±0.0	11.0 ±0.0
NL28	-	-	-	-	-	-	-	5.0 ±0.0	-	2.0 3.0* ±0.0	-	-	-	2.0 ±0.0	2.0 ±0.0	4.0 ±0.0	12.0 ±0.0	14.0 ±0.0
(C) Representative isolates from the litter and mineral horizons of the southern slope of the inland dune of the pine forest																		
SA4	14.0 ±1.0	-	7.0 ±0.6	-	4.3 ±0.6	5.0* ±0.0	3.7 ±0.6	-	7.7 ±0.6	-	2.7 ± 0.6	-	4.3 ±0.6	-	6.0 ±0.0	5.0* ±0.0	-	-
SA7	-	-	3.3 ±0.6	2.0* ±0.0	3.3 ±0.6	7.0 ±0.0	3.7 ±0.6	14.0* ±0.0	-	-	2.3 ± 0.6	-	3.3 ±0.6	4.0 ±0.0	4.7 ±1.2	7.0 ±0.0	-	5.0 ±0.0
SA10	-	8.0* ± 0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SA16	-	-	-	-	-	7.0* ±0.0	-	-	-	-	-	-	-	-	-	7.0* ±0.0	-	-
SF4	12.0 ±0.0	-	-	-	3.3 ±1.2	-	3.3 ±0.6	-	-	-	-	-	-	-	14.3 ±0.6	7.0 ±0.0	-	-
SF8	-	-	3.7 ±0.6	4.0 ±0.0	3.0 ±0.0	4.0 ±0.0	6.0 ±0.0	11.0* ±0.0	-	-	2.7 ± 0.6	-	3.0 ±0.0	3.0 ±0.0	4.7 ±0.6	5.0 ±0.0	9.7 ±0.6	5.0 ±0.0
SF10	-	-	-	-	-	-	-	-	-	2.0* ±0.0	-	-	-	-	7.0 ±0.0	-	-	-
SF13	-	-	-	-	-	-	-	-	-	2.0* ±0.0	-	-	-	-	-	-	-	-
SF15	10.7 ±0.6	4.0 ± 0.0	4.7 ±0.6	-	4.3 ±0.6	7.0* ±0.0	4.3 ±0.6	-	-	-	2.0 ±0.0	-	3.3 ±1.2	-	7.7 ±0.6	7.0* ±0.0	-	-
SF17	-	-	-	-	-	5.0* ±0.0	-	-	-	-	-	-	-	-	7.0 ±1.0	5.0* ±0.0	-	-
SF28^{T} **	9.7 ±1.2	-	3.0 ±1.0	-	2.0 ±0.0	-	3.0 ±0.1	-	-	-	3.0 ±0.1	-	3.3* ±0.6	-	6.2 ±0.6	-	-	-
SH11	10.3 ±1.2	-	3.0 ±1.0	5.0 ±0.0	2.3 ±0.6	7.0 ±0.0	4.0 ±0.0	13.0* ± 0.0	-	6.0 ±0.0	2.3 ±0.6	-	3.0 ±1.0	-	4.3 ±0.6	7.0 ±0.0	10.0 ±0.0	7.0 ±0.0
SH15	3.0 ±0.0	7.7 ±0.6	-	3.0 5.0* ±0.0	-	5.0* ±0.0	5.0 ±0.0	12.7 ±0.6	-	3.0 ±0.0	-	-	-	3.0 ±0.0	-	5.0* ±0.0	10.0 ±0.0	15.0 ±0.0

SH20	-	-	-	-	-	6.0 ±0.0	-		-	-	-	-	-	-	-	6.0 ±0.0	-	-
SH24	11.0 ±1.0	-	5.3 ±1.2	-	4.0 ±0.0		3.3 ±0.6		8.3 ±1.5	-	2.3 ±0.6	-	4.3 ±0.6	4.0 ±0.0	7.3 ±1.2	-	-	-
SH56	-	-	-	-	-	-	2.0* ±0.0	7.0* ±0.0	4.0 ±0.0	-	-	-	8.0 ±1.2	2.0* ±0.0	-	-	3.7 ±0.6	-
SH57	2.0* ±0.0	-	-	-	-	-	2.3 ±0.6	2.0* ±0.0	-	-	-	-	-	-	-	-	-	10.0 ±0.0
SL3	2.0 ±0.0	-	4.0* ±0.0	3.0 4.0* ±0.0	-	-	5.0 ±0.0	13.0 ±0.0	-	5.0 ±0.0	-	-	-	3.0 ±0.0	-	4.0* ±0.0	13.0 ±0.0	13.0 ±0.0
SL4	10.0 ±0.0	-	-	-	-	-	2.7 ±0.6	-	-	-	-	-	-	-	13.3 ±0.6	9.0 ±0.0	-	-
SL5	5.0 ±0.0	3.0 ±0.0	-	-	-	-	6.0 ±0.0	6.0 ±0.0	-	-	-	-	-	-	5.0 ±0.0	3.0 ±0.0	-	-
SL7	4.0* ±0.0	5.0 ±0.0	-	-	6.0* ± 0.0	-	4.0 ±0.0	5.0 ±0.0	-	-	-	-	-	-	4.0* ±0.0	2.0 ±0.0	6.0 ±0.0	6.0 ±0.0
SL10	9.0 ±0.0	-	3.0 ±0.0	-	-	-	7.3 ±1.2	6.0 ±0.0	-	-	-	-	-	-	9.0 ±0.0	5.0 ±0.0	-	-
SL19	-	8.0 ±0.0	-	-	-	-	-	9.0 ±0.0	-	-	-	-	-	-	-	8.0 ±0.0	-	-
SL22	5.0 ±0.0	6.7 ±0.6	-	-	-	-	6.0 ±0.0	10.0 ±0.0	-	-	-	-	-	-	5.0 ±0.0	5.0 ±0.0	-	-
SL24	-	-	-	4.0* ±0.0	-	8.0 ±0.0	-	-	-	-	-	-	-	6.0* ±0.0	-	-	-	-
SL55	3.0* ±0.0	2.0 ±0.0	4.0 ±0.0	-	-	2.0* ±0.0	-	-	-	-	-	-	-	-	8.0 ±0.0	2.0* ±0.0	-	-

Inhibition zones of growth were measured in mm, the values are the mean of three replicates ± standard deviations.

-, no inhibition; *isolates showing poor growth; **Antimicrobial activity of *C. pinisilvae* NH11^{T} and NF3, and *S. pinistramenti* SF28^{T} was mentioned previously (Świecimska et al. 2021a, 2022). ^{T}, type strain.

Isolates which did not inhibit the growth of any of the strains:

(a) Atacama Desert: 1G4^{T}, 1G14, 1G51, 1G52, 1HA3, 1R2A1, 1R2A7, 2G7, 2R2A4, 2SCA1, 3G5 and 3G6; (b) northern slope of the inland dune: NA19a, NF10, NF20, NF23, NF27, NH5, NH14, NH15, NL3, NL13 and NL35; (c) southern slope of the inland dune: SA8, SA20, SA23, SF9, SF23, SL13, SL16, SL52 and SL54.

Table 3. Antimicrobial activity of isolates from the saline soil adjacent to Lake Lonar using a standard plug assay.

Strain	*B. subtilis* PCM 2021		*M. luteus* ATCC 10240		*P. aeruginosa* ATCC 10145		*S. infantis* (SES)		*C. albicans* ATCC 10231	
	HA	ISP3	HA	ISP3	HA	ISP3	HA	ISP3	HA	ISP3
IF7	-	-	5.3 ±0.6	-	-	-	-	-	-	-
IF11	-	-	2.3 ±2.1	-	-	-	-	-	-	-
IF15	-	-	-	-	-	-	-	2.0* ±0.0	-	-
IT2	3.0 ±0.0	-	-	-	-	-	-	-	-	-
OF1^T	-	-	8.3 ±0.6	-	2.0 ±0.0	-	-	-	2.0 ±0.0	-
OF2	-	-	2.7 ±2.5	13.0 ± 0.0	2.3 ±0.6	-	-	-	2.0 ±0.0	-
OF3	-	-	-	14.0 ± 0.0	-	-	-	-	2.7 ±0.6	-
OF5	-	-	5.0 ±0.0	-	4.0 ±0.0	-	-	-	2.7 ±0.6	-
OF6	-	-	4.0 ±0.0	-	-	-	-	-	-	-
OF7	-	-	17.3 ±0.6	12.0 ± 0.0	3.0 ±0.0	-	-	-	2.0 ±0.0	4.0 ±0.0
OF8	-	-	15.7 ±0.6	11.0 ± 0.0	2.0 ±0.0	-	-	-	3.0 ±0.0	-
OT1	-	-	4.3 ±1.2	-	-	-	-	-	-	-

Inhibition zones of growth were measured in mm, the values are the mean of three replicates ± standard deviations.

-, no inhibition; *isolates showing poor growth; Isolates IF12, IF17, IF19 and OF4 did not inhibit the growth of any of the strains.

None of the isolates, irrespective of whether they were cultivated on HA or ISP3 media inhibited the growth of *E. coli* PCM 2057, *K. pneumoniae* ATCC 700603, *P. mirabilis* (CM NCU) or *S. aureus* PCM 2054.

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Supplementary Material

Table S1. Assignment of isolates to colour-groups based on environmental samples from the four sampling locations following growth on oatmeal agar (ISP3) at 28°C for 4 weeks. The numbers given in parenthesis denotes colour codes from the ISCC-NBS charts (Kelly, 1958). Codes in bold indicate representatives of colour-groups included in the 16S rRNA gene sequencing, antimicrobial and enzyme screening studies.

Colour groups	Aerial spore mass/aerial hyphal pigments	Substrate mycelial pigments	Colour of diffusible pigments	Number of isolates	Isolate codes
(A) Isolates from the hyper-arid Atacama Desert soils					
1	Medium gray (265)	Moderate yellow green (106)	None	7	5G1, 5HA5, 5R2A4, 5R2A6, 5SCA3, **5SCA5, 5SCA7**
2	Absent	Strong orange yellow (68)	None	7	1SCA3, 1SCA8, 3HA4, 3R2A2, **5R2A3, 5R2A7^{T}**, 5R2A8
3	White (263)	Pale greenish yellow (104)	None	6	**1HA3**, 2SCA3, 3G3, **3G5**, 3HA16, **4R2A1**
4	Absent	Black (267)	None	6	**1G4^{T}**, **1G6^{T}**, **1G14**, **1G50**, **1G51**, **1G52**
5	White (263)	Brilliant greenish yellow (98)	None	5	**1HA1**, **2G7**, **2SCA1**, 4G1, 4G6,
6	Dark gray (266)	Dark greenish yellow (103)	None	5	1G3, 1R2A9, 1SCA15, 1SCA17, **1SCA21**
7	Dark gray (266)	Moderate yellow green (120)	None	5	**1G2**, 2G12, **3G6**, **5SCA4**, 5SCA8
8	Greenish gray (155)	Moderate greenish yellow (102)	None	4	3G7, 3HA9, **3HA10**, 3HA13
9	Strong bluish green (160)	Brilliant yellow green (116)	None	4	1G16, **1SCA19**, 1SCA20, 2G17
10	Medium gray (265)	Deep greenish yellow (100)	None	3	**2G9**, 2R2A1, **5HA2**
11	Medium gray (265)	Moderate olive (107)	None	3	1G8, **1R2A1**, 3HA19
12	Medium gray (265)	Strong greenish yellow (99)	None	3	**1R2A7**, 2G6, **4SCA5**
13	Medium gray (265)	Moderate greenish yellow (102)	None	1	**2R2A4**
(B) Isolates from the saline soil adjacent to Lake Lonar					
1	Absent	Moderate yellow (87)	Pale purple (227)	5	**OF1^{T}**, **OF2**, **OF3**, **OF7**, **OF8**
2	Light gray (264)	Moderate olive (107)	None	3	**IF11**, **IF17**, **IF19**
3	Yellowish white (92)	Moderate yellowish brown (77)	Light yellowish brown (76)	2	**IF7, OF5**
4	White (263)	Strong yellow (84)	Grayish yellow (90)	2	**OF4**, **OF6**
5	White (263) scant	Yellowish white (92)	None	1	**IF12**

6	Absent	Yellowish gray (93)	None	1	**IT2**
7	Pale orange yellow (73)	Moderate olive brown (95)	Dark grayish yellow (91)	1	**IF15**
8	White (263)	Strong orange yellow (68)	Light orange yellow (70)	1	**OT1**
(C) Isolates from the litter and mineral horizons of the northern slope of the inland dune of the pine forest					
1	Absent	Yellowish white (92)	None	9	NA2, NA12, NA14, NA25, NA28, **NF10**, NF37a, NH26, NL25
2	Pale green (149)	Dark greenish yellowish green (151)	Grayish yellowish green (122)	9	NF5, NF15, **NF24**, NF35, NF36, NF37, NF40, **NH9**, NH18
3	Strong purplish red (255)	Greenish white (153)	None	9	**NF3**, NF13, **NF23**, NF32, **NH11^{T}**, NH26a, **NL8^{T}**, **NL13**, NL37
4	Pale yellowish green (121)	Dark grayish yellow (91)	Dark yellow (88)	8	**NA24,** NL3a, NL5, NL14, **NL21**, NL30*, NL34, NL38
5	Light greenish gray (154)	Dark grayish brown (62)	Grayish olive (110)	4	**NH15, NH5**, **NH16**, **NL35**
6	Greenish white (153)	Grayish yellow (90)	None	3	**NA4**, **NA21, NL15**
7	Light bluish gray (190)	Dark bluish gray (192)	Light grayish olive (109)	2	**NF27**, **NL23**
8	Greenish white (153)	Light olive gray (112)	Grayish greenish yellow (105)	2	**NH21**, **NH27**
9	Greenish white (153)	Light olive gray (112)	Dark grayish yellow (91)	2	NL27, **NL28**
10	Greenish white (153)	Grayish olive (110)	Light grayish olive (109)	2	**NF39**, NL20
11	Pinkish gray (10)	Grayish reddish brown (46)	Dark red (16)	2	**NH28a**, **NL16**
12	Greenish white (153)	Grayish greenish yellow (105)	None	2	**NF20**, NH4
13	Light greenish gray (154)	Light greenish gray (154)	Light olive (106)	2	**NA10a**, **NH17**
14	Greenish white (153)	Yellowish gray (93)	Grayish yellow (90)	2	NA27, **NH14**
15	Greenish white (153)	Light brown gray (63)	Grayish greenish yellow (105)	1	**NA13**
16	Bluish white (189)	Dark bluish gray (192)	Light grayish olive (109)	1	**NA19a**
17	Yellowish white (92)	Grayish yellow (90)	Grayish yellow (90)	1	**NF22**
18	White (263)	Grayish yellow (90)	Strong greenish yellow (99)	1	**NH7**
19	White (263)	Grayish yellow (90)	Moderate greenish yellow (102)	1	**NH28**
20	Yellowish white (92)	Moderate yellow (87)	Moderate yellow (87)	1	**NH22**
21	Greenish white (153)	Light yellowish brown (76)	None	1	**NL3**
(D) Isolates from the litter and mineral horizons of the southern slope of the inland dune of the pine forest					
1	Moderate yellow green (120)	Brilliant greenish yellow (98)	Brilliant yellow green (116)	24	SA1, SA3, SA5, **SA7**, SA18, SF1, SF2, **SF8**, SF18, SF20, SF21, SF25, SH3, SH6,

					SH9, **SH11**, SH12, SH13, SH23, SH25, SH26, SH28, SH55, **SL3**
2	White (263)	Vivid yellow (82)	None	12	**SA4,** SF12, **SF15, SH24, SL4**, SL6, SL9, SL15, SL20, **SL24** SL26*, **SL55**
3	Moderate yellowish green (136)	Deep yellow green (118)	None	8	**SA8,** SA13, SA14, **SA16**, SA25, SA26, SA27, SA28
4	Dark yellowish green (137)	Moderate olive green (125)	None	7	SA9, SA11, SA12, SA17, SA19, SA21, **SF28^{T}**
5	White (263)	Dark greenish yellow (103)	Brilliant yellow green (116)	7	**SF23**, SF30, SL1, SL8, **SL16, SL19**, SL27
6	White (263)	Strong yellow green (117)	None	4	**SA10**, SA24, SH16, SH17,
7	Moderate yellowish green (136)	Strong greenish yellow (99)	Brilliant yellowish green (130)	4	**SA23**, SA30, **SF17,** SF29
8	Light pink (4)	Deep orange (51)	Strong orange (50)	3	**SL5, SL10, SL22**
9	White (263)	Very light yellowish green (134)	None	3	**SF9, SF13, SH20**
10	Moderate green (145)	Dark grayish olive green (128)	Grayish yellow (90)	3	**SL7**, SL29, **SL52**
11	Moderate yellowish green (136)	Light olive brown (94)	None	3	SL12, **SL13**, SL21
12	White (263)	Brilliant yellow green (116)	None	2	SL28, **SL54**
13	White (263)	Vivid greenish yellow (99)	None	1	**SF4**
14	White (263)	Moderate greenish yellow (102)	None	1	**SH57**
15	Moderate yellowish green (136)	Moderate yellow green (120)	None	1	**SA20**
16	Moderate yellowish green (136)	Strong yellow green (117)	None	1	**SF10**
17	White (263)	Moderate yellowish green (136)	None	1	**SH15**
18	Pale pink (7)	Deep orange (51)	Strong orange (50)	1	**SH56**

*Isolates that produced melanin pigments. ^{T}, type strain.

Table S2. Classification of representative isolates assigned to colour-groups based on the distribution of reference strains included in the 16S rRNA gene sequence analysis. Isolates in bold are shown with their closest phylogenetic neighbours. Those with sequence similarities at or below the threshold of 99.0% can be considered to be members of putatively novel species.

(A) Colour-groups composed of isolates from the hyper-arid Atacama Desert soils	
1	**5SCA5** and **5SCA7** (*Streptomyces marokkonensis* Ap1^{T}, both 99.50%), 5G1, 5HA5, 5R2A4, 5R2A6 and 5SCA3
2	***Micromonospora acroterricola* 5R2A7^{T}** and **5R2A3** (*M. acroterricola* 5R2A7^{T}, 99.72%), 1SCA3, 1SCA8, 3HA4, 3R2A2 and 5R2A8
3	**1HA3** (*Pseudonocardia khuvsgulensis* MN08-A0297^{T}, 99.36%), **3G5** *Pseudonocardia xinjiangensis* AS 4.1538^{T}, 97.81%), **4R2A1** (*Pseudonocardia rhizophila* YIM 67013^{T}, 99.64%), 2SCA3, 3G3 and 3HA16
4	***Modestobacter altitudinis* 1G4^{T}**, **1G51** and **1G52** (*M. altitudinis* 1G4^{T}, both 100%), ***Modestobacter excelsi* 1G6^{T}**, **1G14** and **1G50** (*M. excelsi* 1G6^{T}, 100 and 99.86% respectively)
5	**2G7** (*Kribbella flavida* DSM 17836^{T}, 99.43%), **1HA1** (*Kribbella italica* BC637^{T}, 99.36%), **2SCA1** (*Kribbella turkmenica* 16K104^{T}, 98.94%), 4G1 and 4G6
6	**1SCA21** (*Streptomyces tendae* ATCC 19812^{T}, 99.15%), 1G3, 1R2A9, 1SCA15, 1SCA17
7	**5SCA4** (*Streptomyces albogriseolus* NRRL B-1305^{T}, 99.43%), **3G6 (***Streptomyces bungoensis* DSM 41781^{T}, 99.51%), **1G2 (***Streptomyces flaveolus* NBRC 3715^{T}, 99.72%), 2G12 and 5SCA8
8	**3HA10** (*Streptomyces galbus* DSM 40089^{T}, 99.37%), 3G7, 3HA9 and 3HA13
9	**1SCA19** (*Streptomyces purpurascens* NBRC 13077^{T}, 99.15%), 1G16, 1SCA20 and 2G17
10	**5HA2** (*Streptomyces mutabilis* NBRC 12800^{T}, 99.79%), **2G9** (*Streptomyces paradoxus* NBRC 14887^{T}, 98.79%) and 2R2A1
11	**1R2A1** (*Streptomyces flaveolus* NBRC 3715^{T}, 99.72%), 1G8 and 3HA19
12	**4SCA5** (*Streptomyces camponoticapitis* 2H-TWYE14^{T}, 97.58%), **1R2A7** (*Streptomyces aquilus* GGCR-6^{T}, 99.28%) and 2G6
13	**2R2A4** (*Pseudonocardia rhizophila* YIM 67013^{T}, 99.72%)
(B) Colour-groups composed of isolates from the saline soil adjacent to Lake Lonar	
1	***Streptomyces alkaliterrae* OF1^{T}**, **OF2**, **OF3**, **OF7** and **OF8** (*S. alkaliterrae* OF1^{T}, all 100%)
2	**IF11**, **IF17**, **IF19** (*Streptomyces alkaliphilus* DSM 42118^{T}, 99.42, 99.52, 99.50%, respectively)
3	**IF7** (*Streptomyces cahuitamycinicus* 13K301^{T}, 97.24%) and **OF5** (*S. alkaliterrae* OF1^{T}, 99.79%)
4	**OF4** and **OF6** (*Nocardiopsis metallicus* KBS6^{T}, 100 and 99.86%, respectively)
5	**IF12** (*Nocardiopsis halotolerans* DSM 44410^{T}, 98.72%)
6	**IT2** (*Nocardiopsis flavescens* CGMCC 4.5723^{T}, 97.45%)
7	**IF15** (*S. alkaliphilus* DSM 42118^{T}, 99.36%)
8	**OT1** (*Nocardiopsis valliformis* DSM 45023^{T}, 99.50%)
(C) Colour-groups composed of isolates from the litter and mineral horizons of the northern slope of the inland dune of the pine forest	
1	**NF10** (*Streptacidiphilus torunensis* NF37^{T}, 100%), NA2, NA12, NA14, NA25, NA28, NF37a, NH26 and NL25
2	**NF24** and **NH9** (*Kitasatospora herbaricolor* NBRC 12876^{T}, 99.71, 99.43%, respectively), NF5, NF15, NF35, NF36, NF37, NF40 and NH18
3	***Catenulispora pinisilvae* NH11^{T}**, **NF3** and **NL13** (*C. pinisilvae* NH11^{T}, 100 and 99.79%, respectively), ***Catenulispora pinistramenti* NL8^{T}** and **NF23** (*C. pinistramenti* NL8^{T} 99.86%), NF13, NF32, NH26a and NL37
4	**NA24** and **NL21** (*Streptomyces xanthochromogenes* NRRL B-5410^{T}, 100 and 99.93%, respectively), NL3a, NL5, NL14, NL30, NL34, NL38
5	**NH5** and **NH16** (*Streptomyces atratus* NRRL B-16927^{T}, 100 and 99.79%, respectively), **NH15** and **NL35** (*Streptomyces yanii* NBRC 14669^{T}, 99.20 and 99.12%, respectively)

6	**NA4** (*Streptacidiphilus albus* NBRC 100918^T, 97.73%), **NL15** (*Streptacidiphilus carbonis* DSM 41754^T, 98.44%) and **NA21** (*Streptacidiphilus hamsterleyensis* HSCA 14^T, 92.01%)
7	**NL23** (*Actinacidiphila paucisporea* CGMCC 4.2025^T, 98.62%) and **NF27** (*Actinacidiphila yanglinensis* 1307^T, 98.14%)
8	**NH21** and **NH27** (*Actinacidiphila yanglinensis* 1307^T, 99.07 and 99.22%, respectively)
9	**NL28** (*Pilimelia columellifera* subsp. *pallida* MB-SK 8^T, 99.64%) and NL27
10	**NF39** (*Kitasatospora herbaricolor* NBRC 12876^T, 99.64%) and NL20
11	**NH28a** (*Actinacidiphila alni* D65^T, 98.50%) and **NL16** (*Actinacidiphila bryophytorum* NEAU-HZ10^T, 98.48%)
12	**NF20** (*Streptacidiphilus torunensis* NF37^T, 99.86%) and NH4
13	**NA10a** (*Streptomyces celluloflavus* NRRL B-2493^T, 100%) and **NH17** (*Streptomyces luteireticuli* NBRC 13422^T, 92.32%)
14	**NH14** (*Streptacidiphilus torunensis* NF37^T, 100%) and NA27
15	**NA13** (*Streptacidiphilus durhamensis* FSCA67^T, 99.64%)
16	**NA19a** (*Streptacidiphilus durhamensis* FSCA67^T, 99.86%)
17	**NF22** (*Streptacidiphilus hamsterleyensis* HSCA 14^T, 99.56%)
18	**NH7** (*Streptomyces celluloflavus* NRRL B-2493^T, 99.86%)
19	**NH28** (*Streptomyces paludis* GSSD-12^T, 98.13%)
20	**NH22** (*Streptacidiphilus neutrinimicus* DSM 41755^T, 99.78%)
21	**NL3** (*Streptomyces celluloflavus* NRRL B-2493^T, 100%)
(D) Colour-groups composed of isolates from the litter and mineral horizons of the northern slope of the inland dune of the pine forest	
1	**SF8**, **SH11** and **SL3** (*Streptomyces celluloflavus* NRRL B-2493^T, all 100%), **SA7** (*Streptomyces celluloflavus* NRRL B-2493^T, 99.79%), SA1, SA3, SA5, SA18, SF1, SF2, SF18, SF20, SF21, SF25, SH3, SH6, SH9, SH12, SH13, SH23, SH25, SH26, SH28 and SH55
2	**SA4**, **SF15**, **SH24**, **SL4** and **SL24** (*Pilimelia columellifera* subsp. *pallida* MB-SK 8^T, all 99.71 or 99.72%), **SL55** (*Pilimelia columellifera* subsp. *pallida* MB-SK 8^T, 99.0%), SF12, SL6, SL9, SL15, SL20 and SL26
3	**SA8** and **SA16** (*Streptomyces sanglieri* NBRC 100784^T, 100 and 99.79%), SA13, SA14, SA25, SA26, SA27 and SA28
4	***Streptomyces pinistramenti* SF28^T**, SA9, SA11, SA12, SA17, SA19 and SA21
5	**SF23**, **SL16** and **SL19** (*Pilimelia columellifera* subsp. *pallida* MB-SK 8^T, all 99.71%), SF30, SL1, SL8 and SL27
6	**SA10** (*Actinospica acidiphila* GE134766^T, 98.07%), SA24, SH16 and SH17
7	**SA23** and **SF17** (*Actinacidiphila yanglinensis* 1307^T, 99.36 and 99.50%, respectively), SA30 and SF29
8	**SL5**, **SL10** and **SL22** (*Actinacidiphila yanglinensis* 1307^T, 98.70, 98.84 and 98.78%, respectively)
9	**SF9** (*Nocardia nova* NBRC 15556^T, 98.85%), **SF13** and **SH20** (*Nocardia vinacea* NBRC 16497^T, 98.22 and 98.26%)
10	**SL7** and **SL52** (*Actinacidiphila bryophytorum* NEAU-HZ10^T, 98.33 and 98.61%) and SL29
11	**SL13** (*Actinacidiphila rubida* 13C15^T, 97.49%), SL12 and SL21
12	**SL54** (*Streptomyces ferralitis* SFOp68^T, 97.65%) and SL28
13	**SF4** (*Streptomyces celluloflavus* NRRL B-2493^T, 100%)
14	**SH57** (*Streptomyces halstedii* NBRC 12783^T, 99.79%)
15	**SA20** (*Streptomyces atratus* NRRL B-16927^T, 99.79%)
16	**SF10** (*Streptacidiphilus torunensis* NF37^T, 100%)
17	**SH15** (*Streptomyces celluloflavus* NRRL B-2493^T, 99.79%)
18	**SH56** (*Streptomyces cocklensis* BK168^T, 99.15%)

^{T}, type strain.

Table S3. Colour-groups composed of isolates from the sampling sites which produce hydrolytic enzymes and compounds that support plant growth. Codes in bold are isolates that show 16S rRNA sequence similarities with their nearest neighbours at or below the 99.0% sequence similarity threshold.

Isolates	Compounds promoting plant growth					Production of					
	Production of			Siderophore activity*	Phosphate solubilization*						
	Ammonia	HCN	IAA			Cellulases	Chitinases	Lipases	Pectinases	Proteases	Ureases
(A) Representative isolates from the hyper-arid Atacama Desert soils											
1G2	-	-	+	13.2	0.0	24.1	0.0	4.9	9.4	9.7	-
1G4^{T}	-	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
1G6^{T}	++	-	+	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
1G14	-	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
1G50	-	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
1G51	-	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
1G52	-	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
1HA1	-	-	+++	3.1	0.0	1.1	0.0	6.0	0.0	8.2	-
1HA3	-	-	-	2.1	0.0	0.0	0.0	16.0	0.0	0.0	-
1R2A1	++	-	-	17.7	0.0	18.7	0.6	6.3	5.6	9.7	+
1R2A7	++	-	-	0.0	0.5	4.6	0.0	7.9	21.4	5.8	++
1SCA19	-	-	+	0.0	0.0	4.6	0.0	1.8	0.0	5.0	-
1SCA21	+	-	++	15.9	0.7	9.0	0.0	3.6	12.6	8.0	++
2G7	-	-	-	0.6	0.0	1.4	0.0	12.5	0.0	12.5	-
2G9	**-**	**-**	**-**	**9.3**	**0.0**	**3.6**	**0.0**	**3.2**	**17.3**	**12.0**	+
2R2A4	-	-	-	1.3	0.0	0.0	0.0	19.6	0.0	0.0	-
2SCA1	**-**	**-**	+	**4.6**	**0.0**	**13.4**	**0.6**	**10.7**	**8.3**	**11.6**	+
3G5	**-**	**-**	**-**	**0.6**	**0.0**	**0.0**	**0.0**	**13.4**	**0.0**	**0.0**	**-**
3G6	-	-	-	0.3	0.0	0.0	0.0	0.0	0.0	0.0	-
3HA10	+	-	+	0.0	0.0	27.3	0.0	2.2	18.7	5.8	-
4R2A1	-	-	-	0.3	0.0	0.0	0.0	10.0	0.0	0.0	-

4SCA5	**-**	-	+	**0.0**	**0.0**	**0.0**	**0.8**	**3.1**	**19.0**	**8.7**	**-**
5HA2	-	-	-	14.4	0.0	17.5	0.0	19.4	4.6	4.5	+
5R2A3	+	-	+++	0.0	0.1	18.0	0.0	10.7	0.0	10.7	-
5R2A7^{T}	++	-	++	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-
5SCA4	-	-	-	7.4	0.0	5.8	1.1	7.0	4.0	7.0	-
5SCA5	-	-	+	0.0	0.0	15.0	0.8	4.3	2.7	4.3	+
5SCA7	-	-	-	11.5	0.0	16.5	0.8	5.1	3.0	5.1	+
(B) Representative isolates from the saline soil adjacent to Lake Lonar											
IF7	**-**	-	-	**0.3**	**0.0**	**17.0**	**0.0**	**1.4**	0.0	**9.8**	-
IF11	-	-	-	0.0	0.0	9.5	0.0	3.0	0.0	7.1	**-**
IF12	**-**	-	-	**0.0**	**0.0**	**6.8**	**0.0**	**0.9**	0.0	**3.1**	-
IF15	-	-	-	0.0	0.0	13.9	0.0	2.0	0.0	11.1	**-**
IF17	-	-	-	1.9	0.0	6.3	0.7	1.8	0.0	5.0	**-**
IF19	-	-	-	0.0	0.0	12.4	0.0	2.0	0.0	7.1	**-**
IT2	**-**	-	-	**0.0**	**0.0**	**9.4**	**0.0**	**0.4**	0.0	**1.4**	-
OF1^{T}	-	-	-	0.0	0.0	0.0	0.0	2.7	0.0	2.7	++
OF2	-	-	-	0.0	0.0	0.0	0.0	3.6	0.0	6.3	+
OF3	-	-	-	0.0	0.0	0.0	0.0	3.6	0.0	10.1	+
OF4	-	-	-	0.0	0.0	17.1	0.0	1.8	0.0	5.7	-
OF5	-	-	-	2.5	0.0	0.0	0.0	2.9	0.0	4.9	-
OF6	-	-	-	0.0	0.0	15.6	0.0	2.4	0.0	4.6	-
OF7	-	-	-	0.0	0.0	0.0	0.0	2.7	0.0	3.6	+
OF8	-	-	-	0.0	0.0	0.0	0.0	2.3	0.0	6.3	-
OT1	+	-	-	0.0	0.0	5.4	0.0	1.9	0.0	3.1	-
(C) Representative isolates from the litter and mineral horizons of the northern slope of the inland dune of the pine forest											
NA4	**-**	-	-	**0.0**	**0.0**	**0.0**	**0.0**	**4.5**	**0.0**	**0.0**	**+++**
NA10a	+++	-	-	5.7	0.0	0.0	0.0	3.6	1.1	3.4	+++
NA13	+	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	+++
NA19a	++	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	++

NA21	-	-	-	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	+++
NA24	++	-	+	0.0	0.0	0.0	0.0	0.0	0.0	4.9	+++
NF3	-	-	+	9.6	0.0	0.0	0.7	10.9	0.0	0.0	+
NF10	-	-	++	0.3	0.0	0.0	0.0	0.0	0.0	0.0	-
NF20	+	-	++	0.3	0.0	0.0	0.0	0.0	0.0	0.0	-
NF22	-	-	+	0.3	0.0	0.0	0.0	0.5	0.0	0.0	+
NF23	-	-	++	0.0	0.0	0.0	0.0	0.9	0.0	0.0	++
NF24	+	-	-	4.1	1.4	0.0	0.0	0.0	0.0	17.1	+
NF27	-	-	++	**0.0**	**0.0**	**2.2**	**0.0**	**1.0**	**0.0**	**0.0**	-
NF39	-	-	-	3.7	1.4	0.0	0.0	3.8	0.0	13.4	-
NH5	+	-	-	0.0	0.0	5.8	1.3	4.5	0.0	9.9	+++
NH7	-	-	-	8.6	0.0	0.0	1.5	3.6	0.0	12.0	+++
NH9	++	-	-	2.8	0.6	0.0	0.0	2.1	0.0	13.0	++
NH11^{T}	-	-	++	1.1	0.0	0.0	0.0	1.0	0.0	0.0	+
NH14	+	-	+	0.0	0.0	0.0	0.0	0.9	0.0	0.0	+
NH15	-	-	-	0.0	0.0	2.9	1.7	4.5	0.0	20.6	+++
NH16	-	-	-	3.3	0.0	2.1	1.4	3.2	0.0	17.8	+++
NH17	+	-	-	**8.6**	**0.0**	**0.0**	**0.7**	**4.0**	**0.0**	**6.7**	+++
NH21	-	-	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	+++
NH22	-	-	-	0.0	0.0	0.0	0.0	0.5	0.0	0.0	++
NH27	-	-	-	0.0	0.0	0.0	0.0	1.0	0.0	0.0	-
NH28	+++	-	-	**0.8**	**0.0**	**0.0**	**1.3**	**3.6**	**0.0**	**5.4**	+++
NH28a	-	-	-	**0.3**	**0.0**	**9.0**	**1.3**	**3.6**	**0.0**	**4.4**	+++
NL3	++	-	+	0.3	0.0	50.0	0.0	4.7	0.0	0.0	-
NL8^{T}	-	+	++	0.0	0.0	0.0	0.0	0.9	0.0	0.0	+
NL13	-	-	++	0.0	0.0	0.0	0.0	0.0	0.0	0.0	++
NL15	+++	-	+	**0.3**	**0.0**	**0.0**	**0.0**	**1.8**	**0.0**	**0.0**	++
NL16	+	-	++	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	++
NL21	++	-	++	0.0	1.1	3.5	0.0	8.3	0.0	10.3	++
NL23	-	-	-	**0.0**	**0.0**	**8.3**	**0.0**	**0.0**	**0.0**	**0.0**	+

NL28	-	-	-	0.0	0.0	0.0	0.4	3.4	0.0	2.4	++
NL35	++	-	-	0.7	0.0	15.4	1.3	3.3	0.0	10.5	+++
D) Representative isolates from the litter and mineral horizons of the southern slope of the inland dune of the pine forest											
SA4	-	-	+	0.0	0.0	0.0	0.0	4.0	0.0	3.2	+++
SA7	++	-	-	8.8	0.0	0.0	0.0	2.8	0.0	0.9	+++
SA8	-	-	+++	0.0	0.0	0.0	1.0	3.1	0.0	10.9	+++
SA10	**-**	**-**	**-**	**0.3**	**0.0**	**2.0**	**0.0**	**8.2**	**0.0**	**0.0**	**+++**
SA16	+++	-	-	6.7	0.0	0.0	0.8	3.2	0.0	13.7	+++
SA20	-	-	-	0.0	0.0	3.6	3.1	4.0	0.0	6.5	+++
SA23	+	-	+	1.4	0.0	7.4	0.0	0.5	0.0	2.7	+++
SF4	-	-	-	1.3	0.0	0.0	0.0	7.4	0.0	3.2	+
SF8	+	-	-	6.6	1.6	0.0	0.0	3.0	0.0	10.9	+++
SF9	**-**	**-**	**-**	**0.0**	**0.0**	**0.0**	**0.0**	**4.5**	**0.0**	**0.0**	**++**
SF10	+	-	-	0.3	0.0	0.0	0.0	0.0	0.0	0.0	+
SF13	**+**	**-**	**-**	**0.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**-**
SF15	+	-	+	3.5	0.0	0.0	0.0	5.3	0.0	2.7	-
SF17	+	-	++	0.0	0.0	2.7	0.0	1.8	0.0	0.0	+
SF23	+	-	-	1.2	0.0	0.0	0.6	2.7	0.0	11.9	+++
SF28^{T}	+++	-	-	11.3	0.0	0.0	0.9	4.5	0.0	10.8	+++
SH11	+	-	-	9.6	0.0	0.0	0.0	2.9	0.0	9.9	+++
SH15	+	-	-	4.7	1.1	0.0	0.0	0.0	0.6	9.6	+++
SH20	**+**	**-**	**-**	**0.0**	**0.0**	**0.0**	**0.0**	**3.6**	**0.0**	**0.0**	**+++**
SH24	-	-	-	6.8	0.0	0.0	0.0	4.1	0.0	4.1	+++
SH56	-	-	+	0.6	0.0	25.2	0.0	0.5	12.8	4.7	+
SH57	-	-	-	14.2	0.0	9.9	1.0	1.9	2.1	8.0	-
SL3	+	-	-	5.8	1.5	0.0	1.0	2.5	0.0	7.7	+++
SL4	+	-	+	0.0	0.0	0.0	0.0	6.7	0.0	1.9	+++
SL5	**-**	**-**	**-**	**0.3**	**0.0**	**2.0**	**0.0**	**0.0**	**0.0**	**1.8**	**+++**
SL7	**+**	**-**	**-**	**0.3**	**0.0**	**0.0**	**0.0**	**0.4**	**0.0**	**0.0**	**+++**

SL10	-	-	-	**0.3**	**0.0**	**0.0**	**0.0**	**4.0**	**0.0**	**0.0**	+++
SL13	+	-	-	**0.0**	**0.0**	**0.0**	**0.0**	**0.4**	**0.0**	**0.0**	+++
SL16	-	-	-	0.0	0.0	9.0	0.0	0.0	0.0	8.1	+++
SL19	+	-	+	0.0	0.0	0.0	0.8	3.1	0.0	12.7	+++
SL22	-	-	-	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	+++
SL24	+	-	-	0.0	0.0	0.0	0.8	2.2	0.0	13.9	+++
SL52	+	-	-	**0.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**4.5**	+++
SL54	-	-	+	**0.3**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	+++
SL55	+	-	-	**3.8**	**0.0**	**0.0**	**0.0**	**3.8**	**0.0**	**7.5**	+++

Key: -, no production; +, low production; ++, moderate production; +++, strong production.
*Hydrolysis, siderophore and phosphate solubilization values are given as activity indices that were calculated as follows: Wact = Sh^2 (Sc × t) where Sh indicates the diameter of the hydrolysis zones, Sc the colony diameters and t the time of incubation (Hrynkiewicz et al., 2010).
Wact <1, very low activity; 1-5, low activity; >5-10, good activity; >10-20, high activity; >20, very high activity.

Atacama Desert isolates giving negative results: 1G4^T, 1G14, 1G50, 1G51, 1G52. ^{T}, type strain.

Table S4. Inhibition of fungal and oomycete plant pathogens by selected isolates from the pine forest sites based on a co-culture method. Bold print denotes isolates which showed 16S rRNA sequence similarity values below the 99.0% threshold for delineating actinomycete species.

Pathogens	**Extent of inhibition shown by isolates**		
	>50-70%	**>70-90%**	**>90%**
Representative isolates from the litter and mineral horizons of the northern slope of the inland dune of the pine forest			
(A) Fungi			
Alternaria alternata IOR 1783	NA24, NF20,	NA10a, NH7, **NH17**, **NH28**	None
Botrytis cinerea IOR 1873	NF24, NH9, **NH17**, **NL16**, NL21	NA10a, NA24, NH7, **NH28**	None
Chalara fraxinea	**NA4**, NA13, **NA21**, NF24, NH5, NH9, NH15, NH16, NH22, **NH28a**, **NL15**, NL28, NL35	NA24, NF39, **NH28**	NA10a, NH7, **NH17**
Colletotrichum acutatum IOR 2153	NF20, NF24, NF39, NH7, NH9	NA10a, **NH17**, **NH28**	None
Fusarium culmorum IOR 2333	NA10a, NF24, NF39, NH7, NL21	**NH17**	None
Fusarium culmorum D	NA10a, NA24, NF20, NF24, NF39, NH7, **NH28**, NL21	**NH17**	None
Fusarium graminearum A	NA13, **NA21**, NF3, NF24, **NF27**, NF39, NH9, NH11^{T}, NH15, NH27, **NH28**, NL13, **NL15**, NL21	NA10a, NA24, NH7, **NH17**	None
Fusarium oxysporum IOR 342	NA10a, NH7, **NH17**, **NH28**, NL28	None	None
Fusarium oxysporum D	NF24, NF39, **NH17**	None	None
Fusarium poae A	NA10a, NA24, NF24, NF39, **NH28**, NL21	NH7, **NH17**	None
Fusarium solani IOR 825	NA24	None	None
Fusarium tricinctum A	NA24, NF20, NF39, NH7, **NH17**, NH22, **NH28**, **NL16**, NL21	NA10a	None
Phoma lingam IOR 2284	NA13, NA24, NF22, NH5, NH15, NL35	NA10a, NF39, NH7, NH9, NH14, **NH17**, **NH28**	NF24
Rhizoctonia solani 13	NA13, NF24, NH5, NH9, NH15, NH16	NA10a, NF39, NH7, **NH17**, **NH28**	None
Sclerotinia sclerotiorum IOR 2242	NH7, NH14, NH15, NH27, **NH28**, **NL16**	NA10a, NA24, **NH28a**, NL21	**NA21**, NF3

(B) *Oomycetes*			
Phytophthora cactorum IOR 1925	NF20, **NF27**, NH15, NL35	NA10a, NA24, NF24, NF39, NH7, NH9, **NH17**, **NH28**, NL21	None
Phytophthora cryptogea IOR 2080	NA10a, **NH28**	None	None
Phytophthora megasperma IOR 404	**NH28**	None	None
Phytophthora plurivora IOR 2303	NA24, NF24, NH9, NH21, NH22, NL21	NA10a, NA19a, NF39, NH7, NH16, **NH17**, **NH28**	None
Representative isolates from the litter and mineral horizons of the southern slope of the inland dune of the pine forest			
(A) Fungi			
Alternaria alternata IOR 1783	SA7, **SF9**, SF10, SH11, SH15, SL3, **SL13**	SF8, SF28^{T}	None
Botrytis cinerea IOR 1873	SF23, SH24, SH56, SH57, SL3, **SL10**	SA7, SA16, SF8, **SF9**, SF10, SF28^{T}, SH11, SH15, **SL13**, **SL54**	None
Chalara fraxinea	SA4, SA16, SA20, SF4, SF10, SF15, SF17, SF23, SF28^{T}, SL4, SL19, SL24, **SL55**	SH11, SH56	SA7, SF8, SH15, SL3
Colletotrichum acutatum IOR 2153	SA7, SF8, SH11, SH15, SF10, **SL13**, **SL52**	**SF9**, SL3	None
Fusarium culmorum IOR 2333	SA20, SA23, SF4, SF8, SF15, SF17, SH11, SH24, SH56, SH57	SA7, SH15, SL3	SF28^{T}
Fusarium culmorum D	SA8, SA20, SF17, SH11, SH15, SL3, **SL54**	SA7, SF8, SH56	SF28^{T}
Fusarium graminearum A	SA8, SA20, SA23, SF4, SF15, SF17, SH11, SL4, **SL13**, **SL54**, **SL55**	SA7, SF8, SH15, SH56, SL3	SF28^{T}
Fusarium oxysporum IOR 342	SA7, SF8, **SF9**, SH15, SL3	SF23, SH57	SF28^{T}
Fusarium oxysporum D	SA20, SH56	None	None
Fusarium poae A	SA20, SF10, SF28^{T}, SH56	SA7, SF8, SH11, SH15, SL3	None
Fusarium solani IOR 825	None	SF28^{T}	None
Fusarium tricinctum A	SA7, SA8, SA20, SF8, SF17, SF28^{T}, SH11, SH15, SL3, **SL54**	SH56	None

Phoma lingam IOR 2284	SA16, SA20, SA23, **SL54**	SA7, SF8, **SF9**, SF10, SF28^{T}, SH11, SH15, SL3, **SL13**	None
Rhizoctonia solani 13	SA20, SH11, **SL54**	SA7, SF8, SH15, SH56, SL3	SF28^{T}
Sclerotinia sclerotiorum IOR 2242	SA7, SA16, SF8, **SF9**, SH56, SL3	SA8, SH11, **SL10**, **SL54**	SF28^{T}
(B) Oomycetes			
Phytophthora cactorum IOR 1925	SA4, SH15	SA7, **SA10**, SF8, **SF9**, SF10, SF17, SL3, **SL13**	SF28^{T}
Phytophthora cryptogea IOR 2080	SA7, SF8, SF10, SF28^{T}, SH11, SH15, SH56, SL3, **SL13**	None	None
Phytophthora megasperma IOR 404	SA7, SF8, SF10, SF28^{T}, SH11, SH15, SL3	None	None
Phytophthora plurivora IOR 2303	SA23, SH57	SA8, SA20, SF4, SF8, SF17, SH15, SH56, SL3, **SL5**, **SL22**	SA4, SA7, SF15, SF28^{T}, SH11, SH24, SL4

*The % inhibition of the growth of the fungi and oomycetes was calculated using the following formula: I (%) = (C-T/C) × 100, where C is the diameter of pathogen growth in the control sample and T the diameter of pathogen growth in each of the co-cultures.

Antifungal and oomycete activities of *C. pinisilvae* NH11^{T} and NF3, and *S. pinistramenti* SF28^{T} were reported previously by Świecimska et al. (2021a and 2022). Activity against *Chalara fraxinea* was not tested for strains NF3, **NF27**, NH11^{T}, NL3, NL13, **NL16**, NL21, **NL23** (from the northern slope of the pine forest) or SH57, **SL5**, **SL10**, **SL13**, **SL22**, **SL52**, **SL54** (from the southern slope of the pine forest) as they not grown on the medium used for cultivation of this pathogen. ^{T}, type strain.

Systematic and Applied Microbiology 43 (2020) 126051

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: http://www.elsevier.com/locate/syapm

Modestobacter excelsi sp. nov., a novel actinobacterium isolated from a high altitude Atacama Desert soil

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ARTICLE INFO

Article history:
Received 10 August 2019
Received in revised form
10 December 2019
Accepted 16 December 2019

Keywords:
Modestobacter excelsi
Polyphasic taxonomy
Phylogeny
Whole genome sequence
Atacama desert
Stress tolerance

ABSTRACT

A polyphasic study was undertaken to establish the taxonomic status of three *Modestobacter* strains isolated from a high altitude Atacama Desert soil. The isolates, strains 1G6^T, 1G14 and 1G50, showed chemotaxonomic and morphological properties characteristic of members of the genus *Modestobacter*. The peptidoglycan contained *meso*-diaminopimelic acid, the whole cell sugars were glucose and ribose (diagnostic sugars) and arabinose, the predominant menaquinone was MK-9(H_4), polar lipid patterns contained diphosphatidylglycerol, glycophosphatidylinositol, phosphatidylethanolamine (diagnostic component), phosphatidylglycerol and phosphatidylinositol while whole cellular fatty acid profiles consisted of complex mixtures of saturated, unsaturated iso- and *anteiso*-components. The isolates were shown to have different BOX-PCR fingerprint and physiological profiles. They formed a distinct phyletic line in *Modestobacter* 16S rRNA gene trees, were most closely related to the type strain of *Modestobacter italicus* (99.9 % similarity) but were distinguished from this and other closely related *Modestobacter* type strains using a combination of phenotypic properties. Average nucleotide identity and digital DNA:DNA hybridization similarities between the draft genome sequences of isolate 1G6^T and *M. italicus* BC 501^T were 90.9 % and 42.3 %, respectively, indicating that they belong to different species. Based on these phenotypic and genotypic data it is proposed that the isolates be assigned to a novel species in the genus *Modestobacter*, namely as *Modestobacter excelsi* with isolate 1G6^T (=DSM 107535^T =PCM 3004^T) as the type strain. Analysis of the whole genome sequence of *M. excelsi* 1G6^T (genome size of 5.26 Mb) showed the presence of genes and gene clusters that encode for properties that are in tune with its adaptation to extreme environmental conditions that prevail in the Atacama Desert biome.

Abbreviations: A_2pm, diaminopimelic acid; AL, aminolipids; APL, aminophospholipids; ANI, average nucleotide identity; ATP, adenosine triphosphate; BLAST, Basic Local Alignment Search Tool; CO, carbon monoxide; dDDH, digital DNA–DNA hybridization; DDH, DNA–DNA hybridization; DNA, deoxyribonucleic acid; DPG, diphosphatidylglycerol; GGDC, genome to genome distance calculator; GL, glycolipid; GPI, glycophosphoinositol; ECLs, equivalent chain lengths; IF, inoculating fluid; IMG/M, Integrated Microbial Genomes and Microbiomes System; ISP, International *Streptomyces* Project; IUPAC, International Union of Pure and Applied Chemistry; L, unidentified lipid; M., *Modestobacter*; MIDI, Microbial Identification System; MK, menaquinones; NCBI, National Center for Biotechnology Information; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphoinositolmannoside; PL, unidentified phospholipid; PYGV, peptone-yeast extract-glucose-vitamins agar; rRNA, Ribosomal RNA; T, transmitance; TLC, thin-layer chromatography TYGS Type (Strain) Genome Server; UV, ultraviolet.

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https://doi.org/10.1016/j.syapm.2019.126051

Introduction

The actinobacterial genus *Modestobacter* was proposed by Mevs et al. [46] and the description of the taxon subsequently emended by Reddy et al. [61], Xiao et al. [81], Qin et al. [59] and Montero-Calasanz et al. [49]. The genus is classified in the family *Geodermatophilaceae* [53] of the order *Geodermatophilales* [69] together with the genera *Blastococcus* [1], *Cumulibacter* [26], *Klenkia* [48] and *Geodermatophilus* [40]. *Modestobacter* strains can be distinguished from members of all of these taxa using a combination of genotypic and phenotypic features [48,49].

In general, *Modestobacter* strains are aerobic, Gram-stain-positive, non-spore-forming, heterotrophic, psychrotolerant actinobacteria which form rod- and coccoid-like elements that have a tendency to remain aggregated and produce short, multiseptate filaments; the diagnostic diamino acid is *meso*-diaminopimelic acid, the major fatty acid iso-$C_{16:0}$, the predominant respiratory quinone tetrahydrogenated menaquinone with nine isoprene units, a polar lipid profile containing diphospatidylglycerol, phosphatidylethanolamine and phosphatidylinositol, the characteristic whole-cell sugar pattern consists of glucose and ribose while the presence of arabinose, galactose, mannose and rhamnose is variable, and the genomic G + C content falls with the range 70–74 mol% [10,49].

The genus *Modestobacter* currently encompasses nine validly published species: *Modestobacter multiseptatus* [46,49,61], the type species, *Modestobacter caceresii* [10], *Modestobacter italicus* [49], *Modestobacter lacusdianchii* [85], *Modestobacter lapidis* [75], *Modestobacter marinus* [49,81], *Modestobacter muralis* [75], *Modestobacter roseus* [49,59] and *Modestobacter versicolor* [49,61]. Members of these taxa have been recovered from diverse habitats through they seem to be associated with extreme biomes, as exemplified by the isolation of *M. caceresii* from extreme hyper-arid Atacama Desert soils [10], *M. lapidis*, *M. muralis* and *M. italicus* from deteriorating sandstone [49,74], *M. marinus* from deep-sea sediment [81] and *M. multiseptatus* from an Antarctic surface soil [46]. Culture-independent surveys show that *Modestobacter* strains are a common feature of the Atacama Desert landscape [9,27] while interrogation of whole-genome sequences of *Geodermatophilaceae* strains, including that of the type strain of *M. caceresii*, show the presence of stress-related genes that provide an insight into how these microorganisms adapt to harsh environmental conditions associated with this desert biome [10,11,12]. It has been shown that *M. italicus* BC 501^T, previously misclassified as *M. multiseptatus* [23,70] and as *M. marinus* [55], is highly resistant to desiccation and to γ- and UV-radiation [23,70]. The genomes of *Modestobacter* strains contain multiple copies of several genes, such as *coxSML* (carbon monoxide dehydrogenase), *katA* (manganese-containing catalase) and *uvrACD* [10,55,70].

The present study, a continuation of our previous surveys on actinobacterial diversity in the Atacama Desert biome [8–12], was designed to establish the taxonomic status of three presumptive *Modestobacter* strains isolated from an arid high altitude soil. The strains, isolates 1G6^T, 1G14 and 1G50, were compared with reference strains of *Modestobacter* species using a combination of genotypic and phenotypic properties known to be of value in the delineation of *Modestobacter* species. The isolates were found to belong to a novel species of *Modestobacter*; the name proposed for this taxon is *Modestobacter excelsi* with isolate 1G6^T as the type strain.

Materials and methods

Isolation of strains

Three presumptive *Modestobacter* strains were isolated from a surface soil sample (2 cm depth) collected at 3018 m above sea level on Cerro Chajnantor (23°04′39″S/67°57′43″W), adjacent to the Atacama Large Millimeter Array (ALMA), east of San Pedro de Atacama, Chile in November 2012 [27]. The physicochemical properties of the soil sample (pH 7.08, 360 mv, moisture content 0 % and organic matter content 1.67 %) were determined by Bull et al. [9]. One gram of soil sample was diluted ten-fold with ¼ strength Ringer's solution (Oxoid) prior to preparing a 10^{-2} dilution using the same diluent. Aliquots (100 μl) of each dilution were spread, in triplicate, over plates of Gauze's No. 1 medium [84] supplemented with cycloxehimide and nystatin (each at 50 μg ml^{-1}), which had been dried for 15 min at room temperature prior to inoculation as recommended by Vickers and Williams [79]. After incubation at 28 °C for 3 weeks, the presumptive *Modestobacter* isolates were subcultured onto Gauze's agar plates to check their purity.

Maintenance and cultural conditions

Isolates 1G6^T, 1G14 and 1G50 were maintained on modified Bennett's agar slopes [28], pH 7.5, at room temperature and as suspensions of cells in 20 %, v/v glycerol at −80 °C. Biomass for most of the chemotaxonomic analyses and for the molecular systematic studies were harvested from 500 ml of modified Bennett's broth cultures which had been shaken at 150 rpm at 28 °C for 7 days following inoculation with 1 ml of each of the isolates prepared in the same medium. The resultant biomass preparations were washed three times with sterile distilled water and those for the molecular systematic analyses were stored at room temperature; biomass preparations for the chemotaxonomic studies were freeze dried. Biomass preparations of the isolates and *M. caceresii* KNN 45-2b^T, *M. italicus* BC 501^T, *M. lacusdianchii* KCTC 39600^T, *M. lapidis* MON 3.1^T, *M. marinus* DSM 45201^T, *M. multiseptatus* DSM 44406^T, *M. muralis* MDVD1^T, *M. roseus* DSM 45764^T and *M. versicolor* DSM 16678^T for the fatty acid analyses were prepared from plates of peptone-yeast extract-glucose-vitamins agar (PYGV, DSM medium 621) that had been incubated for 16 days at 20 °C.

Phylogenetic analyses

Genomic DNA was extracted from isolates 1G6^T, 1G14 and 1G50 and PCR-mediated amplification of 16S rRNA genes carried out following the procedures described by Golinska et al. [19,20]. PCR-products were purified using a PCR purification kit (Qiagen), according to the protocol of the manufacturer, checked for quality by using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) and sequenced at the Institute of Biochemistry and Biophysics (IBB) of the Polish Academy of Sciences, Warsaw, Poland using an ABI 3730xl Genetic Analyzer (Applied Biosystems). The resultant 16S rRNA gene sequences (1400–1526 bp) were aligned using CLUSTAL W [33] against corresponding sequences of the type strains of *Modestobacter* species retrieved from the GenBank database using the EzBioCloud server [82].

Maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic trees based on the 16S rRNA gene sequences were inferred using the genome-to-genome distance calculator (GGDC) web server [44] adapted to single genes; the server was also used to calculate pairwise sequence similarities [42,45]. Multiple sequence alignments were generated using MUSCLE software [17]. The ML tree was inferred from the alignments with RAxML [72] 81) using rapid bootstrapping together with the auto maximal-relative-error (MRE) bootstrapping criterion [58]. The MP tree was inferred from the alignments with the Tree Analysis using New Technology (TNT) program [21] using 1000 bootstraps together with tree bisection and reconnection branch swapping and 10 random sequence replicates. The sequences

were checked for computational bias using the x^2 test implemented in PAUP* [73]. A third phylogenetic tree was inferred using the neighbour-joining [66] algorithm with 1000 bootstrap replicates [18] and the MEGA 7 software package [32]. Evolutionary distances were calculated using the two-parameter model of Kimura [30]. The phylogenetic positions of the isolates, in all trees, were established by comparison with corresponding sequences from representative members of the family *Geodermatophilaceae*.

BOX typing

BOX-PCR fingerprints were generated from DNA extracted from the three isolates using the BOXAIR primer [78] and the experimental conditions described by Trujillo et al. [75].

Whole-genome sequencing

A colony of strain 1G6^T was used to inoculate 50 ml of modified Bennett's broth [28] and the preparation incubated at 28 °C in a shake flask (180 rpm) for 72 h. Genomic DNA was extracted from the harvested biomass and sequenced at MicrobesNG (http://www.microbesng.uk) on a MiSeq instrument (Illumina). The reads were trimmed using Trimmomatic [5] and their quality assessed using in-house scripts and Samtools [35,37], BedTools [60] and bwa-mem [36] software. The reads were assembled into contigs with Spades 3.6.2 software [57], contigs under 200 bp were discarded. The draft genome assembly was annotated using the RAST server [2] with default options and submitted to GenBank (accession no. SJEX00000000). The genome sequences of its closest phylogenetic neighbours, *M. italicus* BC 501^T and *M. caceresii* KNN 45-2b^T, were obtained from GenBank (accession numbers: FO203431 and JPMX00000000, respectively), that of *M. roseus* DSM 45764^T from the Integrated Microbial Genomes and Microbiomes System (IMG/M) [13] (IMG genome ID: 2585427561) while the sequence of *M. marinus* DSM 45201^T was provided by Vartul Sangal from Northumbria University, UK (IMG genome ID: 2820994125).

The digital DNA-DNA hybridization (dDDH) value between the draft genome of strain 1G6^T and that of the type strain of *M. italicus*, its closest phylogenetic neighbour, was determined using the GGDC server [42] and the corresponding values between the draft genome of strain 1G6^T and those of the three remaining strains mentioned above were calculated using the TYGS platform [43]. The average nucleotide identity (ANI) values between the draft genome of strain 1G6^T and those of its four closest phylogenetic neighbours were calculated according to Rodriguez and Konstantinidis [63]. Gene sequences encoding proteins involved in stress responses were sought in the genome of strain 1G6^T using the SEED server with default settings [3]. In addition, the genome of strain 1G6^T was examined for gene clusters encoding for natural products using anti-SMASH 4.0 software [4].

Chemotaxonomy

Standard chromatographic procedures were used to detect diagnostic chemical markers of the isolates from freeze-dried biomass. Analysis of the isomers of diaminopimelic acid (A_2pm) was achieved after Lechevalier & Lechevalier [34] and Hasegawa et al. [24]. Menaquinones extracted following Collins et al. [15] were analysed by high performance liquid chromatography [31] and extracted polar lipids identified by two-dimensional thin-layer chromatography (TLC), as described by Minnikin et al. [47]. Extracted whole-organism sugars were analysed following the protocol of Hasegawa et al. [24]; the sugars were separated by TLC on Merck cellulose plates and detected by spraying with acid aniline phthalate. Fatty acids extracted from the isolates and the reference strains of their closest neighbours were methylated and analysed using the protocol of the Sherlock Microbial Identification (MIDI) system, version 5 [68] and the resultant peaks named using the ACTIN6 database. The annotation of the fatty acids in the ACTIN6 peak naming table is consistent with IUPAC nomenclature (i.e. double bond positions identified with reference to the carboxyl group of the fatty acid), but to be in line with other publications this has been changed to numbering from the aliphatic end of the molecule (i.e. $C_{16:1}$ CIS 9 becomes $C_{16:1}$ ω7c and $C_{17:1}$ CIS 9 becomes $C_{17:1}$ ω8c).

Cultural and morphological properties

The isolates were examined for motility, Gram stain, cultural and morphological properties using procedures described by Trujillo et al. [74]. Cultural properties of the isolates were recorded on tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic salts-starch, glycerol-asparagine, peptone-yeast extract iron and tyrosine agar plates (ISP media 1–7) [71] following incubation at 28 °C for 14 days. Colony pigments were determined by comparison against chips from Inter-Society Color Council National Bureau of Standard Colour charts [29]. Growth of the isolates at different temperatures (4, 10, 15, 20, 25, 28, 30, 35, 40 and 45 °C), from pH 4.0–12.0 (at intervals of 0.5 pH unit) and in the presence of 0–10 % NaCl (w/v) were determined on glucose yeast extract-malt extract (ISP2) agar medium [71]; KH_2PO_4/HCl, KH_2PO_4/K_2HPO_4 (0.1 M) and K_2HPO_4/NaOH buffer systems were used to adjust pH values.

Phenotypic properties

The isolates and the type strains of *Modestobacter* species were screened for a combination of biochemical, degradation and physiological properties found to be of value in the circumscription of *Modestobacter* species [10,49,74]. All of these tests were carried out in duplicate using a standard inoculum equivalent to 5.0 on the McFarland scale [51]. The ability of the strains to oxidise diverse carbon and nitrogen sources and to show resistance to inhibitory compounds were determined, in duplicate, using GEN III microplates in an Omnilog device (BIOLOG Inc., Haywood, USA) following incubation at 28 °C for 7 days. Briefly, the isolates were grown on GYM *Streptomyces* agar plates at 28 °C, cell suspensions prepared in a viscous inoculating fluid (IF C) at 83 % transmittance (T) for the type strains, at 92 % T for isolate 1G6^T, at 80 % T for isolate 1G14 and at 91 % T for isolate 1G50 and the microplates inoculated according to the protocol of the manufacturer. Data were exported and analysed using the opm package for R v.1.0.6 [76,77]. In addition, the enzymatic activities of the isolates were determined using API ZYM kits (bioMerieux) by following the manufacturer's instructions. In all cases conflicting results between duplicated cultures were scored as variable.

Results and discussion

Phylogeny

Nearly complete 16S rRNA gene sequences of isolates 1G6^T, 1G14 and 1G50 (1526, 1416 and 1417 nucleotides [nt], respectively) were determined (GenBank accession numbers: MH430528, MH430529 and MH430530, respectively). Isolates 1G6^T and 1G14 were found to have identical 16S rRNA gene sequences and shared a 99.86 % sequence similarity with isolate 1G50, a value corresponding to two nt differences at 1414 and 1416 locations. The isolates formed a distinct lineage within the evolutionary radiation occupied by the genus *Modestobacter* that was supported by

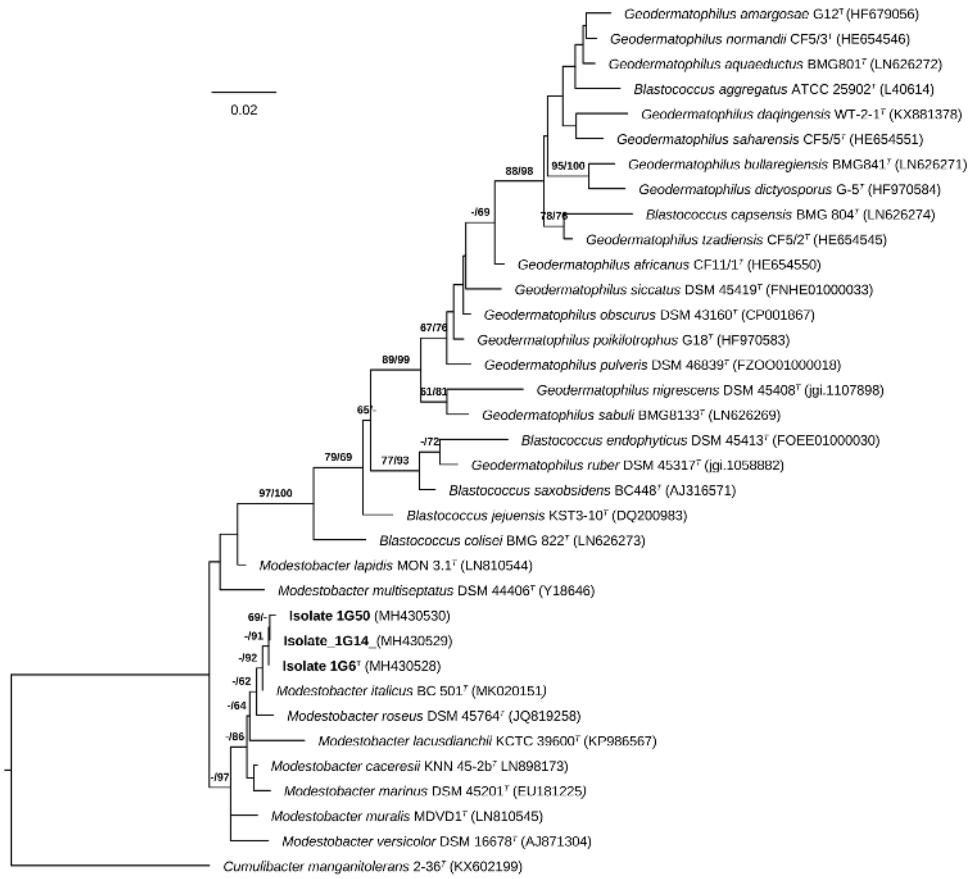

Fig. 1. Maximum-likelihod and maximum-parsimony tree based on nearly complete 16S rRNA gene sequences showing relationships between isolates 1G6^T, 1G14 and 1G50 and between them and the type strains of *Modestobacter* species. The numbers above the branches are support values when larger than 60% from ML (left) and MP (right) bootstrapping. Bar; 0.02 substitutions per nucleotide position. The root position of the tree was determined using *Cumulibacter manganitolerans* 2-36^T as the outgroup.

the maximum-likelihood, maximum-parsimony and neighbour-joining algorithms (Fig. 1, Fig. S1). The isolates showed diverse BOX-PCR profiles (Fig. S2) indicating that they are not clones.

All of the isolates were most closely related to *M. italicus* BC 501^T sharing a 99.8–99.9 % 16S rRNA gene similarity with the latter, this corresponds to 1–3 nt differences at between 1416–1508 locations. The isolates were also closely related to *M. caceresii* KNN 45-2b^T (99.6 % similarity), *M. roseus* DSM 45764^T (99.4–99.5 % similarity) and *M. marinus* DSM 45201^T (99.3–99.5% similarity) but less so to *M. versicolor* DSM 16678^T (98.6–98.7% similarity), *M. muralis* MDVD1^T (98.5–98.7% similarity) and *M. lacusdianchii* KCTC 39600^T (98.2–98.6 % similarity). According to Meier-Kolthoff et al. [42] an actinobacteria-specific 16S rRNA threshold of 99.0 % with a 1.0 % maximum probability of error is equivalent to a DNA–DNA hybridization (DDH) value at the 70 % threshold recommended for the assignment of closely related strains to the same species [80]. Information drawn from whole-genome sequences is being used to distinguish between members of closely related prokaryotic species [42,56,67,83], as exemplified by ANI and dDDH values used to distinguish between closely related strains classified in the genera *Blastococcus* and *Geodermatophilus* [11,12].

Comparison of the draft genome of isolate 1G6^T with those of its closest phylogenetic neighbours

The draft assembly of the genome sequence of isolate 1G6^T (GenBank accession number: SJEX00000000) was composed of 178 contigs giving a total genome size of 5255906 bp with a digital DNA G + C content of 73.7 mol%. The total number of reads was 725876 (N50 = 48913, L50 = 32) and the genome sequencing coverage 60 ×. The whole genome was annotated to include 5273 protein coding sequences and 50 RNA genes. Digital DDH values between isolate 1G6^T and the type strains of *M. caceresii, M. italicus*, *M. marinus* and *M. roseus*, its closest phylogenetic relatives, were 26.8 %, 42.3 %, 26.9 % and 25.6 % respectively, values well below the previously mentioned 70 % threshold introduced by Wayne et al. [80]. Similarly, the corresponding ANI similarities between isolate 1G6^T and the type strains of *M. italicus*, *M. caceresii, M. marinus* and *M. roseus* were 90.7 %, 84.28 %, 84.1 % and 82.5 % respectively, values well below the 95–96 % threshold used to distinguish between members of closely related species [14,22].

Chemotaxonomic, cultural and morphological properties

In general, the chemotaxonomic, cultural and morphological properties of the isolates were in accord with their classification in the genus *Modestobacter* [46,49,54,74]. The isolates were found to be aerobic, Gram-stain-positive, non-motile, formed short rods and coccoid-shaped cells which tended to remain aggregated. The isolates grew well on all of the ISP agar media, mainly forming black, greenish-yellow, olive and brown colonies (Table 2). They produced whole-organism hydrolysates rich in *meso*-A_2pm, glucose and ribose (diagnostic sugars) and arabinose, contained MK-9(H_4) as the predominant isoprenologue (77.4–91.3 % of total menaquinone composition) with minor amounts of MK-8(H_4) (8.7–22.5 %) and polar lipid patterns containing diphosphatidylglycerol, glycophosphatidylinositol, phosphatidylethanolamine (diagnostic component), phosphatidylglycerol, phosphatidylinositol, and as exemplified in Fig. S3. Glycophosphatidylinositol, previously annotated as phosphatidylinositol mannoside (PIM) [10,59,74], is a

Table 1
Fatty acid profiles (%) of the isolates and their closest relatives after growth on peptone-yeast extract-glucose-vitamins agar plates for 16 days at 20 °C. Values are percentages of total fatty acids; -, not detected.

Modestobacter strains							
Fatty acids	1G6^T	1G14	1G50	*M. caceresii* KNN 45-2b^T	*M. italicus* BC 501^T	*M. marinus* DSM 45201^T	*M. roseus* DSM 45764^T
$C_{9:0}$	–	–	–	–	0.1	–	–
$C_{10:0}$	–	–	–	–	0.1	–	–
$C_{12:0}$	–	–	0.4	–	–	–	–
$C_{12:0}$ 3OH	–	–	–	–	–	–	0.1
$C_{14:0}$	0.4	0.9	1.5	0.3	0.3	0.2	0.2
$C_{15:0}$	–	–	–	0.4	0.1	0.2	2.4
$C_{15:0}$ 2OH	–	–	–	–	–	–	0.1
$C_{16:0}$	1.6	1.7	7.9	3.1	1.0	4.1	0.3
$C_{16:0}$ 2OH	–	–	–	0.1	–	–	–
$C_{17:0}$	0.3	0.3	1.5	0.3	0.2	1.1	1.7
$C_{18:0}$	0.9	3.9	4.5	–	0.4	0.2	–
$C_{14:1}$ ω5c	0.1	0.2	–	–	–	–	–
$C_{15:1}$ ω5c	–	0.2	–	–	–	–	–
$C_{15:1}$ ω6c	0.5	0.4	–	–	–	0.5	–
$C_{15:1}$ B	–	–	–	0.5	0.1	–	1.2
$C_{16:1}$ B	–	–	–	–	0.2	–	–
$C_{16:1}$ cis 9	–	–	–	23.5	5.7	–	5.4
$C_{17:0}$ cyclo	–	–	–	2.6	–	1.7	1.5
$C_{17:1}$ ω8c	3.3	3.0	5.2	–	–	7.6	–
$C_{17:1}$ cis 9	–	–	–	7.6	4.5	–	10.9
$C_{18:1}$ ω6c	0.4	0.7	1.7	–	–	–	–
$C_{18:1}$ ω9c	7.3	8.0	12.2	–	–	4.1	–
$C_{18:1}$ cis 9	–	–	–	3.8	17.3	–	0.4
$C_{20:1}$ ω9c	0.2	–	–	–	–	–	–
$C_{20:1}$ cis 11	–	–	–	0.1	–	–	–
$C_{16:0}$ 9-methyl	–	–	–	1.8	1.5	–	4.4
$C_{17:0}$ 10-methyl	–	–	–	1.8	4.4	6.0	0.9
$C_{18:0}$ 10-methyl, TSBA	–	–	1.3	–	–	–	–
anteiso-$C_{11:0}$	–	–	0.5	–	0.2	–	–
anteiso-$C_{13:0}$	–	0.2		–	–	–	–
anteiso- $C_{15:0}$	5.8	12.5	14.5	0.4	1.2	0.7	0.9
anteiso-$C_{15:0}$ A	0.6	0.5	0.5	–	–	–	–
anteiso-$C_{15:0}$ 2OH	–	–	–	–	–	–	0.4
anteiso-$C_{15:1}$ A	–	–	–	–	0.2	0.2	–
anteiso-$C_{16:0}$	0.3	0.7	0.9	0.1	–	–	–
anteiso- $C_{17:0}$	–	–	–	0.5	1.9	0.7	0.5
anteiso- $C_{17:1}$	6.3	7.6	11.6	–	–	–	–
anteiso- $C_{17:1}$ C	–	–	–	0.3	0.5	–	1.0
anteiso-$C_{17:1}$ ω9c	3.8	4.3	1.3	–	–	0.7	–
iso-$C_{10:0}$	–	–	–	–	1.3	–	–
iso-$C_{11:0}$	–	–	–	–	0.4	–	–
iso-$C_{12:0}$	–	–	–	–	0.1	–	–
iso-$C_{13:0}$	0.1	0.2	–	–	0.1	–	0.1
iso-$C_{14:0}$	1.4	1.0	1.1	1.7	1.4	2.3	3.1
iso-$C_{15:0}$	18.7	20.8	10.7	3.2	6.9	2.9	16.0
iso-$C_{15:1}$ G	8.1	6.1	2.3	3.1	4.4	3.0	–
iso-$C_{16:0}$	16.3	7.4	7.5	28.1	32.8	32.5	25.7
iso-$C_{16:0}$ 2OH	–	–	–	–	2.0	–	1.7
iso-$C_{16:1}$ G	–	–	–	–	5.6	–	–
iso-$C_{16:1}$ H	5.8	2.3	–	14.9	–	16.7	9.4
iso-$C_{17:0}$	1.7	1.4	2.7	0.6	1.9	1.4	2.4
iso-$C_{17:0}$ 2OH	–	–	–	0.6	0.3	–	3.7
iso-$C_{17:1}$ ω9c.	5.9	4.5	0.9	–	–	1.7	–
iso-$C_{18:0}$	0.1	–	–	–	0.9	0.4	–
iso-$C_{18:1}$ H	–	–	–	–	0.3	0.4	–
iso-$C_{19:1}$ I	–	–	–	–	0.1	–	–
Sum in feature 2	–	–	–	–	–	–	0.5
Sum in feature 3	9.1	10.2	5.2	–	–	8.5	–
Sum in feature 4	–	–	–	–	–	–	2.6
Sum in feature 6	–	–	–	0.4	–	–	–
Sum in feature 7*/**	1.0*	1.2*	4.2*	0.3**	0.8**	0.6*	0.1**
Sum in feature 9	–	–	–	0.2	0.8	–	–

*As indicated by Montero-Calasanz et al. [50] summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete ECLs as well as those where the ECLs are not reported separately.
Summed feature 2 comprises iso-$C_{15:1}$ I and $C_{13:0}$ 3OH, summed feature 3 $C_{16:1}$ ω7c and $C_{16:1}$ ω6c; summed feature 4 iso-$C_{15:0}$ 2OH and $C_{16:1}$ t9; summed feature 6 anteiso-$C_{18:0}$ and $C_{18:2}$ cis; summed feature 7* $C_{19:0}$ cyclo ω10c and/or 19ω6; summed feature 7** $C_{18:1}$ trans 9 and $C_{18:1}$ trans 6 and $C_{18:1}$ cis; summed feature 9 $C_{19:0}$ cyclo C9-10 and undefined fatty acid.

Table 2
Cultural and phenotypic properties that distinguish between the isolates.

Characteristics	Isolates					
	1G6^T		1G14		1G50	
Growth on media:						
Tryptone - yeast extract agar (ISP 1)	++	Dark olive	++	Black	+++	Light olive
Yeast extract - malt extract agar (ISP 2)	+++	Black	+++	Black	++	Strong yellow
Oatmeal agar (ISP 3)	++	Black	++	Black	++	Black
Inorganic salts starch agar (ISP 4)	+	Black	+	Black	+	Brown
Glycerol-asparagine agar (ISP 5)	+	Dark olive	+	Black	+	Pale greenish yellow
Peptone - yeast extract iron agar(ISP 6)	+++	Strong brown	+++	Deep brown	++	Pale greenish yellow
Tyrosine agar (ISP 7)	+	Black	+	Black	+	Pale greenish yellow
API-ZYM tests:						
α-Fucosidase	–		–		+	
α-Galactosidase	–		–		+	
β-Galactosidase	–		–		+	
Lipase (C14)	–		–		+	
α-Mannosidase	–		–		+	
BIOLOG GEN III microplate tests						
Assimilation of:						
D-Cellobiose	+		–		+	
D-Fructose	+		+		–	
L-Fucose	+		–		–	
L-Galactonic acid-γ-lactone	–		+		–	
β-Gentiobiose	+		–		+	
D-Gluconic acid	–		+		–	
L-Glucuronic acid	+		–		–	
D-Glucose-6-phosphate	+		+		–	
D-Mannose	+		+		–	
Pectin	–		+		+	
D-Turanose	+		–		+	
Citric acid	+		–		+	
Sodium lactate (1 %)	+		+		–	
Gelatin	–		+		–	
Temperature growth range (°C)	4-30		10-35		15-35	

Growth: +++, abundant; ++, good; +, weak. None of the isolates formed diffusible pigments on the growth media. +, positive; -, negative.
The isolates were positive for acid and alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), *N*-acetyl-β-glucosaminidase, α- and β-glucosidases, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase (API-ZYM tests); oxidised D-serine#2 (amino acid), acetic acid, acetoacetic acid, butyric acid, α- and β-*hydroxy*-butyric acid, α-*keto*-butyric acid, D-galacturonic acid, α-*keto*-glutaric acid, L-malic acid and propionic acid (organic acids), dextrin, D-fructose-6-phosphate, D-glucose, glucuronamide, D-maltose, D-raffinose, D-sorbitol and D-trehalose (sugars), Tween 40 (polymer) and grew in the presence of aztreonam, fusidic acid, guanidine hydrochloride, lincomycin, lithium chloride, minocycline, nalidixic acid, niaproof, potassium tellurite, rifamycin SV, sodium bromate, sodium chloride (1, 4 and 8 %), tetrazolium blue, tetrazolium violet, troleandomycin and vancomycin (Biolog tests). In contrast, the isolates were negative for β-glucuronidase (API-ZYM tests), did not metabolise L-alanine, L-arginine, D- and L-aspartic acid, bromo-succinic acid, γ-amino-*n*-butyric acid, L-glutamic acid, L-histidine, D-serine#1 or L-serine (amino acids), L-lactic acid, D-lactic acid methyl ester, D-malic acid, mucic acid, *N*-acetyl-neuraminic acid, *p*-*hydroxy*-phenylacetic acid, glycyl-L-proline, pyroglutamic acid, methyl-pyruvate, quinic acid or D-saccharic acid (organic acids), D-arabitol, D-fucose, *N*-acetyl-D-galactosamine, D-galactose, *N*-acetyl-D-glucosamine, 3-*O*-methyl-D-glucose, β-methyl-D-glucoside, glycerol, inosine, *myo*-inositol, α-D-lactose, D-mannitol, *N*-acetyl-β-D-mannosamine, D-melibiose, L-rhamnose, D-salicin, D-stachyose or D-sucrose (sugars) or grow in the presence of sodium formate (Biolog tests).

common component in chromatographic profiles of *Modestobacter* species, except for *M. multiseptatus* DSM 44406^T, which it contains an unidentified glycolipid [49]. In this context, the polar lipid profiles of the isolates are in accordance with polar lipid patterns found in the type strains of *M. caceresii, M. muralis, M. lapidis, M. roseus* and *M. versicolor* [10,49,74].

In general, the isolates had fatty acid profiles that distinguish them from those of their closest phylogenetic neighbour, as exemplified by relatively high proportions of $C_{18:1}$ ω9c (7.3–12.2 %), anteiso-$C_{15:0}$ (5.8–14.5 %), anteiso-$C_{17:1}$ (6.3–11.6 %), iso-$C_{15:0}$ (10.7–20.8 %) and summed feature 3 (5.2–10.2 %). In contrast, *M. caceresii, M. italicus, M. marinus* and *M. roseus* were characterized by much higher proportions of iso-$C_{16:0}$ (25.7–32.8 %); the corresponding range for the isolates was 7.4–16.3 %. Qualitative and quantitative differences were also found between the isolates; the predominant fatty acid in isolates 1G6^T and 1G14 was iso-$C_{15:0}$ (18.7–20.8 %, respectively) whereas the predominant component in isolate 1G50 was anteiso-$C_{15:0}$ (14.5 %); much lower levels of this fatty acid were found in isolates 1G6^T and 1G14 (with 5.8 and 12.5 %, respectively). In general, the polar lipid profiles of the isolates are in line with these found in the type strains of *Modestobacter* species, as shown in Table 3.

Phenotypic tests

The duplicated cultures of the isolates gave the same results for all of the API-ZYM tests though variable results were recorded for some of the Biolog tests, as shown in Table 2.

It can also be seen from Table 2 that the isolates have many phenotypic properties in common, namely 83 %, though several features can be weighted to distinguish between them thereby providing further evidence that the isolates are not clones. Isolate 1G6^T, for instance, can be separated from the other two strains by its ability to metabolize L-fucose and L-glucuronic acid while only isolate 1G50 was positive for α-fucosidase, α- and β-galactosidase, lipase (C14) and α-mannosidase. Only isolate 1G14 was found to be positive for D-gluconic acid and gelatin and negative for the assimilation of D-cellobiose, citric acid β–gentiobiose and D-turanose (Table 2).

It can be seen from Table 3 that although some of the duplicated cultures gave variable results, a combination of phenotypic properties can be weighted to distinguish between the isolates and the type strains of *Modestobacter* species. In particular, the isolates can be separated from *M. italicus* BC 501^T, their nearest phylogenetic neighbour, by an ability to oxidise acetic acid, acetoacetic acid, α-*hydroxy*-butyric acid, D-fructose-6-phosphate, D-galacturonic acid,

Table 3
Phenotypic properties that distinguish the isolates from the type strains of *Modestobacter* species.

	1	2	3	4	5	6	7	8	9	10
BIOLOG GEN III microplate tests Assimilation of:										
Acetic acid	+	+	–	+	+	–	+	+	+	+
Acetoacetic acid	+	+	–	+	+	–	+	+	+	+
Bromo-succinic acid	–	–	–	–	–	–	–	–	+	–
Butyric acid	+	–	+	+	–	–	–	–	+	–
γ-amino-*n*-Butyric acid	–	+	–	–	–	–	+	–	–	+
α-*hydroxy*-Butyric acid	+	+	–	–	+	–	–	–	–	–
β-*hydroxy*-Butyric acid	+	+	–	–	–	–	+	–	–	+
Dextrin	+	+	+	+	+	+	–	–	+	–
D-Fructose-6-phosphate	+	+	–	–	–	+	–	+	–	+
D-Fucose	–	+	–	–	+	–	+	–	–	+
D-Galactose	–	+	–	+	+	+	+	+	–	+
D-Galacturonic acid	+	–	–	+	+	–	+	–	–	–
N-Acetyl-D-glucosamine	–	+	–	+	+	+	+	+	+	+
β-Methyl-D-glucoside	–	+	–	+	+	+	+	–	–	–
Glucuronamide	+	+	–	–	+	–	+	–	–	–
Glycerol	–	+	+	+	–	+	+	+	+	+
Glycyl-L-proline	–	–	–	–	–	–	+	–	–	–
Inosine	–	–	–	–	–	+	–	–	–	–
D-Lactic acid methyl ester	–	+	–	–	–	–	–	–	–	+
α-D-lactose	–	–	–	+	+	+	+	+	+	–
D-Malic acid	–	–	–	+	–	–	+	+	+	–
L-Malic acid	+	–	–	+	+	+	+	+	+	+
D-Maltose	+	+	–	–	+	+	+	+	+	–
D-Melibiose	–	–	–	–	+	–	+	+	–	–
p-hydroxy-Phenylacetic acid	–	+	–	–	–	–	–	–	+	–
Propionic acid	+	+	–	+	+	–	+	–	+	+
L-Pyroglutamic acid	–	–	–	–	–	+	+	–	–	–
Methyl pyruvate	–	–	–	–	–	–	–	–	+	–
Quinic acid	–	–	–	–	–	–	–	–	+	–
D-Raffinose	+	–	–	+	+	–	–	+	–	–
L-Rhamnose	–	+	+	+	+	+	+	+	+	+
D-Saccharic acid	–	–	–	–	–	+	–	–	+	–
D-Serine#2	+	–	–	–	–	–	–	–	–	–
L-Serine	–	–	–	+	–	+	–	–	–	–
D-Sorbitol	+	+	–	+	+	+	–	+	–	+
D-Stachyose	–	–	–	–	+	+	+	–	–	–
D-Sucrose	–	+	–	+	+	+	+	+	+	+
D-Trehalose	+	+	–	+	+	+	+	+	+	+
Tween 40	+	+	+	+	+	–	+	+	+	–
Growth in presence of inhibitory compounds:										
Fusidic acid	+	–	–	–	–	–	–	–	–	–
Guanidine hydrochloride	+	–	–	–	–	–	–	–	–	–
Lincomycin	+	–	–	–	–	–	–	–	–	–
Minocycline	+	–	+	–	–	–	–	–	–	–
Nalidixic acid	+	–	+	+	+	+	–	+	+	+
Niaproof	+	–	–	–	–	–	–	–	–	–
Sodium bromate	+	–	+	+	–	–	–	+	+	–
Sodium chloride 1 %	+	–	+	+	+	+	–	+	+	–
Sodium chloride 4 %	+	–	–	+	–	–	–	–	+	–
Sodium chloride 8 %	+	–	–	+	–	–	–	–	–	–
Tetrazolium blue	+	–	–	–	–	–	–	–	–	–
Tetrazolium violet	+	–	–	+	–	–	–	–	–	–
Troleandomycin	+	–	–	–	–	–	–	–	–	–
Vancomycin	+	–	–	–	–	–	–	–	–	–
growth at pH 6	+	–	–	–	–	+	–	–	–	–
Polar lipids[a]	DPG, PG, PE, PI, GPI, 2L	DPG, PG, PE, PI, PIM	DPG, PE, PI, GPI	DPG, PE, PI, PIM, PL	DPG, PG, PE, PI, PIM	DPG, PG, PE, PI, APL, GL, PL	DPG, PE, PI, GL, 2APL, 3L	DPG, PG, PE, PI, PIM	DPG, PG, PE, PI, GPI, AL, 2APL	DPG, PG, PE, PI, PL, GPI, 4L

Strains: 1, Isolates 1G6^T, 1G14 and 1G50; 2, *M. caceresii* KNN 45-2b^T; 3, *M. italicus* BC 501^T; 4, *M. lacusdianchii* KCTC 39600^T; 5, *M. lapidis* MON 3.1^T; 6, *M. marinus* DSM 45201^T; 7, *M. multiseptatus* DSM 44406^T; 8, *M. muralis* MDVD1^T; 9, *M. roseus* DSM 45764^T; 10, *M. versicolor* DSM 16678^T. All data are from this study. +, positive; –, negative.
Codes: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; GPI, glycophosphoinositol; PIM, phosphoinositol-mannoside; AL, aminolipids; APL, aminophospholipids; GL, glycolipid; PL, unidentified phospholipid; L, unidentified lipid.
All of the strains oxidized D-glucose, but not D-arabitol, D-aspartic acid, L-histidine, mucic acid, *N*-acetyl-neuraminic acid or D-serine#1, and did not grow in the presence of sodium formate or at pH 5.
Between one and three of the strains gave variable results for the following duplicated tests: L-alanine, L-arginine, L-aspartic acid, α-*keto*-butyric acid, *N*-acetyl-D-galactosamine, 3-*O*-methyl-D-glucose, L-glutamic acid, α-*keto*-glutaric acid, *myo*-inositol, L-lactic acid, D-mannitol, *N*-acetyl-β-D-mannosamine, D-salicin, and for growth in the presence of aztreonam, lithium chloride, pottasium tellurite and rifamycin SV.

[a] Data for strain 2 are from Busarakam et al. [10]; for 3, 6, 7 and 9 from Montero-Calasanz et al. [49]; for 4 from Zhang et al. [85]; for 5 and 8 from Trujillo et al. [74].

glucuronamide, L-malic acid, D-maltose, propionic acid, D-raffinose, D-sorbitol and D-trehalose and by a pronounced ability to grow in the presence of inhibiting compounds. In contrast, the *M. italicus* strain, unlike the isolates, assimilated L-rhamnose and glycerol. Similar combinations of phenotypic properties can be used to distinguish the isolates from the remaining *Modestobacter* type strains and the latter from one another.

Genes associated with stress responses

It is evident from the SEED analyses that the draft genome of isolate 1G6^T contains 59 stress-related genes, as exemplified by the presence of *dnaK* and *dnaJ* gene clusters and *hrcA*, *grpE* and *Hsp* genes that are linked to heat shock responses [38] and by genes encoding for the Csp family of proteins which are associated with cold shock responses [16]. The draft genome also contains a *KatA* and a *KatE* gene encoding for enzymes associated with protection against reactive oxygen species [55], as well as a *sox* gene cluster and several genes coding for betaine biosynthesis and for betaine and choline uptake, all of these genes are involved in responses to oxidative stress [6,7,52]. In turn, the presence of a carbon storage regulator gene (*Csr*) (fig|6666666.388060.peg.4837) is associated with multiple biosynthetic pathways, including glycogen biosynthesis [64,65]. Two copies of an *uvrD* gene encode ATP dependent DNA helicase were detected; these genes are involved in the UV-resistance of *M. italicus* BC 501^T (formerly *M. marinus*) [55]. Genes linked with responses to desiccation stress were not found though multiple copies of genes involved in the uptake of trehalose and associated with tolerance to desiccation and temperature in bacteria [62] were detected. Similarly, genes linked with stabilizing genomes, namely *recO*, *recF* and *recQ* helicase [25,41], were found. Two copies of *coxD*, *coxE* and *coxG* genes and a *coxLSM* cluster which encode for different subunits of carbon monoxide (CO) dehydrogenase were highlighted in a BLAST search of the 1G6^T genome; these genes encode for the uptake of carbon monoxide indicating that this isolate may have a chemolithoautotrophic lifestyle [39]. Similar profile of stress-related genes have been detected in the genomes of the type strains of *Blastococcus atacamensis* [11], *G. chilensis* [12] and *M. caceresii* [10], all of which were isolated from Atacama Desert soils.

Biosynthetic gene clusters

Mining of the draft genome of isolate 1G6^T using the antiSMASH server [4] revealed the presence of several putative biosynthetic gene clusters. Two of the clusters coded for type 2 and type 3 polyketide synthases while others were associated with the synthesis of betalactone and terpenes; these results are in line with corresponding studies on the type strains of *G. chilensis* [12] and *M. caceresii* [10]. However, improved genome alignments are needed to achieve a better understanding of the genomes of isolate 1G6^T and those of the type strains of *G. chilensis* and *M. caceresii*.

Conclusions

The chemotaxonomic, cultural, morphological and phenotypic data show that isolates 1G6^T, 1G14 and 1G50 are not clones but are *bone fide* members of the genus *Modestobacter* and belong to the same species. The isolates can be separated readily from the type strains of *Modestobacter* species by a wealth of genomic and phenotypic data. In addition, isolate 1G6^T can be distinguished from *M. italicus* BC 501^T, its closest phylogenetic neighbour, by low ANI and dDDH values. Consequently, it is proposed that the three isolates be recognised as a novel species of *Modestobacter*, namely as *Modestobacter excelsi*. It is also interesting that the genome of isolate 1G6^T is rich in stress-related genes associated with cold and heat stress responses, oxidative stress, resistance to UV-radiation and with an ability to use carbon monoxide as a source of carbon and energy while the presence of a few natural product biosynthetic gene clusters show that it has the capacity to produce specialized metabolites; these observations provide support for the view that the competitive success of *Geodermatophilaceae* strains in extreme, sparsely populated biomes may be more related to stress resistance than to antibiosis [8].

Description of *Modestobacter excelsi* sp. nov

Modestobacter excelsi *(ex.cel' si. L. gen. n.* excelsi *of a high place)*

Aerobic, Gram-stain-positive, non-motile actinobacteria which form short rods and coccoid-like elements that tend to remain aggregated. Grows between 4 and 35 °C, optimally around 28 °C, from pH 6 to 8.5, optimally around pH 8 and in presence of up to 8 % (w/v) NaCl. Aesculin is hydrolysed but not allantoin, arbutin or urea. Tweens 40 and 60 are degraded but not adenine, casein, chitin, elastin, gelatin, guanine, hypoxanthine, starch, Tweens 20 and 80, L-tyrosine, uric acid, xanthine or xylan. Positive for acid and alkaline phosphatase, α-chymotrypsin, cystine arylamidase esterase (C4), esterase lipase (C8), *N*-acetyl-β-glucosaminidase, α- and β-glucosidases, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin, valine arylamidase and negative for β-glucuronidase. Oxidizes dextrin, D-fructose-6-phosphate, D-glucose, glucuronamide, D-maltose, D-raffinose D-sorbitol and D-trehalose, but not D-arabitol, D-fucose, *N*-acetyl-D-galactosamine, D-galactose, *N*-acetyl-D-glucosamine, 3-*O*-methyl-D-glucose, β-methyl-D-glucoside, glycerol, inosine, *myo*-inositol, α-D-lactose, D-mannitol, *N*-acetyl-β-D-mannosamine, D-melibiose, L-rhamnose, D-salicin, D-stachyose or D-sucrose. Acetic acid, acetoacetic acid, butyric acid, α- and β-*hydroxy*-butyric acid, α-*keto*-butyric acid, D-galacturonic acid, α-*keto*-glutaric acid, L-malic acid and propionic acids are metabolised, but not L-alanine, L-arginine, D- or L-aspartic acid, bromo-succinic acid, γ-amino-*n*-butyric acid, L-glutamic acid, L-histidine, L-lactic acid, D-lactic acid methyl ester, D-malic acid, mucic acid, *N*-acetyl-neuraminic acid, *p-hydroxy*-phenylacetic acid, glycyl-L-proline, L-pyroglutamic acid, methyl pyruvate, quinic acid, D-saccharic acid, D-serine#1 or L-serine. Grows in the presence of aztreonam, fusidic acid, guanidine hydrochloride, lincomycin, lithium chloride, minocycline, nalidixic acid, niaproof, potassium tellurite, rifamycin SV, sodium bromate, tetrazolium blue, tetrazolium violet, troleandomycin and vancomycin, but not in the presence of sodium formate. Additional phenotypic properties are cited in the text and in Tables 1–3. The cell wall peptidoglycan contains *meso*-A_2pm, the whole-cell sugars are arabinose, glucose and ribose, the major fatty acids are anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, anteiso-$C_{17:1}$and and $C_{18:1}\omega$9c, the predominant menaquinone is MK-9(H_4) and the diagnostic phospholipid phosphatidylethanolamine. The genome size of the type strain is around 5.25 Mbp and the genomic DNA G + C content 73.7 mol%.

The type strain 1G6^T (=PCM 3004^T, =DSM 107535^T) was isolated from a surface soil sample (2 cm depth) collected from a high altitude Atacama Desert soil. The GenBank 16S rRNA gene sequence accession number for isolate 1G6^T is MH430528 and the corresponding GenBank NCBI accession number of the genome sequence SJEX00000000. The digital protologue number is TA01023.

Acknowledgements

This work was supported by Grant No. 2017/01/X/NZ8/00140 from the National Science Centre (NCN) of Poland. Genome sequencing was provided by MicrobesNG (https://www.

microbesng.uk) which is supported by the Biotechnology and Biological Sciences Research Council, UK (BBSRC; grant number BB/L024209/1). We are grateful to Tiago Zucchi (Agrivalle, Agricultural Biotechnology, Salto, Brazil) for suggesting the species epithet of the strain.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.syapm.2019.126051.

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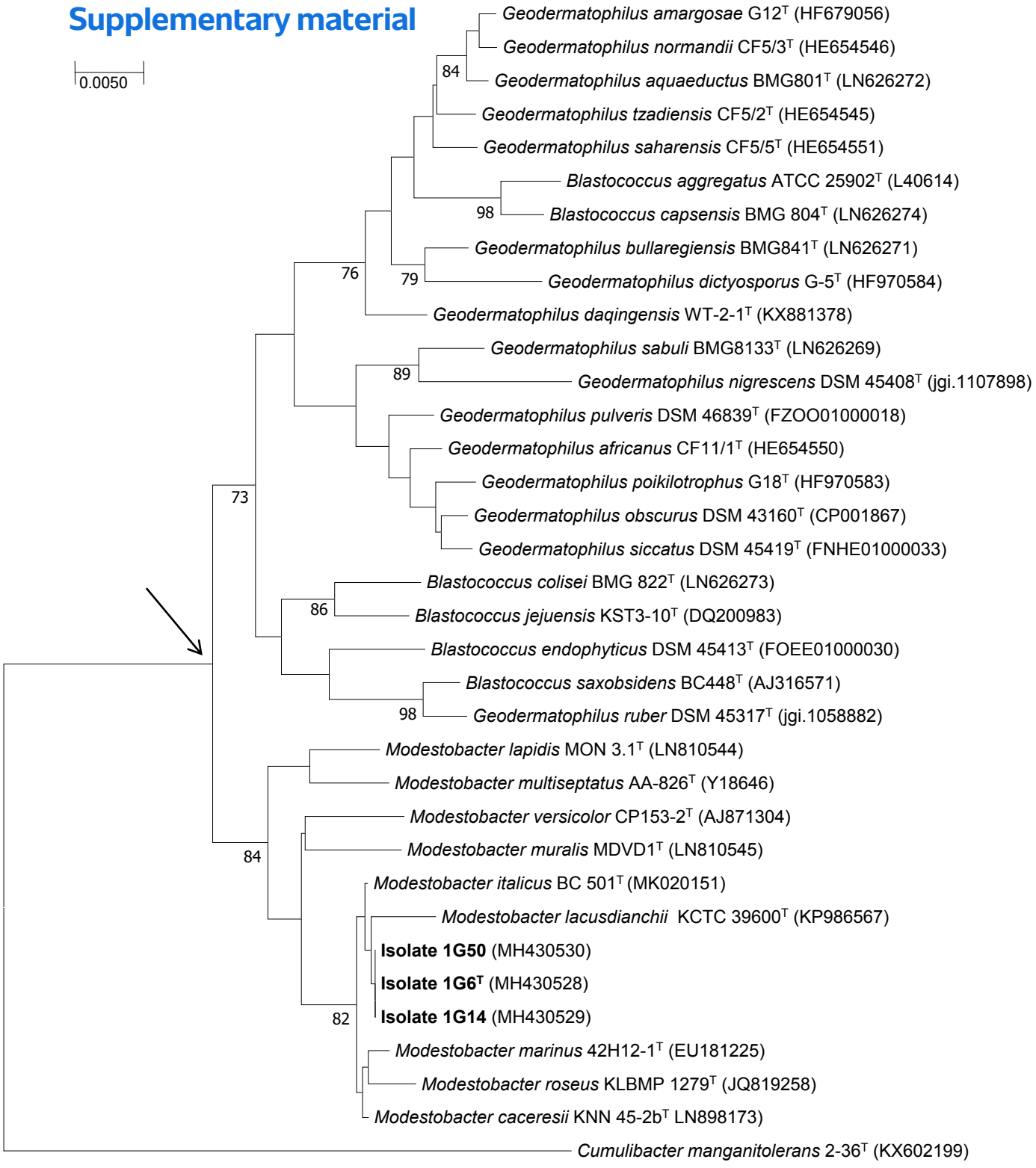

Fig. S1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between isolates 1G6^T, 1G14 and 1G50 and between them and the type strains of *Modestobacter* species. The numbers at the nodes are bootstrap support values at or above 70%. Bar; 0.005 substitutions per nucleotide position. The root position of the tree was determined using *Cumulibacter manganitolerans* 2-36^T as the outgroup.

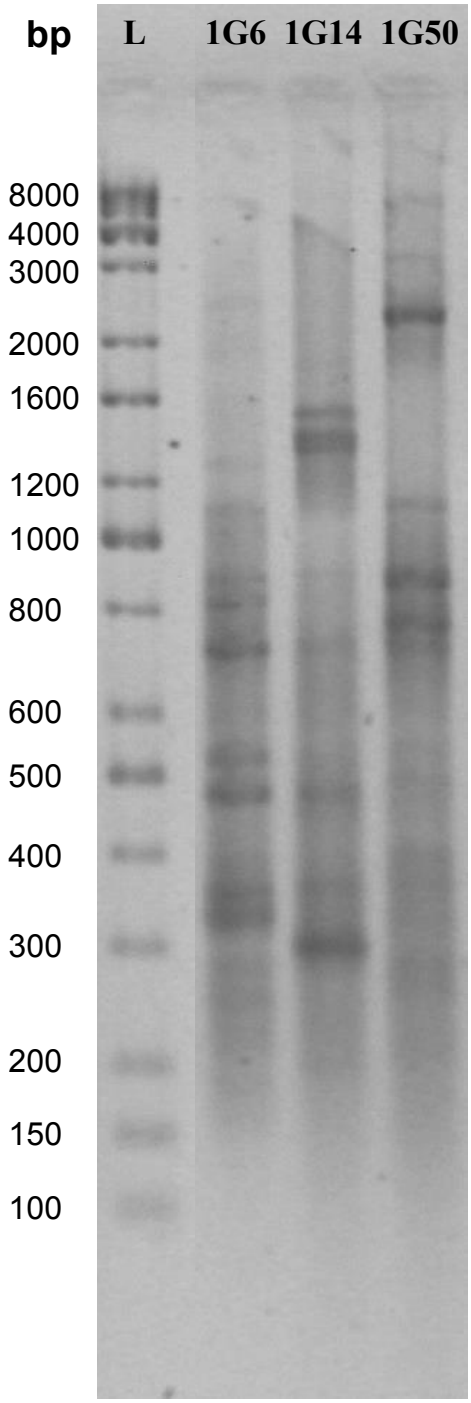

Fig. S2. BOX PCR fingerprint patterns of genomic DNA extracted from isolates: 1, 1G6^{T}; 2, 1G14; and 3; 1G50.

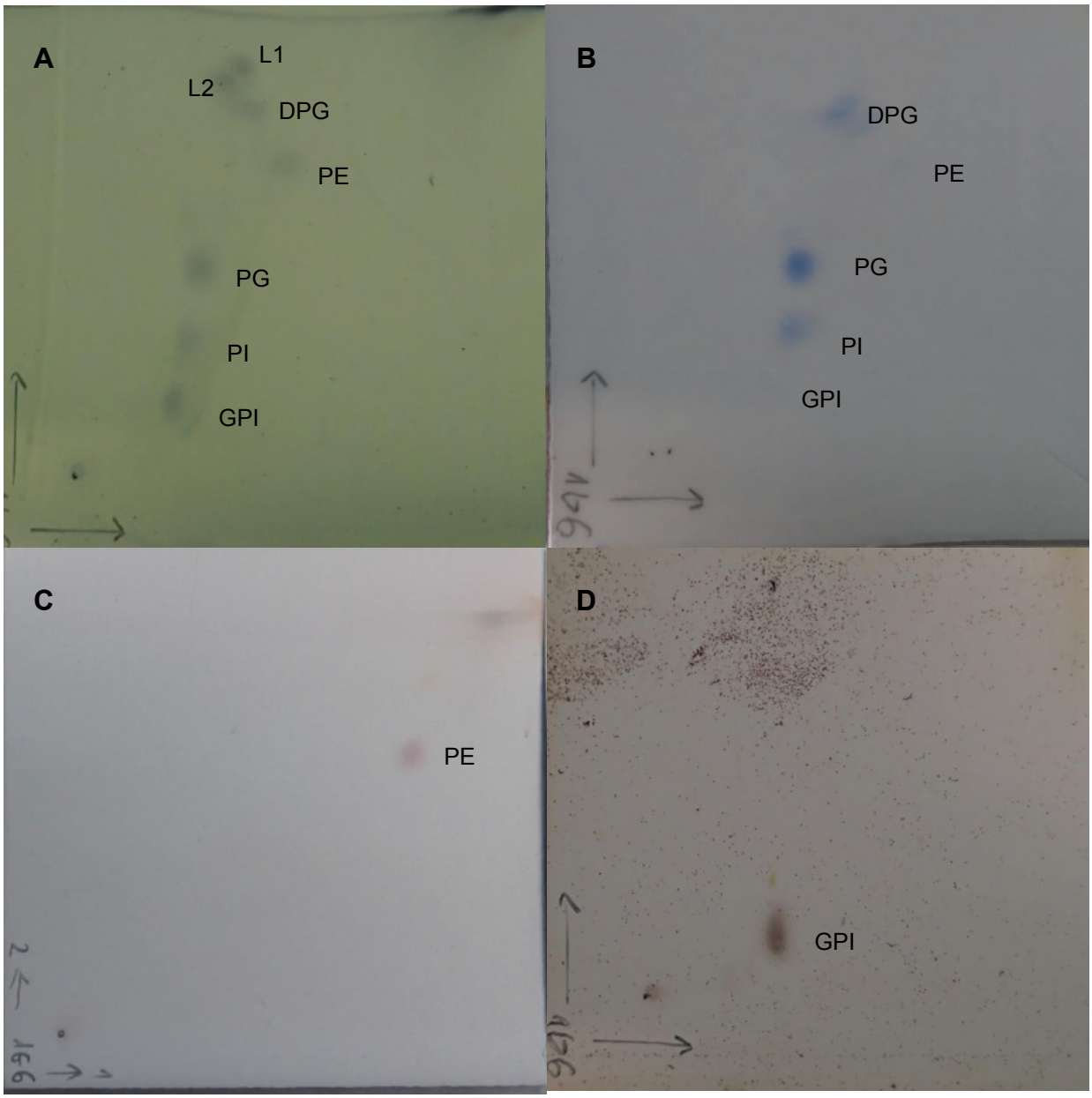

Fig. S3. Polar lipids profile of strain 1G6^T after separation by two-dimensional TLC using solvents chloroform:methanol:water (65 : 25 : 4; by volume) in the first dimension and chloroform:methanol:acetic acid:water (80 : 12 : 15 : 4; by volume) in the second dimension. Plates were sprayed with molybdophosphoric acid (A), molybdenum blue (Sigma) (B), ninhidrin (C) and α-naphtosulfuric acid (D) for the detection of total polar lipids. PE, phosphatidethanolamine; PG, phosphadidylglycerol; DPG, diphosphadidylglycerol; PI, phosphatidylinositol; GPI, glycophosphatidylinositol; L1–2, unidentified lipids.

Publikacja III

INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Golińska *et al.*, *Int. J. Syst. Evol. Microbiol.*

DOI 10.1099/ijsem.0.004212

MICROBIOLOGY SOCIETY

Modestobacter altitudinis sp. nov., a novel actinobacterium isolated from Atacama Desert soil

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Abstract

Three presumptive *Modestobacter* strains isolated from a high altitude Atacama Desert soil were the subject of a polyphasic study. The isolates, strains 1G4^T, 1G51 and 1G52, were found to have chemotaxonomic and morphological properties that were consistent with their assignment to the genus *Modestobacter*. They formed a well supported clade in *Modestobacter* 16S rRNA gene trees and were most closely related to the type strain of *'Modestobacter excelsi'* (99.8–99.9% similarity). They were also closely related to the type strains of *Modestobacter caceresii* (99.6% similarity), *Modestobacter italicus* (99.7–99.9% similarity), *Modestobacter lacusdianchii* (98.4–99.2% similarity), *Modestobacter marinus* (99.4–99.5% similarity) and *Modestobacter roseus* (99.3–99.5% similarity), but were distinguished from their closest relatives by a combination of phenotypic features. Average nucleotide identity and digital DNA:DNA hybridization similarities drawn from comparisons of draft genome sequences of isolate 1G4^T and its closest phylogenetic neighbours mentioned above, were well below the threshold used to assign closely related strains to the same species. The close relationship between isolate 1G4^T and the type strain of *M. excelsi* was showed in a phylogenomic tree containing representative strains of family *Geodermatophilaceae*. The draft genome sequence of isolate 1G4^T (size 5.18 Kb) was shown to be rich in stress related genes providing further evidence that the abundance of *Modestobacter* propagules in Atacama Desert habitats reflects their adaptation to the harsh environmental conditions prevalent in this biome. In light of all of these data it is proposed that the isolates be assigned to a novel species in the genus *Modestobacter*. The name proposed for this taxon is *Modestobacter altitudinis* sp. nov., with isolate 1G4^T (=DSM 107534^T=PCM 3003^T) as the type strain.

The actinobacterial genus *Modestobacter* [1] currently encompasses nine validly named and one effectively published species which share a broad range of chemotaxonomic, morphological and physiological features [2–4] and form a well supported 16S rRNA gene clade which distinguish them from corresponding clades of other genera classified in the family *Geodermatophilaceae* [5, 6] of the order *Geodermatophilales* [7]. *Modestobacter* strains are associated with extreme biomes as exemplified by *Modestobacter multiseptatus*, the type species of the genus, isolated from soils from the Asgard Range in Antarctica [1], *Modestobacter italicus*, *Modestobacter lapidis* and *Modestobacter muralis* from deteriorated sandstone

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Keywords: Atacama Desert; *Modestobacter altitudinis*; polyphasic taxonomy; phylogeny; stress tolerance; whole genome sequence.
Abbreviations: AL, aminolipid; ANI, average nucleotide identity; APL, aminophospholipid; A_2pm, diaminopimelic acid; dDDH, digital DNA–DNA hybridization; DNA, deoxyribonucleic acid; DPG, diphosphatidylglycerol; DSM, German Collection of Microorganisms; DSMZ, German Collection of Microorganisms and Cell Cultures; ECLs, equivalent chain lengths; GBDP, Genome BLAST Distance Phylogeny; G+C, guanine and cytosine; GGDC, genome to genome distance calculator; GL, glycolipid; GPI, glycophosphoinositol; GYM, glucose-yeast extract-malt extract medium; HPLC, high-performance liquid chromatography; IF, inoculating fluid; IMG, Integrated Microbial Genomes; ISP, International *Streptomyces* Project; KCTC, Korean Collection of Type Cultures; L, unidentified lipid; *M.*, *Modestobacter*; Mbp, mega base pair; MEGA, Molecular Evolutionary Genetic Analysis; MIDI, Microbial Identification System; MK, menaquinones; ML, maximum-likelihod; MP, maximum-parsimony; MRE, maximal-relative-error; NP-BGCs, natural product biosynthetic gene clusters; nt, nucleotide; PCM, Polish Collection of Microorganisms; PCR, polymerase chain reaction; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphoinositolmannoside; PL, unidentified phospholipid; PYGV, peptone-yeast extract-glucose-vitamins medium; RAST, Rapid Annotation using Subsystem Technology; rRNA, ribosomal ribonucleic acid; T, transmittance; TLC, thin-layer chromatography; TNT, Tree analysis using New Technology; TYGS, Type (Strain) Genome Server; UV, ultraviolet.
The GenBank accession numbers for the 16S rRNA gene and the whole genome sequence of strain 1G4^T are MH430521 and SJEW00000000, respectively.
Three supplementary figures are available with the online version of this article.

Table 1. Cultural and phenotypic properties that distinguish between the isolates

	Isolates					
Characteristics	**1 G4^T**		**1 G51**		**1 G52**	
Growth		Colony colour		Colony colour		Colony colour
Glycerol-asparagine agar (ISP 5)	+	Dark olive	+	Light yellow	+	Dark olive
Inorganic salts starch agar (ISP 4)	+	Black	+	Moderate olive brown	+	Black
Oatmeal agar (ISP 3)	++	Black	++	Black	++	Black
Peptone – yeast extract iron agar (ISP 6)	+++	Dark brown	+++	Light olive	++	Light olive
Tryptone-yeast extract agar (ISP 1)	++	Black	++	Black	+++	Black
Tyrosine agar (ISP 7)	+	Black	+	Pale orange yellow	+	Light olive
Yeast extract - malt extract agar (ISP 2)	+++	Black	+++	Black	++	Black
Hydrolysis of:						
Arbutin		+		–		+
Degradation of:						
Tween 20		–		+		–
API-ZYM tests:						
Cystine arylamidase		+		–		–
α-Fucosidase		–		–		+
α-Galactosidase		–		–		+
β-Galactosidase		+		–		+
N-acetyl-β-Glucosaminidase		+		+		–
Lipase (C14)		–		–		+
α-Mannosidase		–		–		+
Naphthol-AS-BI-phosphohydrolase		+		+		–
BIOLOG GEN III microplate tests:						
Oxidise:						
Cellobiose		–		–		+
Dextrin		+		+		–
D-Fructose		–		+		+
β-Gentiobiose		–		–		+
3-*O-methyl*-D-Glucose		–		+		+
D-Glucose-6-phosphate		–		+		+
β-*methyl*-D-Glucoside		–		+		–
Maltose		–		–		+
D-Mannose		–		–		+
Melibiose		–		–		+
L-Rhamnose		–		–		+
D-Sorbitol		+		–		+
D- Stachyose		–		–		+
Sucrose		–		–		+

Continued

Table 1. Continued

	Isolates		
Characteristics	**1 G4^T**	**1 G51**	**1 G52**
Trehalose	–	+	+
Turanose	–	–	+
L-Arginine	–	–	+
L-Aspartic acid	–	–	+
Glycyl-L-proline	–	–	+
L-Glutamic acid	–	–	+
L-Serine	+	–	+
Bromo-succinic acid	–	+	–
Butyric acid	+	+	–
α-*keto*-Glutaric acid	+	+	–
L-Lactic acid	+	–	+
L-Malic acid	+	+	–
Methyl-pyruvate	+	–	+
N-acetyl-Neuraminic acid	–	–	+
Propionic acid	+	+	–
Growth in presence of inhibitory compounds:			
Fusidic acid	+	+	–
Guanidine hydrochloride	+	+	–
Lincomycin	+	+	–
Minocycline	+	+	–
Niaproof	+	+	–
Troleandomycin	+	+	–
Vancomycin	+	+	–
D-Serine	+	+	–
Sodium bromate	+	+	–
Sodium formate	+	+	–
Tetrazolium violet	+	+	–
Tetrazolium blue	+	+	–
Temperature growth range (°C)	10–35	10–35	10–30
pH growth range	5.0–9.0	5.0–9.0	6.0–8.0
NaCl tolerance (% w/v)	0–8	0–8	0–3

Key: Growth: +++, abundant; ++, good; +, weak.
Physiological tests: +, positive; -, negative.
All of the isolates reduced nitrate, hydrolysed aesculin but not allantoin or urea; degraded Tweens 40 and 60 but, not adenine, casein, chitin, elastin, guanine, hypoxanthine, starch, Tween 80, L-tyrosine, uric acid, xanthine or xylan. They were positive for acid and alkaline phosphatases, esterase (C4), esterase lipase (C8), α- and *β*-glucosidases, leucine arylamidase, valine arylamidase and negative for trypsin, α-chymotrypsin and *β*-glucuronidase (API-ZYM tests) and oxidised D-fructose-6-phosphate, D-glucose, glucuronamide, raffinose and D-salicin (sugars), acetic acid, acetoacetic acid, *p-hydroxy*-phenylacetic acid and quinic acid (organic acids), degraded gelatin, pectin and Tween 40 (polymers), and grew in the presence of aztreonam, lithium chloride, nalidixic acid, potassium tellurite, rifamicin SV, sodium chloride (1% w/v), sodium lactate (1% w/v) and at pH 6. In contrast, they did not oxidise L-alanine, D-aspartic acid, L-histidine, L-pyroglutamic acid or D-serine (amino acids), D-arabitol, D- or L-fucose, *N-acetyl*-D-galactosamine, D-galactose, *N-acetyl*-D-glucosamine, glycerol, *myo*-inositol, lactose, D-mannitol and *N-acetyl-β*-mannosamine (sugars), α- and *β-hydroxy*-butyric acid, α-*keto*-butyric acid, γ-amino-*n*-butyric acid, citric acid, L-galactonic acid-γ-lactone, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, D-lactic acid methyl ester, D-malic acid, mucic acid, D-saccharic acid (organic acids) or inosine (nucleoside).

[4, 8], *Modestobacter marinus* from deep-sea sediment [9] and *Modestobacter caceresii* and *'Modestobacter excelsi'* from hyper-arid Atacama Desert soils [3, 10]. Culture-independent studies show that *Modestobacter* strains are key components of Atacama Desert soils [11, 12] characterised by scarcity of liquid water, low concentrations of organic carbon and high solar irradiation [13–15]. In light of such considerations it is interesting that the genomes of the type strains of *M. caceresii* and *'M. excelsi'* are rich in stress genes that provide an insight into how these organisms have adapted to the prevailing environmental conditions in hyper-arid Atacama Desert soils [3, 10]. Moreover, genomes of representative type strains of the family *Geodermatophilaceae* revealed many biosynthetic gene clusters (BGCs) related to the biosynthesis of secondary metabolites, as exemplified by the genomes of the type strains of *Blastococcus atacamensis* [16], *Geodermatophilus chilensis* [17], *M. caceresii* [10] and '*M. excelsi*' [3].

The present study, a continuation of our previous studies on actinobacterial diversity in Atacama Desert habitats [12, 13, 18], was designed to establish the taxonomic status of three presumptive *Modestobacter* strains isolated from a high altitude, hyper-arid soil of the Central-Andes in Chile. The strains, isolates 1G4^T, 1G51 and 1G52, were compared with reference strains of *Modestobacter* species using an combination of genomic, genotypic and phenotypic properties. The isolates formed a new species within the genus *Modestobacter*, the proposed name for which is *Modestobacter altitudinis* sp. nov. with isolate 1G4^T as the type strain.

Isolates 1G4^T, 1G51 and 1G52 were recovered from a hyper-arid, surface soil sample (2 cm depth, pH 7.1, 360 mV, 0% moisture content, 1.7% organic matter content [19]) collected at a high altitude (3018 m above sea level) on Cerro Chajnantor (23° 04′ 39″ S 67° 57′ 43″ W), east of San Pedro de Atacama in Chile [11] using a standard dilution plate procedure [3]. Aliquots (100 µl) of 10^{-1} and 10^{-2} dilutions prepared in ¼ strength Ringer's solution (Oxoid) prepared from a gram of the soil sample were spread, in triplicate, over dried plates of Gauze's No. 1 agar [20, 21] supplemented with cycloheximide and nystatin (each at 50 µg ml^{-1}) prior to incubation at 28 °C for 21 days. Colonies of the three isolates were checked for purity on Gauze's agar plates and maintained on slopes of modified Bennett's agar [22] at room temperature and as cell suspensions in 20%, v/v glycerol at −80 °C. Similarly, strains of *M. caceresii* KNN 45-2b^T, *'M. excelsi'* DSM 107535^T, *M. italicus* BC 501^T, *Modestobacter lacusdianchii* KCTC 39600^T [23], *M. lapidis* MON 3.1^T, *M. marinus* DSM 45201^T, *M. muralis* MDVD1^T, *M. multiseptatus* DSM 44406^T, *Modestobacter roseus* DSM 45764^T [24] and *Modestobacter versicolor* DSM 16678^T [25] were maintained on GYM *Streptomyces* medium (DSMZ medium 65) at room temperature and as cell suspensions in 20%, v/v glycerol at −80 °C.

Biomass for most of the chemotaxonomic and molecular systematic analyses were harvested from 500 ml of modified Bennett's broth cultures (pH 7.5) which had been shaken at 150 r.p.m. at 28 °C for 7 days following inoculation with 1 ml of each isolate prepared in modified Bennett's broth under the same conditions. Harvested cells were washed three times in sterile distilled water; those for the chemotaxonomic studies were freeze dried and the ones for the 16S rRNA gene sequencing and genomic analyses were kept at room temperature. Biomass for the fatty acid analyses was scrapped from peptone-yeast extract-glucose-vitamins agar (PYGV, DSM medium 621) plates following incubation for 16 days at 20 °C.

The isolates were examined for chemotaxonomic, cultural and morphological properties known to be of value in the classification of *Modestobacter* species [3, 4]. Motility and micromorphological features were acquired according to Trujillo *et al.* [8] and cultural properties recorded from tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic-salts starch, glucose-asparagine, peptone-yeast extract-iron and tyrosine agar (International *Streptomyces* Project [ISP] media 1–7 respectively [26]) plates following incubation at 28 °C for 14 days; colony colours were determined by comparison against colour charts [27]. In turn, chemotaxonomic properties of the isolates were established using standard procedures. Whole-cell amino acids and sugars prepared after Lechevalier and Lechevalier [28] were analysed by thin-layer chromatography (TLC), as described by Staneck and Roberts [29]; menaquinones extracted from freeze dried cells with methanol [30] were separated by high-performance liquid chromatography (HPLC) [31] while polar lipids were extracted from freeze dried cells, separated by two-dimensional TLC and identified after Minnikin *et al.* [32] using modifications introduced by Kroppenstedt and Goodfellow [33]. Fatty acids extracted from the isolates and the type strains of *M. caceresii, 'M. excelsi', M. italicus, M. marinus* and *M. roseus* harvested under the same growth conditions were analysed using the Sherlock Microbial Identification (MIDI) system version 5 [34] and the resultant acids identified using the ACTIN6 database, as described previously [3].

The chemotaxonomic, cultural and morphological properties of the isolates were consistent with their classification in the genus *Modestobacter* [1–4, 8]. The isolates were aerobic, Gram-stain-positive, non-motile, formed short rods and coccoid-shaped cells which tended to remain aggregated (Fig. S1, available in the online version of this article), produced whole-organism hydrolysates rich in *meso*-A_2pm, glucose and ribose (diagnostic sugars), MK-9(H_4) as the predominant menaquinone (81–93.1%) and polar lipid patterns containing diphosphatidylglycerol, glycophosphatidylinositol, phosphatidylethanolamine (diagnostic component), phosphatidylglycerol and phosphatidylinositol, as exemplified in Fig. S2.

The isolates grew particularly well on ISP media 2 and 6 producing black colonies on the former and dark brown or light-olive ones the latter (Table 1).

It is worth mentioning that in previously studies [8, 10, 24] glycophosphatidylinositol (GPI) was annotated as phosphatidylinositol mannoside (PIM); this compound has been found in representatives of *Modestobacter* species, apart from *M. multiseptatus* [1, 4]. Consequently, the polar lipid profiles of isolates 1G4^T, 1G51 and 1G52 accord with those found in the

Table 2. Fatty acid profiles (%) of the isolates grown on peptone-yeast extract-glucose-vitamins agar plates after incubation for 16 days at 20 °C. Values are percentages of total fatty acids. All data are from this study

	Modestobacter strains							
Fatty acids	**Isolate 1 G4^T**	**Isolate 1G51**	**Isolate 1 G52**	***M. caceresii* KNN 45-2b^T**	**'*M. excelsi*' DSM 107535^T**	***M. italicus* BC 501^T**	***M. marinus* DSM 45201^T**	***M. roseus* DSM 45764^T**
$C_{14:0}$	1.6	0.8	1.6	TR	TR	TR	TR	TR
$C_{15:0}$	–	–	–	TR	–	TR	TR	2.4
$C_{16:0}$	8.4	3.5	2.9	3.1	1.6	1.0	4.1	TR
$C_{17:0}$	4.4	TR	TR	TR	TR	TR	1.1	1.7
$C_{18:0}$	3.5	1.2	2.3	–	TR	TR	TR	–
$C_{16:1}$ cis 9	–	–	–	23.5	–	5.7	–	5.4
$C_{17:0}$ cyclo	–	–	–	2.6	–	–	1.7	1.5
$C_{17:1}$ ω8c	17.3	4.5	4.7	–	3.3	–	7.6	–
$C_{17:1}$ cis 9	–	–	–	7.6	–	4.5	–	10.9
$C_{18:1}$ ω6c	2.5	TR	TR	–	–	–	–	–
$C_{18:1}$ ω9c	26.2	16.6	15.6	3.8	7.3	17.3	4.1	TR
$C_{16:0}$ 9-methyl	–	–	–	1.8	–	1.5	–	4.4
$C_{17:0}$ 10-methyl	–	–	–	1.8	–	4.4	6.0	TR
anteiso- $C_{15:0}$	1.2	2.9	5.3	TR	5.8	1.2	TR	TR
anteiso- $C_{17:0}$	1.7	3.3	8.3	TR	–	1.9	TR	TR
anteiso- $C_{17:1}$	–	–	–	–	6.3	–	–	–
anteiso- $C_{17:1}$ C	–	–	–	TR	–	TR	–	1.0
anteiso-$C_{17:1}$ ω9c	–	1.1	2.5	–	3.8	–	TR	–
iso-$C_{10:0}$	–	–	–	–	–	1.3	–	–
iso-$C_{14:0}$	TR	2.1	TR	1.7	1.4	1.4	2.3	3.1
iso-$C_{15:0}$	5.0	6.1	16.5	3.2	18.7	6.9	2.9	16.0
iso-$C_{15:1}$ G	1.6	2.8	3.6	3.1	8.1	4.4	3.0	–
iso-$C_{16:0}$	11.9	41.9	11.2	28.1	16.3	32.8	32.5	25.7
iso-$C_{16:0}$ 2OH	–	–	–	–	–	2.0	–	1.7
iso-$C_{16:1}$ G	TR	–	–	–	–	5.6	–	–

Continued

Table 2. Continued

	Modestobacter strains							
Fatty acids	**Isolate 1 G4^T**	**Isolate 1G51**	**Isolate 1 G52**	***M. caceresii* KNN 45-2b^T**	**'*M. excelsi*' DSM 107535^T**	***M. italicus* BC 501^T**	***M. marinus* DSM 45201^T**	***M. roseus* DSM 45764^T**
iso-$C_{16:1}$ h	–	–	1.7	14.9	5.8	–	16.7	9.4
iso-$C_{17:0}$	1.1	1.8	5.6	TR	1.7	1.9	1.4	2.4
iso-$C_{17:1}$ ω9c	–	–	–	–	5.9	–	1.7	–
Summed feature 3	4.1	5.0	5.9	–	9.1	–	8.5	–
Summed feature 4	–	–	–	–	–	–	–	2.6
Summed feature 7*/**	5.5*	2.9*	3.2*	TR**	1.0*	TR**	TR*	TR**
Summed feature 9*/**	TR**	1.1**	4.9**	TR*	–	TR*	–	–

Key: –, not detected. TR, trace (amount below 1 %).

*As indicated by Montero-Calasanz *et al.* [81] summed features are groups of two or three fatty acids that are taken together for the purpose of evaluation in the MIDI system and include both peaks with discrete ECLs as well as those where the ECLs are not reported separately.

Summed feature 3 comprises $C_{16:1}\omega 7c$ and $C_{16:1}\omega 6c$; summed feature 4 iso-$C_{15:0}$ 2OH and $C_{16:1}$ t9; summed feature 7* $C_{19:0}$ cyclo $\omega 10c$ and/or 19 $\omega 6$; summed feature 7** $C_{18:1}$ trans nine and $C_{18:1}$ trans six and $C_{18:1}$ cis; summed feature 9 $C_{19:0}$ cyclo C9-10 and undefined fatty acid.

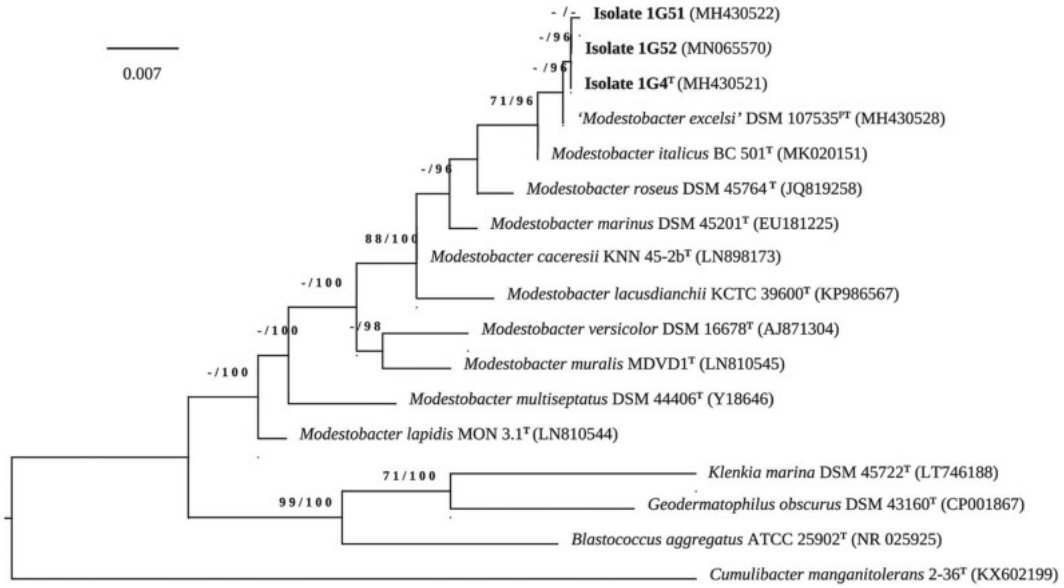

Fig. 1. Maximum-likelihod and maximum-parsimony tree based on nearly complete 16S rRNA gene sequences (1390–1541 nucleotides) showing relationships between isolates 1G4^T, 1G51 and 1G52 and between them and the type strains of *Modestobacter* species. The numbers above the branches are bootstrap support values when larger than 70% from ML (left) and MP (right). Bar; 0.007 substitutions per nucleotide position. The root position of the tree was determined using *Cumulibacter manganitolerans* 2-36^T as the outgroup.

type strains of most *Modestobacter* species [3, 4, 10]. Similarly, the predominance of MK-9(H_4) in the isolates agrees with data previously recorded for members of the genus *Modestobacter* [1–4]. The cellular fatty acid profiles of the isolates contained variable proportions of $C_{17:1}$ ω8c (4.5–17.3% of total fatty acids), $C_{18:1}$ ω9c (15.6–26.2%), iso-$C_{15:0}$ (5.0–16.5%) and iso-$C_{16:0}$ (11.2–41.9%), quantitative differences were also recorded for components found in lesser proportions (Table 2). $C_{17:1}$ ω8c has also been detected in '*M. excelsi*' DSM 107535^T and *M. marinus* DSM 45201^T and $C_{18:1}$ ω9c in the type strains of *M. caceresii*, '*M. excelsi*', *M. marinus* and *M. roseus* albeit in lesser proportions than in the isolates though it was the predominant component in *M. italicus* BC 501^T. Similarly, smaller proportions of $C_{16:0}$ were found in all of the reference strains with only a trace amount found in *M. roseus* DSM 45764^T.

Genomic DNA was extracted from each of the isolates using a GenElute™ Bacterial Genomic Kit (Sigma-Aldrich) following the instructions of the manufacturer and PCR-mediated amplifications of the 16S rRNA genes carried out, as described by Golinska *et al.* [35, 36]. PCR-products were purified using a PCR purification kit (Qiagen) following the protocol of the manufacturer, checked for quality with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) and sequenced at the Institute of Biochemistry and Biophysics (IBB) of the Polish Academy of Sciences, Warsaw, Poland using an ABI3730xl Genetic Analyzer (Applied Biosystems). The 16S rRNA gene sequences of isolates 1G4^T, 1G51 and 1G52 (1526, 1398 and 1394 nucleotides [nt], respectively) were deposited in the GenBank sequence database (accession numbers MH430521, MH430522 and MN065570, respectively). The sequences were compared with corresponding sequences of *Modestobacter* type strains using the Eztaxon server [37]. The 16S rRNA gene sequence similarities were calculated using PHYDIT version 1.0 software [38].

Maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic trees based on the 16S rRNA gene sequences were inferred using the genome-to-genome distance calculator (GGDC) web server [39] adapted to single genes and multiple sequence alignments generated using ClustalW [40]. The ML tree was inferred from the alignments with RAxML [41] using rapid bootstrapping together with the auto maximal-relative-error (MRE) bootstrapping criterion [42]. The MP tree was inferred from the alignments with the Tree Analysis using the New Technology (TNT) program [43] with 1000 bootstraps together with tree bisection and reconnection branch swapping and ten random sequence replicates. The sequences were checked for computational bias using the x^2 test implemented in PAUP* [44]. A third tree was generated with the neighbour-joining [45] algorithm from the MEGA seven software package [46]; an evolutionary distance matrix for the neighbour-joining analysis was prepared using the two-parameter model of Kimura [47]. The topologies of the inferred evolutionary trees were evaluated by bootstrap analyses [48] based on 1000 replicates. The root positions of unrooted trees were estimated using the sequence of *Cumulibacter manganitolerans* 2-36^T (Genbank accession number: KX602199). BOX-PCR fingerprints of the isolates were prepared from extracted DNA using the BOXAIR primer [49] and the experimental conditions described by Trujillo *et al.* [50].

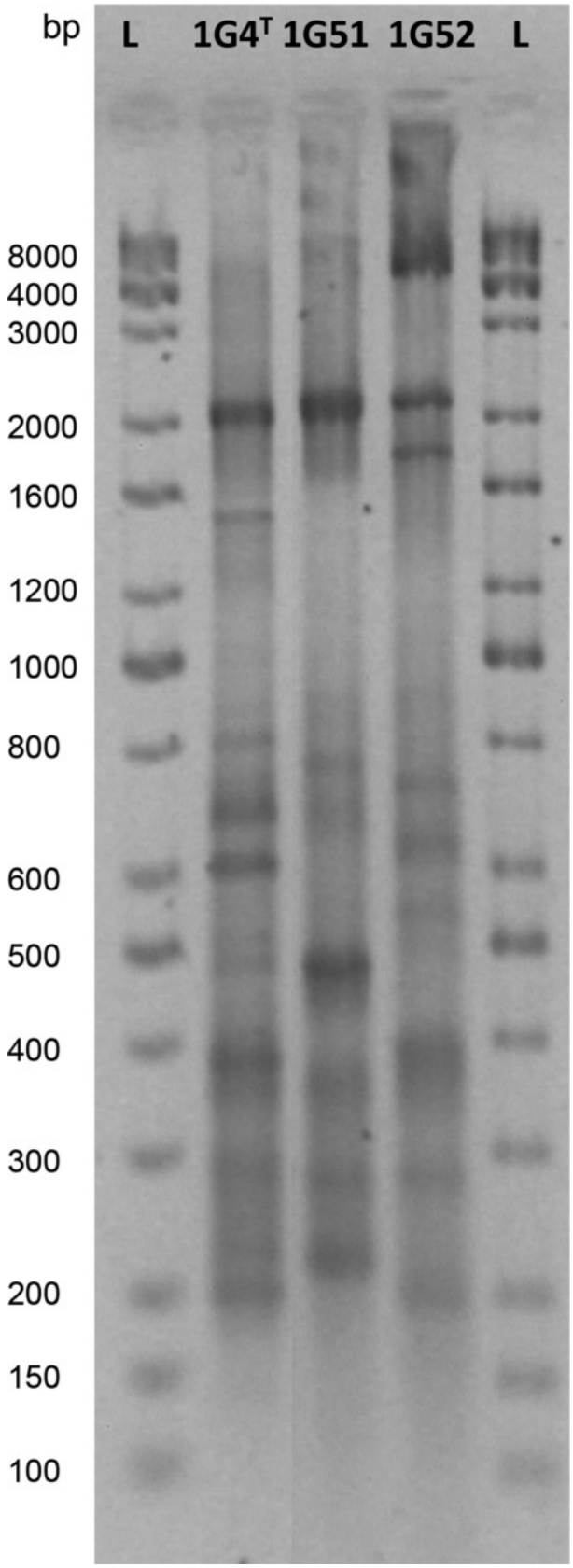

Fig. 2. BOX PCR fingerprint patterns of genomic DNA extracted from the isolates.

The isolates formed a well-supported clade in the *Modestobacter* 16S rRNA gene tree (Figs 1 and S3). Strains 1G4^T and 1G52 have identical 16S rRNA gene sequences and share a 99.9% similarity with isolate 1G51 which corresponds to two and one nt differences at 1411 and 1387 locations, respectively. The isolates were most closely related to *'M. excelsi'* DSM 107535^T sharing 16S rRNA gene sequence similarities with the latter of 99.9% (1G4^T), 99.8% (1G51) and 99.9% (1G52), values equivalent to one and three nt differences. They were also closely related to *M. italicus* BC 501^T (99.7–99.9% similarity), *M. caceresii* KNN 45-2b^T (99.6% similarity), *M. marinus* DSM 45201^T (99.4–99.5% similarity), *M. roseus* DSM 45764^T (99.3–99.5% similarity) and *M. lacusdianchii* KCTC 39600^T (98.4–99.2% similarity). All of these similarity values are above the 98.7% threshold used to recognise novel prokaryotic species [51]. In contrast, the isolates were separated from the remaining *Modestobacter* type strains sharing 16S rRNA gene similarities with them within the range 97.8 to 98.7%, values corresponding to between 18 and 31 nt differences. The isolates showed diverse BOX-PCR profiles indicating that they are not clones (Fig. 2).

Comparative analyses of whole genome sequences are proving to be effective in establishing relationships between closely related species that are difficult to resolve using conventional taxonomic methods [52–54]. In this study, a single colony of isolate 1G4^T was used to inoculate 50 ml of modified Bennett's broth [22] prior to incubating at 28 °C for 72 h in a shaking incubator at 150 r.p.m. Genomic DNA extracted from 1.5 ml of the resultant culture was sequenced at MicrobesNG (http://www.microbesng.uk) using a MiSeq instrument (Illumina). The reads were assembled into contigs with SPAdes 3.6.2 software [55] and contigs under 300 bp discarded. The draft genome assembly was annotated using the RAST server [56, 57] with default options and submitted to GenBank (accession no. SJEW00000000). The GGDC server [39, 58] was used to estimate dDDH values between the draft genome sequence of strain 1G4^T and the type strains of *M. caceresii* (GenBank accession number: JPMX00000000), *'M. excelsi'* (GenBank accession number: SJEX00000000), *M. italicus* (GenBank accession number: FO203431), *M. lacusdianchii* (unpublished, provided by Montero-Calasanz), *M. marinus* (IMG genome ID: 2820994125) and *M. roseus* (IMG genome ID: 2585427561), phylogenetic relatives that showed similarities higher than 98.7% based on 16S rRNA gene sequences. In turn, the OrthoANIu algorithm from the ANI Calculator [59, 60] was used to calculate ANI values between isolate 1G4^T and the type strains of its closest relatives, with the exception of the ANI value between isolate 1G4^T and *M. marinus* DSM 45201^T which was provided by Vartul Sangal from Northumbria University, UK.

The draft genome sequence of isolate 1G4^T contained 161 contigs giving a total size of 5175439 bp with a DNA G+C content of 73.7 mol%. The genome contained 52 RNA genes and was annotated to include 5182 protein coding sequences which were assigned to 324 subsystems. Most of the categories with coding sequences classified into subsystems were amino acids and derivatives (15.7%), followed by carbohydrates

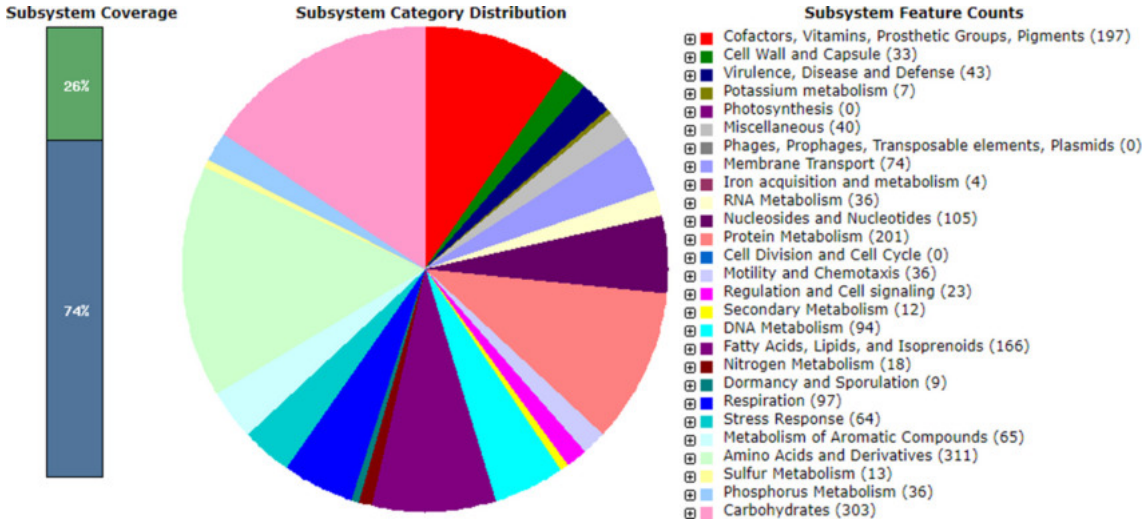

Fig. 3. The subsystem category distribution statistics for isolate1G4^T based on the SEED database. Bar chart shows the percentage of subsystem coverage (green bar corresponds to the percentage of proteins involved). The pie chart with legend shows the fraction and count (parenthesis in legend) of each subsystem feature.

(15.3%), proteins (10.1%), cofactors, vitamins, prosthetic groups and pigments (9.9%) as well as fatty acids, lipids and isoprenoids (8.4%) (Fig. 3).

The dDDH values between isolate 1G4^T and the type strains of *M. caceresii, 'M. excelsi'*, *M. italicus*, *M. lacusdianchii*, *M. marinus* and *M. roseus* were 35.6, 47.6, 41.4, 27.2, 35.8 and 32.5%, respectively, values well below the recommended cut-off point of ≥70% used to assign strains to the same species [61]. Similarly, ANI values of 83.4, 92.2, 90.5, 83.8, 84.1 and 82.2% observed between the genomes of isolate 1G4^T and the reference strains cited above are well below the 95–96% threshold used to distinguish between members of closely related species [62, 63].

The genome sequences of isolate 1G4^T and the type strains of *M. lacusdianchii* and *M. multiseptatus* were uploaded on to Type (Strain) Genome Server (TYGS) [58] and compared against all of the type strain *Geodermatophilaceae* genomes available in the TYGS database using the MASH algorithm which allows a fast approximation of intergenomic relatedness between strains [64]. A phylogenomic tree was inferred with FastME 2.1.4 [65] from GBDP distances calculated from the genome sequences, branch lengths were scaled using GBDP distance formula d_5 [39], numbers above branches are GBDP pseudo-bootstrap support values based on 100 replications. It can be seen from the phylogenomic tree that the isolate forms not only a distinct branch in the *Modestobacter* clade but is most closely related to the '*M. excelsi*' and lesser so to the type strains of *M. italicus* and *M. versicolor* (Fig. 4).

The isolates and *'M. excelsi'* DSM 107535^T, their nearest phylogenetic neighbour, were examined for a broad range of phenotypic properties. They were tested for their ability to grow over a range of pH values (pH 4.0–12.0 at 0.5 unit intervals using phosphate buffers) and temperatures (4, 10, and then at 5 °C intervals up to 45 °C) and in the presence of various concentrations of sodium chloride (0–10% NaCl, w/v) using ISP 2 agar as the basal medium [26]. Apart from the temperature tests, these features were recorded at 28 °C after incubation for 2 weeks. The biochemical and degradation tests were done as recommended by Williams *et al.* [66]. All of these tests were carried out in duplicate using a standard inoculum equivalent to 5.0 on the McFarland scale [67]. Enzyme profiles were established using API-ZYM kits (bioMérieux) following the instructions of the manufacturer. The isolates, and all of the *Modestobacter* type strains, including *'M. excelsi'* DSM 107535^T, were examined for their capacity to oxidise a broad range of carbon and nitrogen compounds and to show resistance to inhibitory compounds using GEN III microplates in an Omnilog device (BIOLOG Inc., Haywood, USA) following incubation of duplicated cultures at 28 °C for 7 days. The strains were grown on GYM *Streptomyces* agar plates at 28 °C and cell suspensions prepared in a viscous inoculating fluid (IF C) at around 83% transmittance (T); the microplates were inoculated by following the protocol of the manufacturer. The resultant data were exported and analysed using the opm package for R v.1.0.6 [68, 69].

The duplicated cultures of the isolates gave the same results for the API-ZYM, biochemical, degradation and physiological tests though variable results were recorded for some of the Biolog tests, as shown in Table 1**.** It is apparent that the isolates have many phenotypic properties in common though some features can be weighted to distinguish between them providing further evidence that they are not clones. Isolate 1G52, for instance, metabolised many more carbon and nitrogen compounds than isolates 1G4^T and 1G51; the former unlike the latter produced α-fucosidase, α-galactosidase, lipase (C14) and α-mannosidase and was unable to oxidise

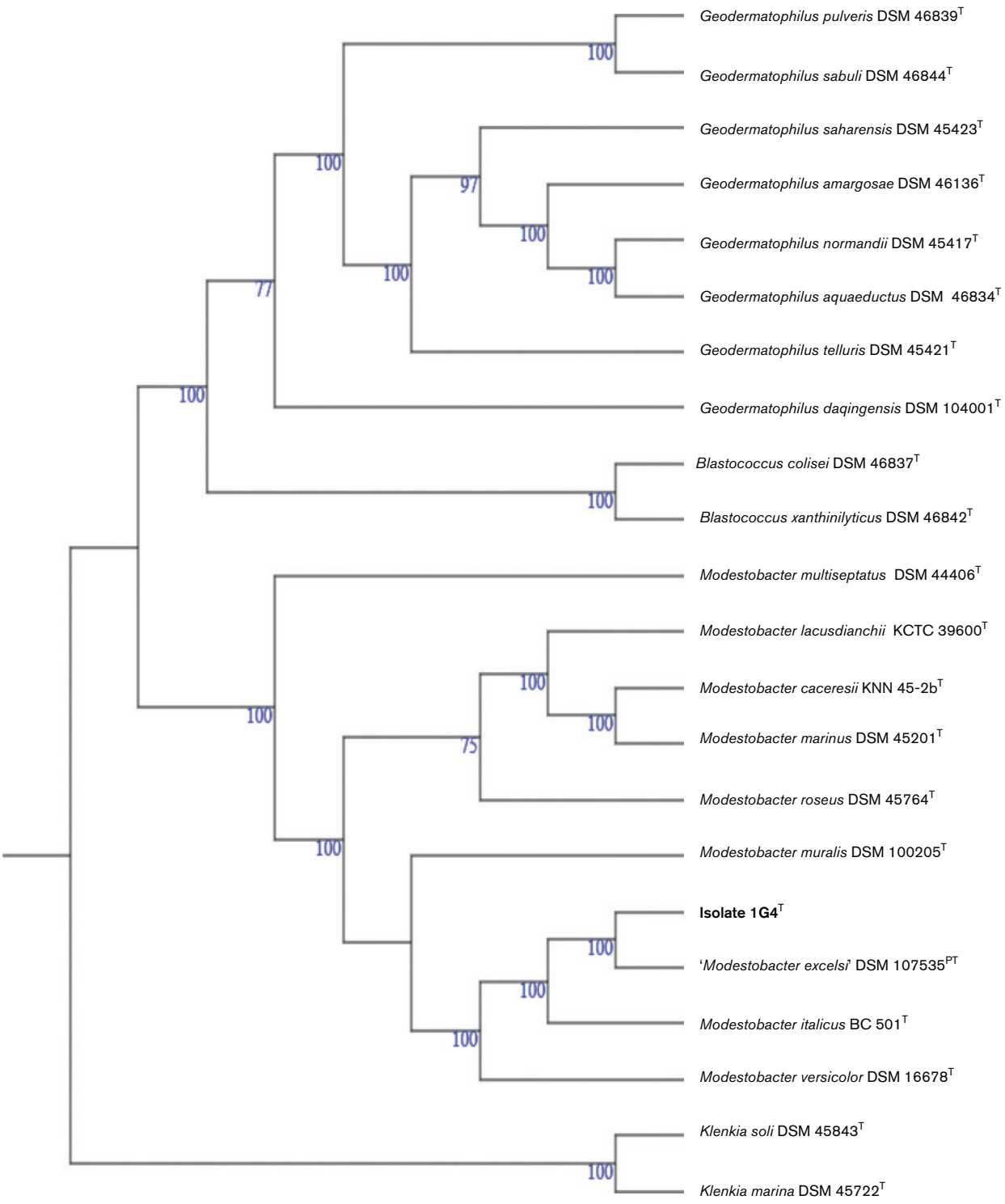

Fig. 4. Phylogenomic tree showing relationship between isolate 1G4^{T} and type strains of species classified in the family *Geodermatophilaceae*. The numbers above the branches are GBDP pseudo-bootstrap support values >70% from 100 replications with an average branch support of 94.7%. The tree was rooted at the midpoint [82].

Table 3. Phenotypic properties that distinguish the isolates from the type strains of *Modestobacter* species

Strains: 1, Isolates 1G4T, 1G51 and 1G52; 2, *M. caceresii* KNN 45-2b^T; 3, *'M. excelsi'* DSM 107535^T; 4, *M. italicus* BC 501^T; 5, *M. lacusdianchii* KCTC 39600^T; 6, *M. lapidis* MON 3.1^T; 7, *M. marinus* DSM 45201^T; 8, *M. multiseptatus* DSM 44406^T; 9, *M. muralis* MDVD1^T; 10, *M. roseus* DSM 45764^T; 11, *M. versicolor* DSM 16678^T.

	***Modestobacter* strains**										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
BIOLOG GEN III microplate tests assimilation of:											
D-Fructose-6-phosphate	+	+	+	−	−	−	+	−	+	−	+
D-Fucose	−	+	−	−	−	+	−	+	−	−	+
L-Fucose	−	+	+	−	+	+	+	+	+	−	+
D-Galactose	−	+	−	−	+	+	+	+	+	−	+
N-acetyl-D-Galactosamine	−	−	−	−	−	+	+	+	+	+	−
N-acetyl-D-Glucosamine	−	+	−	−	+	+	+	+	+	+	+
Glucuronamide	+	+	+	−	−	+	−	+	−	−	−
Glycerol	−	+	−	+	+	−	+	+	+	+	+
Inosine	−	−	−	−	−	−	+	−	−	−	−
Lactose	−	−	−	−	+	+	+	+	+	+	−
D-Mannitol	−	+	−	+	+	+	+	+	+	+	+
N-acetyl-β-D-Mannosamine	−	+	−	−	+	+	+	+	+	+	+
Myo-inositol	−	−	−	−	−	+	−	+	−	−	−
Pectin	+	+	−	−	+	−	+	−	+	+	+
Raffinose	+	−	+	−	+	+	−	−	+	−	−
D-Salicin	+	+	−	+	+	+	+	+	+	+	−
L-Pyroglutamic acid	−	−	−	−	−	−	+	+	−	−	−
Acetic acid	+	+	+	−	+	+	−	+	+	+	+
Acetoacetic acid	+	+	+	−	+	+	−	+	+	+	+
γ-amino-*n*-Butyric acid	−	+	−	−	−	−	−	+	−	−	+
α-*hydroxy*-Butyric acid	−	+	+	−	−	+	−	−	−	−	−
β-*hydroxy*-Butyric acid	−	+	+	−	−	−	−	+	−	−	+
α-*keto*-Butyric acid	−	+	+	−	−	+	−	−	−	+	−
Citric acid	−	−	+	−	+	−	−	+	−	−	+
D-Galacturonic acid	−	−	+	−	+	+	−	+	−	−	−
D-Gluconic acid	−	+	−	+	+	+	+	+	+	+	+
D-Glucuronic acid	−	+	+	−	−	+	−	+	−	−	+
D-Lactic acid methyl ester	−	+	−	−	−	−	−	−	−	−	+
D-Malic acid	−	−	−	−	+	−	−	+	+	+	−
Quinic acid	+	−	−	−	−	−	−	−	−	+	−
p-hydroxy-Phenylacetic acid	+	+	−	−	−	−	−	−	−	+	−
D-Saccharic acid	−	−	−	−	−	−	+	−	−	+	−
Gelatin	+	+	−	−	−	−	+	−	−	−	−
Tween 40	+	+	+	+	+	+	−	+	+	+	−

Continued

Table 3. Continued

	Modestobacter strains										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Growth in presence of inhibitory compounds:											
Aztreonam	+	+	–	+	+	+	+	–	+	+	+
Nalidixic acid	+	–	+	+	+	+	+	–	+	+	+
Potassium tellurite	+	–	+	+	+	–	–	–	+	+	+
Rifamycin SV	+	–	+	+	+	–	–	–	–	–	+
Sodium lactate (1% w/v)	+	–	+	+	–	–	+	–	+	+	+
Growth at pH 6	+	–	+	–	–	–	+	–	–	–	–
Polar lipids*	DPG, PG, PE, PI, GPI 4L	DPG, PG, PE, PI, PIM	DPG, PG, PE, PI, GPI, 2L	DPG, PE, PI, GPI	DPG, PE, PI, PIM, PL	DPG, PG, PE, PI, PIM	DPG, PG, PE, PI, APL, GL, PL	DPG, PE, PI, GL, 2APL, 3L	DPG, PG, PE, PI, PIM	DPG, PG, PE, PI, GPI, AL, 2APL	DPG, PG, PE, PI, PL, GPI, 4L

Key: +, positive; –, negative.
*Polar lipids data for strain two are from Busarakam *et al.* [10]; for three from Golinska *et al.* [3], 4,7, 8 and 10 from Montero-Calasanz *et al.* [4]; for five from Zhang *et al.* [23]; for 6 and 9 from Trujillo *et al.* [8].
Codes: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PI, phosphatidylinositol; GPI, glycophosphoinositol; PIM, phosphoinositolmannoside; AL, aminolipids; APL, aminophospholipids; GL, glycolipid; PL, unidentified phospholipid; L, unidentified lipid.
All of the strains oxidised D-glucose, but not D-arabitol, D-aspartic acid, L-histidine, mucic acid or D-serine. Between one and three of the strains gave variable results in the duplicated tests, involving oxidation of L-alanine and L-galactonic acid-γ-lactone and growth in the presence of lithium chloride.

dextrin, β-*methyl*-D-glucoside, butyric acid, α-*keto*-glutaric acid or L-malic acid and grow in the presence of D-serine.

With few exceptions the duplicated cultures gave the same results in the Biolog tests, as shown in Table 3. It is also evident from this table that the isolates can be distinguished from the type strains of all of the *Modestobacter* species, including those of its closest phylogenetic neighbours, based on a wide range of phenotypic properties. The isolates, unlike *'M. excelsi'* DSM 107535^T, its closest phylogenetic neighbour, oxidised *p-hydroxy*-phenylacetic acid, quinic acid and D-salicin, degraded pectin and gelatin and grew in the presence of aztreonam. In contrast, the *'M. excelsi'* strain, unlike the isolates, oxidised L-fucose, α- and β-*hydroxy*-butyric acids, α-*keto*-butyric acid, citric acid, D-galacturonic acid and D-glucuronic acid. In addition, the isolates can be distinguished from the *'M. excelsi'* strain based on their ability to reduce nitrate. In contrast, *'M. excelsi'* DSM 107535^T, unlike the isolates, was positive for α-chymotrypsin and trypsin. The isolates, unlike *M. italicus* BC 501^T, its closest neighbour with a validly published name, oxidised D-fructose-6-phosphate, glucuronamide, raffinose, acetic acid, acetoacetic acid, quinic acid and *p-hydroxy*-phenylacetic acid, degraded gelatin and pectin and grew at pH 6. In contrast, the *M. italicus* strain, unlike the isolates oxidised glycerol, D-mannitol and D-gluconic acid. Similar combinations of phenotypic features can be used to separate the isolates from the remaining *Modestobacter* type strains.

The genome of isolate 1G4^T was examined for natural product-biosynthetic gene clusters (NP-BGCs) using anti-SMASH software [70] and gene sequences encoding proteins involved in stress responses were detected using the SEED server [71]. The draft genome of the isolate contained NP-BGCs coding for type 2 and type 3 polyketide synthases, betalactone and terpenes thereby underpinning corresponding results on the type strains of *M. caceresii* [10] and *'M. excelsi'* [3]. In turn, many of the stress related genes were shown to be linked with carbon starvation, oxidative and osmotic stress, resistance to UV radiation and temperature fluxes, as exemplified by genes encoding for carbon starvation protein A (CstA), which promotes peptide uptake [72–74]; *sox* genes associated with responses to oxidative stress [75, 76]; *KatE* and *uvrD* genes linked to protection against reactive oxygen and UV-radiation [77]; *dnaK* and *dnaJ*, *hrcA*, *grpE* and *Hsp* genes related to responses to heat shock [78]; a family of proteins associated with cold shock responses [79] and *coxE, coxD* and *coxG* genes along with a *coxSML* cluster coding for utilisation of carbon monoxide indicating that the isolate may have a chemolitho-autotrophic lifestyle [80]. Similar patterns of stress-related genes have been detected in the genomes of the type strains of the *B. atacamensis* [16], *G. chilensis* [17], *M. caceresii* [10] and *'M. excelsi'* [3]. These results go someway towards accounting for the high numbers of *Geodermatophilaceae* propagules present in Atacama Desert habitats where extreme environmental conditions prevail [11, 19]. Indeed, these data lend support to the view that the competitive success of these organisms in extreme, sparsely populated biomes may be a function of stress resistance not antibiosis [12].

It can be concluded from this wealth of genotypic and phenotypic data that isolates 1G4^T, 1G51 and 1G52 belong to a novel species of *Modestobacter* and that isolate 1G4^T can be

distinguished from all of its closest phylogenetic neighbours by low ANI and dDDH values. It is, therefore, proposed that this novel taxon be recognised as *Modestobacter altitudinis* sp. nov.

DESCRIPTION OF *MODESTOBACTER ALTITUDINIS* SP. NOV.

Modestobacter altitudinis (al.ti.tu'di.nis. L. gen. n. *altitudinis* of a high place).

Aerobic, Gram-stain-positive, non-motile actinobacteria that form short rods and coccoid-like elements. Grows from 10 to 35 °C (type strain), optimally around 28 °C, from pH range 5.0–9.0 (type strain), optimally 7.5–8.0, and in presence of up to 8% NaCl, w/v (type strain). Aesculin is hydrolysed but not allantoin or urea. Nitrate is reduced. Tweens 40 and 60 are degraded, but not adenine, casein, chitin, elastin, guanine, hypoxanthine, starch, Tween 80, L-tyrosine, uric acid, xanthine or xylan. Positive for acid and alkaline phosphatases, esterase (C4), esterase lipase (C8), α- and β-glucosidases, leucine arylamidase and valine arylamidase, but negative for α-chymotrypsin, β-glucuronidase and trypsin. Oxidises D-fructose-6-phosphate, D-glucose, glucuronamide, raffinose, D-salicin, actetic acid, acetoacetic acid, *p-hydroxy*-phenylacetic acid and quinic acid, but not D-arabitol, D- or L-fucose, *N-acetyl*-D-galactosamine, D-galactose, *N-acetyl*-D-glucosamine, glycerol, *myo*-inositol, lactose D-mannitol, *N-acetyl*-β-mannosamine, α- and β-*hydroxy*-butyric acids, α-*keto*-butyric acid, γ-amino-*n*-butyric acid, citric acid, L-galactonic acid-γ-lactone, D-galactouronic acid, D-gluconic acid, D-glucuronic acid, D-lactic acid methyl ester, D-malic acid, mucic acid, D-saccharic acid and L-alanine, D-aspartic acid, L-histidine, L-pyroglutamic acid or D-serine and inosine. Resistant to aztreonam, nalidixic acid, rifamycin SV, lithium chloride, potassium tellurite and sodium lactate (1%). Whole cell hydrolysates contain *meso*-diaminopimelic acid, glucose and ribose, the major fatty acids are $C_{17:1}$ ω8c, $C_{18:1}$ ω9c, iso- $C_{16:0}$, MK-9(H_4) is the predominant menaquinone, and phosphatidylethanolamine is the diagnostic phospholipid. The genome size of the type strain is around 5.2 Mbp and its genomic DNA G+C content 73.7 mol%.

The type strain 1G4^T (DSM 107534^T= PCM 3003^T) was isolated from a surface soil sample collected from Cerro Chajnantor, near San Pedro de Atacama, Chile. The accession number of 16S rRNA gene sequence of isolate 1G4^T is MH430521 and the accession number of its genome sequence is SJEW00000000.

Funding information
This work was supported by Grant No. 2017/01/X/NZ8/00140 from the National Science Centre in Poland. Genome sequencing was provided by MicrobesNG (https://www.microbesng.uk), which is supported by the Biotechnology and Biological Sciences Research Council (BBSRC; grant number BB/L024209/1).

Acknowledgements
We are grateful to Tiago Zucchi (Agrivalle, Agricultural Biotechnology, Salto, Brazil) for suggesting the species epithet of the strain.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The authors have not carried out any studies involving human participants or animals.

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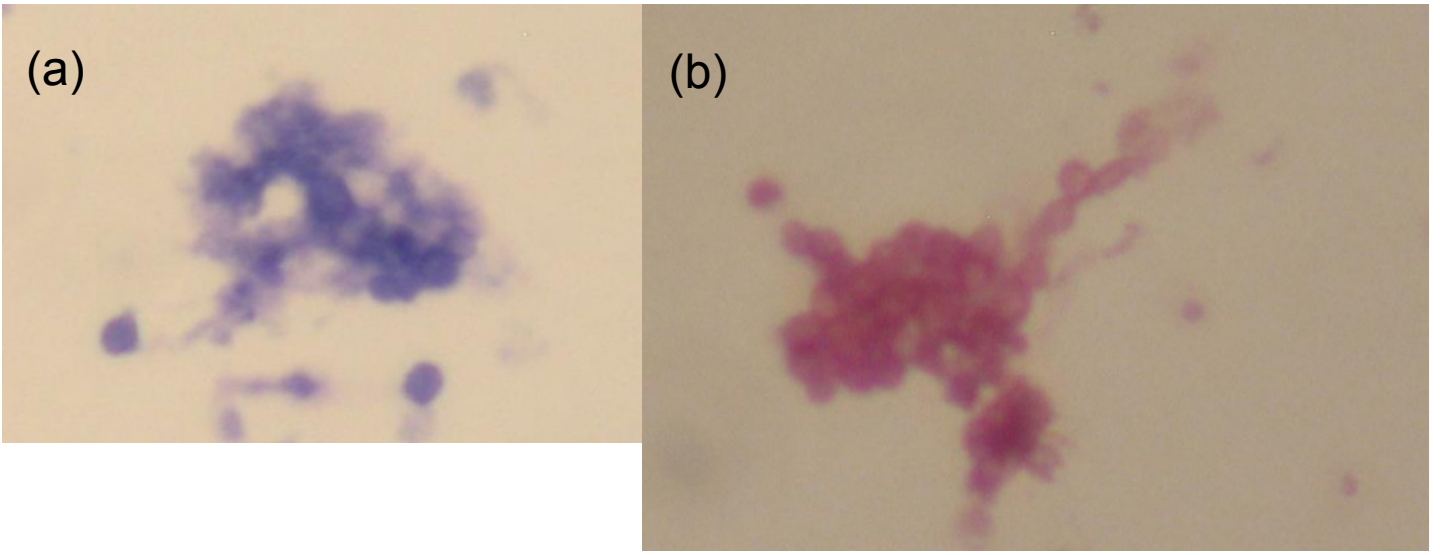

Fig. S1. Micromorphology of isolate 1G4^{T} stained with (a) methylene blue and (b) fuchsin.

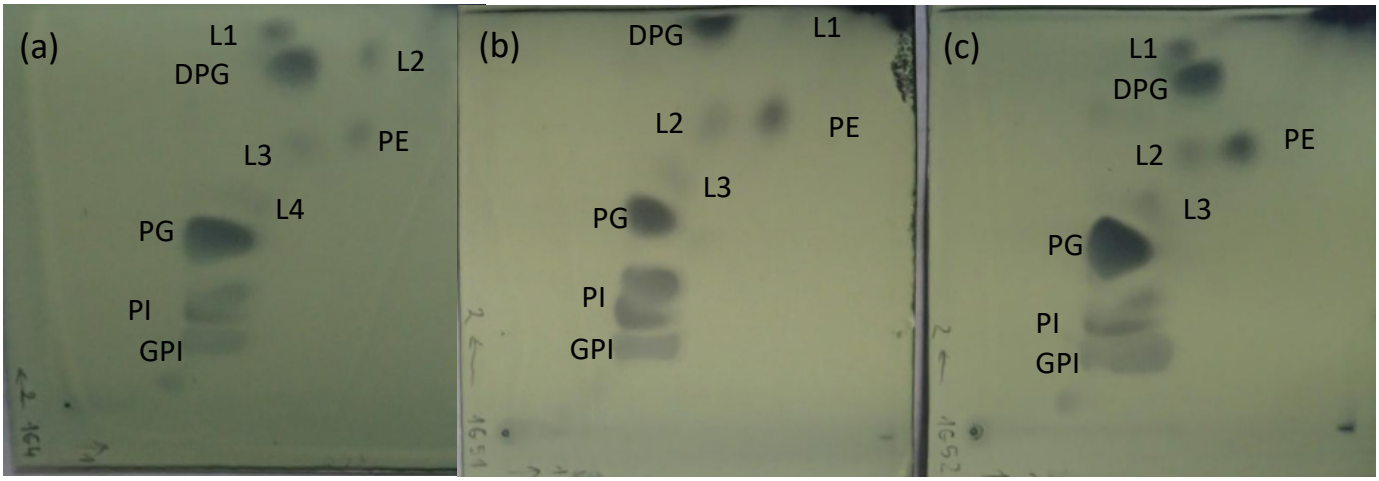

Fig. S2. Polar lipids profile of strains (a) 1G4^{T}, (b) 1G51 and (c) 1G52 after separation by two-dimensional TLC using chloroform:methanol:water (65 : 25 : 4; by volume) in the first dimension and chloroform:methanol:acetic acid:water (80 : 12 : 15 : 4; by volume) in the second dimension. Plates were sprayed with molybdenum blue (Sigma) for the detection of total polar lipids. DPG, diphosphadidylglycerol; GPI, glycophosphatidylinositol; PE, phosphatidethanolamine; PG, phosphadidylglycerol; PI, phosphatidylinositol; L1–4, unidentified lipids.

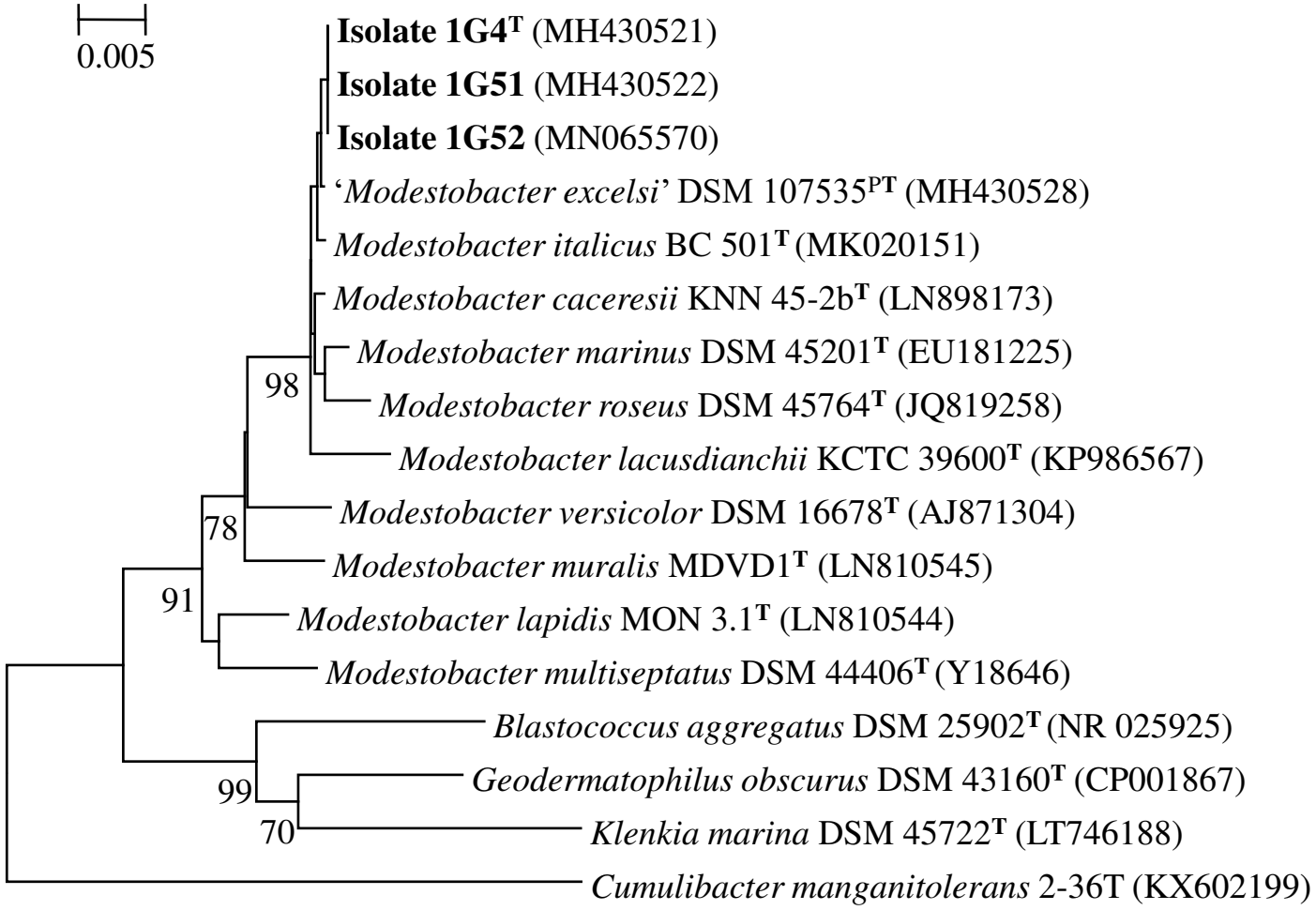

Fig. S3. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences (1390-1541) showing relationships between isolates 1G4^T, 1G51 and 1G52 and between them and the type strains of *Modestobacter* species. The numbers at the nodes are bootstrap support values over 70%. Bar; 0.005 substitutions per nucleotide position. The root position of the tree was determined using *Cumulibacter manganitolerans* 2-36^T as the outgroup.

Systematic and Applied Microbiology 43 (2020) 126153

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: http://www.elsevier.com/locate/syapm

Streptomyces alkaliterrae sp. nov., isolated from an alkaline soil, and emended descriptions of *Streptomyces alkaliphilus*, *Streptomyces calidiresistens* and *Streptomyces durbertensis*

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ARTICLE INFO

Article history:
Received 25 May 2020
Received in revised form 10 October 2020
Accepted 16 October 2020

Keywords:
Alkaliphilic streptomycetes
Polyphasic taxonomy
Genomics
Streptomyces alkaliterrae
Phylogeny
Lonar Lake soil

ABSTRACT

A polyphasic study was undertaken to establish the taxonomic position of six representative streptomycetes isolated from an alkaline soil adjacent to a meteoric alkaline soda lake in India. Chemotaxonomic, cultural and morphological properties of the isolates were consistent with their classification in the genus *Streptomyces*. The isolates formed extensively branched substrate mycelia and aerial hyphae that differentiated in straight chains of spores with smooth surfaces. They contained *LL*-diaminopimelic acid in the wall peptidoglycan, produced either hexa- or octa-hydrogenated menaquinones with nine isoprene units, major amounts of saturated, *iso*- and *anteiso*- fatty acids and phosphatidylethanolamine as the characteristic polar lipid. The isolates grew well at 30 °C, pH 9 and in the presence of 3 to 5% (w/v) sodium chloride. Isolates OF1^T, OF3 and OF8 formed a distinct clade within the *Streptomyces* 16S rRNA gene tree sharing relatively high sequence similarities with the type strains of *Streptomyces durbertensis* (99.3%), *Streptomyces palmae* (98.1%) and *Streptomyces xinghaiensis* (98.3%), but can be distinguished from them using combinations of phenotypic properties. A phylogenomic tree based on draft genome sequences of the isolates and *S. durbertensis* DSM 104538^T confirmed the phylogenetic relationships. Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values calculated from the whole genome sequences of isolate OF1^T and *S. durbertensis* DSM 104538^T were low at 92.0% and 45.2%, respectively, indicating that they belong to different genomic species. Consequently, on the basis of the genomic, phylogenetic and associated phenotypic data it is proposed that isolates OF1^T, OF3 and OF8 be assigned to the genus *Streptomyces* as *Streptomyces alkaliterrae* sp. nov. with strain OF1^T (NCIMB 15195^T =PCM 3001^T) as the type strain. Isolates IF11, IF17 and IF19, and *S. alkaliphilus* DSM 42118^T were shown to belong to the same taxospecies and together with *S. calidiresistens* DSM 42108^T comprised a well supported clade in the *Streptomyces* 16S rRNA gene tree. Isolate IF17 and *S. alkaliphilus* DSM 42118^T formed a well-supported clade in the phylogenomic tree, had almost identical digital G+C similarity values, produced long straight chains of smooth-surfaced spores and shared ANI and dDDH values (98.0 and 79.6%, respectively) consistent with their assignment to the same genomic species. In light of all of the data isolates IF11, IF17 and IF19 should be seen as authentic stains of *S. alkalihilus*. Data acquired in the present study have also been used to emend the descriptions of *S. alkaliphilus*, *S. calidiresistens* and

Abbreviations: A_2pm, diaminopimelic acid; ANI, average nucleotide identity; BGCs, biosynthetic gene clusters; BLAST, Basic Local Alignment Search Tool; dDDH, digital DNA–DNA hybridization; DDH, DNA–DNA hybridization; DNA, deoxyribonucleic acid; GGDC, genome to genome distance calculator; ISP, International *Streptomyces* Project; MIDI, Microbial Identification System; MK, menaquinones; NCIMB, National Collection of Industrial Food and Marine Bacteria; PCM, Polish Collection of Microorganisms; RAST, Rapid Annotation using Subsystem Technology; rRNA, ribosomal RNA; S., *Streptomyces*; TLC, thin-layer chromatography.

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https://doi.org/10.1016/j.syapm.2020.126153

S. durbertensis. The genomes of isolates IF17, and OF1^T, OF3 and OF8 contain relatively high numbers of biosynthetic gene clusters some of which were discontinously distributed indicating ones predicted to express for novel specialised metabolites.

Introduction

Novel filamentous actinobacteria with genomes rich in biosynthetic gene clusters *sensu* Baltz [10] are a key element in the search for new drugs needed in the fight against multi-drug resistant microbial pathogens [31]. Novel neutrophilic streptomycetes (optimal growth around pH 7.0) from deep sea sediments and desert soils are a productive source of new bioactive compounds [15,37,61,65] but, in contrast to these microorganisms, alkaliphilic streptomycetes (optimal pH 9.0) [4] have rarely featured in bioprospecting campaigns despite their biotechnological potential [34]. The realisation that alkaliphilic streptomycetes have a pronounced capacity to synthesize new bioactive compounds [63,71] sparked renewed interest in them leading to the recognition of new species, such as *Streptomyces alkaliphilus* [1], *Streptomyces durbertensis* [86] and *Streptomyces lonarensis* [68] and to the discovery that an alkaliphilic streptomycete inhibits the growth of multi-drug resistant ESKAPE pathogens [78].

Alkaliphilic streptomycetes are common in alkaline soils and soda lakes [70,76], form a distinct taxon in multi-dimensional taxospace [3] and are markedly underspeciated [5]. The discovery that alkaliphilic streptomycetes isolated from sites across a beach and dune sand system could be assigned to *Streptomyces griseus* ecovars was seen to have implications for bioprospecting [4], as do extensive biogeographical studies on global communities of streptomycetes [2,66]. Improved matrices for the recognition of boundaries between prokaryotic species, subspecies and infrasubspecific taxa [9,20,48,55] are bringing greater precision to such studies.

The present investigation was designed to establish the taxonomic status of alkaliphilic streptomycetes isolated from an alkaline soil in India and to show whether representative isolates have the potential to synthesise novel antibiotics. The isolates and the type strains of closely related *Streptomyces* species were the subject of a polyphasic study which drew upon data from draft whole genome sequences of representative strains. Some of the isolates were shown to be members of *S. alkaliphilus* [1] while others formed a new centre of taxonomic variation in the genus *Streptomyces*. The name proposed for this new taxon is *Streptomyces alkaliterrae* sp. nov., with isolate OF1^T as the type strain.

Materials and methods

Isolation, maintenance and biomass preparation

Isolates OF1^T, OF3, OF8, IF11, IF17 and IF19 were recovered from an alkaline soil (pH 9.9–10.4) adjacent to Lake Lonar (19°58′21.4″N/76°30′17.9E), a meteoric alkaline soda lake located in the Buldhana district of Maharashtra, India, using a standard dilution plate procedure [30]. Briefly, one gram of the soil was suspended in 9.0 mL of ¼ strength Ringer's solution (Oxoid), mixed on a tumble shaker for an hour prior to the preparation of serial dilutions. Aliquotes (100 μL) of each serial dilution were spread over the surfaces of starch-casein agar plates [41], adjusted to pH 8.5 with 1 N NaOH, and supplemented with 5% (w/v) sodium chloride and cycloheximide and nystatin (each at 50 μg mL^{-1}); the plates were dried for 15 min prior to inoculation, as recommended by Vickers and Williams [81]. Inoculated plates, three per dilution, were incubated at 28 °C for 4 weeks when typical streptomycete-like colonies were detected; representative isolates were subcultured onto starch-casein agar adjusted to pH 8.5 and supplemented with 5% w/v NaCl. The isolates and *S. alkaliphilus* DSM 42118^T [1], *Streptomyces calidiresistens* DSM 42108^T [25], *S. durbertensis* DSM 104538^T [86], *Streptomyces palmae* CMU-AB204^T [74] and *Streptomyces xinghaiensis* S187^T [87] were maintained on slopes of halophilic nutrient agar [6] at room temperature and as suspensions of spores and hyphae in 20%, v/v glycerol at −80 °C.

Biomass for the molecular systematic and the chemosystematic studies was prepared by growing the isolates in shake flasks of halophilic nutrient broth [6] supplemented with 5% NaCl, w/v (pH 8.5) at 150 revolutions per minute for 2 weeks at 28 °C. Cells were harvested by centrifugation and washed three times in sterile distilled water; biomass for the chemotaxonomic analyses was freeze dried and that for the molecular systematic studies stored at room temperature.

Chemotaxonomic, cultural and morphological properties

Standard chromatographic methods were used to establish the chemotaxonomic profiles of the isolates. They were examined for isomers of diaminopimelic acid [73], isoprenoid quinones [22], polar lipids [53] and whole-organism sugars [33], using appropriate controls.

Cellular fatty acids extracted from isolates OF1^T and IF17 were methylated after Miller [52] with minor modifications from Kuykendall et al. [42]. The fatty acid methyl esters were separated by gas chromatography (Hewlett Packard instrument 6890 N) and the resultant peaks automatically integrated; fatty acid names and percentages were determined using the standard Microbial Identification MIDI System, version 5 [64] and the TSBA40 version of the MIDI database. The G + C mol% of the DNA of isolates OF1^T and IF17 were determined by HPLC following the procedure described by Tamaoka and Komagata [77]; purified DNA prepared, as described later, was hydrolyzed with P1 nuclease, the nucleotides dephosphorylated with bovine alkaline phosphatase [51] then analyzed by HPLC (Shimadzu Corp., Japan). Lambda-DNA and three DNAs with published genome sequences within the G + C range 43–72 mol% were used as standards and G + C values calculated from the ratio of deoxyguanosine and deoxythymidine according to Mesbah et al. [51].

Cultural features were recorded following growth on media adjusted to pH 8.5, as before, and incubated at 28 °C for 3 weeks. The isolates and corresponding reference strains were grown on tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic-salts starch, glucose-asparagine, peptone-yeast extract-iron and tyrosine agar (ISP media 1–7, respectively) [69] and on modified Bennett's [36] and halophilic nutrient [6] agar. Aerial and substrate mycelial colours and those of diffusible pigments were determined by comparison against colour charts [40].

Hyphal and spore chain features of isolates OF1^T, OF3 and OF8 were detected on halophilic nutrient agar [6], adjusted to pH 8.5, after 14 days at 28 °C, using the coverslip technique of Kawato and Shinobu [39]; the same procedure was used for isolates IF11, IF17 and IF19 through in this case the strains were grown on ISP5 agar

[69] at pH 8.5. Spore arrangement and spore surface ornamentation of isolates OF1^T and IF17 were established by examining gold coated dehydrated preparations with growth taken from halophilic nutrient and ISP5 agar plates, respectively, using a scanning electron microscope (Model 1430 VP, LEO Electron Microscopy Ltd, Cambridge, England) and the procedure described by O'Donnell et al. [56].

Box typing

BOX-PCR fingerprints from genomic DNA extracted from the isolates were generated using the BOXA1R primer [80] and experimental conditions described previously [79].

Phylogenetic analyses

Genomic DNA was extracted from each isolate and PCR mediated amplification of 16S rRNA gene sequences achieved following Golinska et al. [28]. PCR products were purified using PCR purification kits (Qiagen) and sequenced at the Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw using an ABI 3730xl Genetic Analyzer (Applied Biosystems). The resultant data were compared with corresponding sequences of the type strains of closely related *Streptomyces* species using the EzBioCloud server [85] and aligned using Clustal W [44]. 16S rRNA gene sequence similarities were calculated using PHYDIT version 1.0 software [19] and a maximum-parsimony (MP) phylogenetic tree generated using the genome-to-genome distance calculator (GGDC) web server [49] adapted for single genes following multiple sequence alignments using MUSCLE software [26]. Similarly, a MP tree was inferred from the alignments with the Tree Analysis using New Technology (TNT) program [29] using 1000 bootstraps together with tree bisection and reconnection branch swapping and 10 random sequence replicates. A ML tree was constructed from alignments with RAxML [72] using rapid bootstrapping and the auto maximal-relative-error (MRE) bootstrapping criterion [60]. The sequences were checked for computational bias using the x^2 test implemented in PAUP* [75].

DNA:DNA relatedness values were estimated, in duplicate, between isolate IF17 and *S. alkaliphilus* DSM 42118^T using the service provided by the DSMZ (Braunschweig, Germany). Wet biomass of each strain was disrupted using a Constant System TS 0.75 KW machine (IUL Instruments, Germany). DNA extracted from the strains using a French pressure cell (Thermo Spectronic) was purified on a hydroxyapatite column [17] and DNA:DNA hybridizations carried out after De Ley et al. [24], albeit using the modifications of Huss et al. [35], on a model Cary 100 BIO UV/VIS spectrophotometer fitted with a Peltier thermostat 6 × 6 multi-well changer and a temperature controller with an *in situ* temperature probe (Varian).

Whole genome sequencing and genome analyses

Genomic DNA was extracted from biomass of isolates OF1^T, OF3, OF8 and IF17 and from *S. alkaliphilus* DSM 42118^T, *S. calidiresistens* DSM 42108^T and *S. durbertensis* DSM 104538^T following growth on ISP 2 agar (pH 8.5) for 7 days at 28 °C using the protocol provided by MicrobesNG (Birmingham, UK; http://www.microbesng.uk). Briefly, genomic DNA was extracted from wet biomass of single colonies of the strains using extraction buffer (EB) with lysozyme and RNase A for 25 min at 37 °C, proteinase K added and the preparations incubated for 5 min at 65 °C. Genomic DNA preparations were purified using equal volumes of SPRI beads, resuspended in EB buffer and DNA quantified, in triplicate, using Quantit dsDNA HS assays in an Ependorff AF2200 plate reader (Eppendorff, Germany). Genomic DNA was sequenced using a HiSeq instrument (Illumina, San Diego, USA) and the 250 bp paired end in house protocol (MicrobesNG). Genomic DNA libraries were prepared using a Nextera XT Library Prep Kit (Illumina, San Diego, USA) following the manufacturer's protocol with the following modifications: two nanograms of DNA instead of one were used as input, and the PCR elongation time was increased to 1 min from 30 s (MicrobesNG). DNA quantification and library preparation were carried out on a Microlab STAR automated liquid handling system (Hamilton, Birmingham, UK) and pooled libraries quantified using the Kapa Biosystems Library Quantification Kit for Illumina on a Roche light cycler 96 qPCR machine (Roche, West Sussex. UK). Reads were trimmed using Trimmomatic 0.30 [14] and those under 250 bp discarded; contigs of each strain were assembled using Spades 3.7 software [11], annotated using Prokka 1.11 [67] and sequence based comparisons achieved using the SEED Viewer [8]. The completeness and contamination of the draft genome sequences of the isolates and the *S. alkaliphilus*, *S. calidiresistens* and *S. durbertensis* strains were estimated using CheckM [59]. The genome sequences of the isolates and the type strains of *S. alkaliphilus*, *S. calidiresistens* and *S. durbertensis* were uploaded onto the Type (Strain) Genome Server (TYGS) [47] and compared using the MASH algorithm which allows fast approximation of intergenomic relatedness between strains [57]. A phylogenomic tree was inferred with FastME 2.1.4 [45] from GBDP distances calculated from genome sequences, branch lengths were scaled using the GBDP distance formula d5 [49], numbers above branches are GBDP pseudo-bootstrap support values based on 100 replications.

Two-way BLAST based average nucleotide identities (ANIs) between the genomes of isolates OF1^T, OF3 and OF8, between these isolates and *S. durbertensis* DSM 42118^T and between isolate IF17 and the type strains of *S. alkaliphilus* and *S. calidiresistens* were calculated using the online resource from the K. Konstantinidis group (http://enve-omics.gatach.edu/; [32]). The corresponding digital DNA:DNA hybridization (dDDH) values between the genomes of the isolates and the reference strains were calculated using the genome-to-genome distance calculator, GGDC [7,49]. The genomes of the isolates and the corresponding reference strains were examined for BGCs using anti-SMASH 4.0 software [13].

Phenotypic properties

The isolates and associated reference strains were examined for a range of biochemical, degradative and physiological properties using media and methods employed in a comprehensive numerical taxonomic study on the genus *Streptomyces* [83]. The tests were carried out, in triplicate, in 12-well plates that were inoculated using a standard inoculum in sterile distilled water that corresponded to 5 on the McFarland scale [54]; the plates were inoculated using a multipoint inoculator (Mast Uri®Dot, Mast Group Ltd., Merseyside, UK) and incubated for 3 weeks at 28 °C, apart from the temperature tests. The enzymatic activities of the strains were determined using API ZYM kits (bioMerieux), according to the manufacturer's instructions. The isolates and reference strains were also tested for their ability to grow over a range of temperature (4, 10, 15, 20, 25, 28, 30, 35, 40, 41, 42, 43, 44, 45 and 50 °C) and pH (4.0–13.0 at single unit intervals) regimes and in the presence of various concentrations of sodium chloride (0–15.0 %, w/v at one unit intervals) using ISP 2 agar as the basal medium; the pH values were achieved using KH_2PO_4/HCl (pH range 4–5), KH_2PO_4/K_2HPO_4 (0.1 M both, pH range 6–8) and K_2HPO_4/NaOH (pH range 9–13) buffer systems.

Results and discussion

The isolates shared chemotaxonomic, cultural and morphological properties consistent with their classification in the genus *Streptomyces* [38,55]. They formed extensively branched substrate

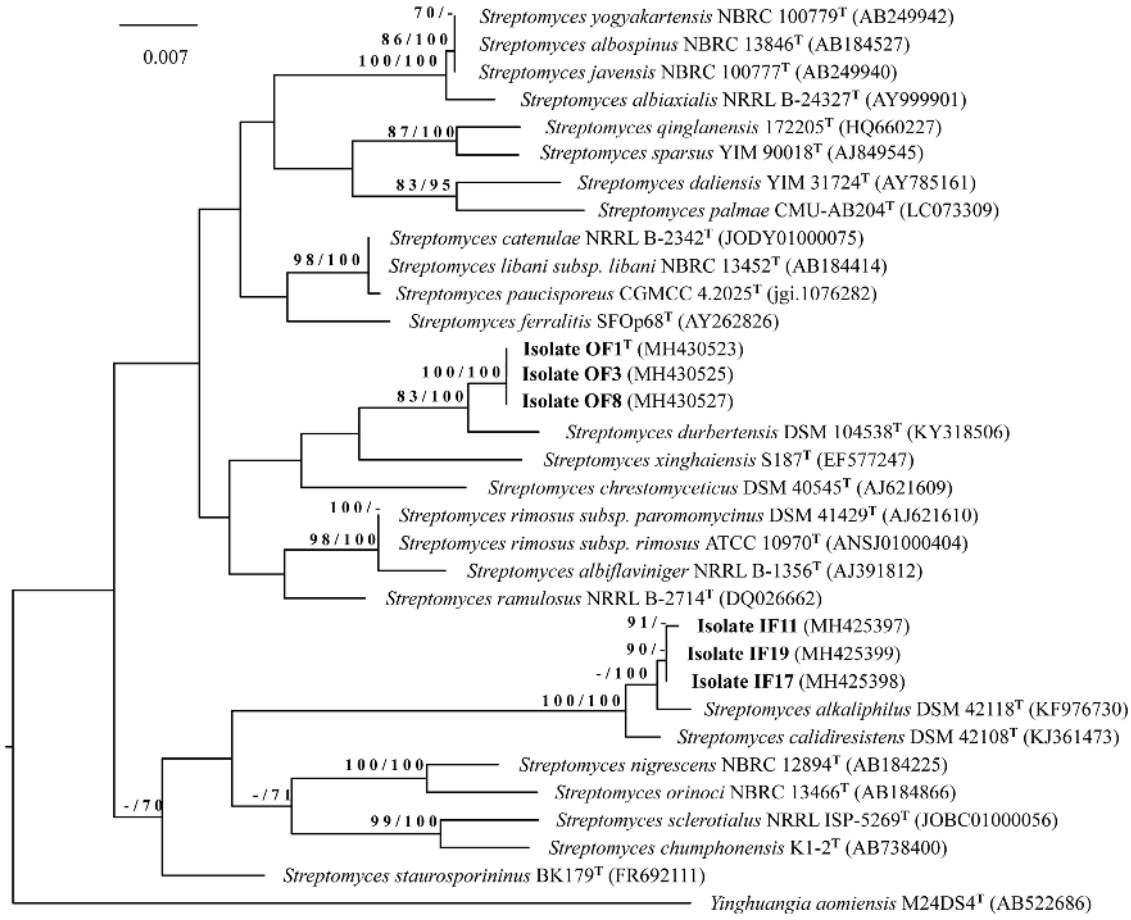

Fig. 1. Maximum-likelihood and maximum-parsimony trees based on nearly complete 16S rRNA gene sequences using the GTR-GAMMA method showing relationships between isolates OF1^T, OF3 and OF8 and IF11, IF17 and IF19 and between them and the type strains of closely related *Streptomyces* species. The numbers at the nodes are bootstrap support values ≥ 70% for ML (left) and MP (right). Bar; 0.007 substitutions per nucleotide position. The root position of the tree was determined using *Yinghuangia aomiensis* M24DS4^T as outgroup.

mycelia and aerial hyphae that differentiated into straight chains of spores. The isolates produced whole organism hydrolysates containing *LL*-A_2pm, glucose and xylose with either ribose (isolates OF1^T, OF3 and OF8) or mannose (isolates IF11, IF17 and IF19), contained either hexa- or octa-hydrogenated menaquinones with nine isoprene units as predominant isoprenologues and complex polar lipid patterns containing diphosphatidylglycerol, phosphatidylethanolamine (diagnostic component), phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides, as shown in **Figure S1**. It is apparent from variations in the BOX-PCR fingerprint data that neither set of isolates are clones though isolates IF11, IF17 and IF19 have very similar fingerprints (**Fig. S2**). In addition, all of the isolates grew well on the nutrient agar media producing a range of pigments (**Table S1**). Isolates IF11, IF17 and IF19 gave identical results on all of the media generally forming reddish brown substrate mycelia and a white to grey aerial spore mass. In contrast, isolates OF1, OF3 and OF8 showed variable results on some ot the media but tended to form brown or yellow substrate mycelia and a white to grey aerial spore mass. Distinctive diffusible pigments were produced on some of the ISP media. Isolates IF17 and OF1^T formed long straight chains of smooth surfaced spores, and complex fatty acid patterns, as exemplified in **Figure S3** and **Table S2**, respectively. Each of the isolates contained major proportions of anteiso-$C_{15:0}$ (38.2 and 22.1%, respectively) and iso-$C_{16:0}$ (20.6 and 23.1%) with iso-$C_{15:0}$ (19%; strain IF17) and anteiso-$C_{17:0}$ (18%; strain OF1^T), profiles consistent with their classification in the genus *Streptomyces* [38,55]. The DNA G + C values of isolates IF17 and OF1^T were 72.4 and 72.0 mol%, respectively.

Nearly complete 16S rRNA gene sequences were obtained for isolates OF1^T, OF3 and OF8 (1534 nucleotides [nt]), the GenBank accession numbers are MH430523, MH430525 and MH430527, respectively. The isolates had identical 16S rRNA gene sequences, formed a well supported branch in the *Streptomyces* 16S rRNA gene tree with *S. durbertensis* DSM 104538^T (Fig. 1) and shared a 99.3% 16S rRNA gene sequence similarity with the latter, which corresponds to 10 nt differences at 1520 locations. The isolates were relatively closely related to the type strains of *S. palmae* (98.1% similarity) and *S. xinghaiensis* (98.3% similarity), values well below the 99% threshold used to assign closely related actinobacteria to the same species [49]. Nearly complete 16S rRNA gene sequences were acquired for isolates IF11, IF17 and IF19 (1389, 1535 and 1402 nt), respectively; the corresponding GenBank accession numbers are MH425397, MH425398 and MH425399. Isolates IF17 and IF19 with identical 16S rRNA gene sequences shared a 99.9% sequence similarity with isolate IF11, a value equivalent to a single nt difference at 1387 and 1385 locations, respectively. It is apparent from Fig. 1 that these isolates form a well supported clade in the *Streptomyces* 16S rRNA gene tree with the type strains of *S. alkaliphilus* and *S. calidiresistens* sharing 16S rRNA gene sequence similarities with the former, their closest phylogenetic neighbour, of either 99.6 or 99.7%; the corresponding range with the *S. calidiresistesns* strain is 99.4 to 99.5%. Isolate IF17 and the type strain of *S. alkaliphilus* shared a mean DNA:DNA relatedness of 63.6 ± 1.1%, a value below the 70% threshold recommended for assigning closely related strains to the same genomic species [82]. However, results such as these need to be interpreted with care as dDDH methods based on comparisons of

Table 1
Average nucleotide identity (ANI) and digital DNA:DNA hybridization (dDDH) values found between representative isolates and between them and their closest phylogenetic neighbours.

Strains		ANI (%)	dDDH (%)
Isolate IF17	*S. alkaliphilus* DSM 42118^T	98.0	79.6
	S. calidiresistens DSM 2108^T	91.5	42.0
Isolate OF1^T	*S. durbertensis* DSM 10538^T	92.0	45.2
Isolate OF1^T	Isolate OF3	99.6	95.8
Isolate OF1^T	Isolate OF8	100	99.9
Isolate OF3	Isolate OF8	99.6	95.7
OF3	*S. durbertensis* DSM 10538^T	92.0	45.1
OF8	*S. durbertensis* DSM 10538^T	92.0	44.9
Isolate OF1^T	Isolate IF17	80.7	23.2
S. alkaliphilus DSM 42118^T	*S. calidiresistens* DSM 42108^T	91.5	42.1

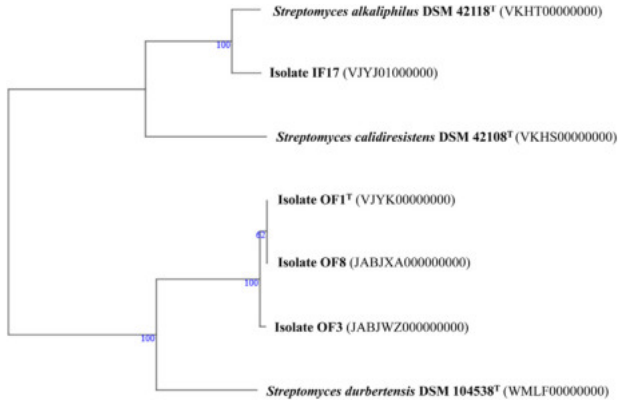

Fig. 2. Phylogenomic tree showing relationships between isolate IF17 and isolates OF1^T, OF3 and OF8 and the type strains of their closest relatives. The numbers above the branches are GBDP pseudo-bootstrap support values >60 % from 100 replications with an average branch support of 90.5 %. The tree was rooted at the midpoint [27].

whole genome sequences provide better quality data for assigning closely related strains to the same or different species than corresponding values derived from the application of experimental methods which are prone to experimental error [7,49,54].

The general features of the draft genome sequences of isolates IF17, OF1^T, OF3 and OF8 and those of the type strains of their closely related phylogenetic neighbours are shown in **Table S3**. The genome sizes of the strains range from 5.8 to 6.2 Mb showing that they are moderately gifted *sensu* Baltz [10]. The completeness of assemblies is high (94.8–97.2%) with very low levels of contamination (0.3–3.8 %), indicating that the draft genomes are of good quality (**Table S3**).

The dDNA G + C similarity values of isolate IF17 and *S. alkaliphilus* DSM 42118^T were 72.4 and 72.5 %, respectively; the corresponding similarity values of isolates OF1^T, OF3 and OF8 and *S. durbertensis* DSM 104538^T ranged from 72.0–72.4%, all of the values are within the 1% range expected of strains belonging to the same species [50]. The relative distribution of the different functional gene classes shown in **Figure S4** are similar to those of the type strain of *Streptomyces leeuwenhoekii* [16] and the model strains, *Streptomyces coelicolor* A3(2) [12] and *Streptomyces lividans* 66 [23].

It is evident from the phylogenomic tree (Fig. 2) that isolate IF17 is closely related to the type strain of *S. alkaliphilus* and less so to *S. calidiresistens* and that isolates OF1^T, OF3 and OF8 have identical or almost identical genomic sequences that distinguish them from *S. durbertensis* thereby confirming the relationships found between all of these strains in the 16S rRNA gene tree. The recommended thresholds used to distinguish between closely related prokaryotic species based on ANI and dDDH similarities are 95 to 96% [21,62] and 70% [20,21,82], respectively. It is evident from Table 1 that on this basis isolates OF1^T, OF3 and OF8 belong to the same genomic species. The dDDH values betwen the genome sequences of these isolates and *S. durbertensis* DSM 10538^T, their closest phylogenomic neighbour, are low at 45.2, 45.1 and 44.9%, respectively; the corresponding shared ANI similarity was 92.0% indicating that the isolates and the *S. durbertensis* strain belong to different species. In contrast, the high ANI and dDDH similarities (98.0 and 79.6%, respectively) found between isolate IF17 and *S. alkaliphilus* DSM 42118^T are well above the recommended thresholds used to assign closely related strains to the same species. This latter value is in sharp contrast to the 63.6% similarity found between these organisms using the laboratory based method though, as mentioned earlier, *in silico* DDH values provide more accurate data than corresponding laboratory based procedures [50,55], as is the case with estimates of dDNA G + C composition [50,55]. Low ANI and DDH similarities clearly separate isolates IF17 and OF1^T (Table 1).

Excellent congruence was found between the replicated phenotypic tests carried out on the isolates and associated reference strains. Each set of isolates had many phenotypic features in common though in each case a few properties can be weighted to distinguish between them (**Table S4**). All of the isolates can be separated from the type strains of their closest phylogenomic neighbours using a combination of phenotypic properties (Tables 2 and 3). Isolates OF1,OF 3 and OF8 can be distinguished from *S. durbertensis* DSM 104538^T based on pH and temperature profiles and by positive results for esterase (C4), esterase lipase (C8) and lipase (C14), nitrate reduction, Tween 80 degradation and use of D-raffinose as a sole carbon source. In contrast, the *S. durbertensis* strain, unlike the isolates, degraded starch, used adonitol, L-arabinose, β-*methyl*-D-glucosamide, D-glucosamine, D-glucose, glycerol, glycogen, D-lactose, D-mannitol, benzoate, butyrate, hippurate and propionate (sodium salts) as sole carbon sources and showed a much greater ability to use sole nitrogen sources. Similarly, the isolates can be separated from the type strains of *S. palmae* and *S. xinghaiensis* using combinations of phenotypic features. Phenotypic features that distinguish the reference strains can be seen in Table 2.

Isolates IF11, IF17 and IF19 and *S. alkaliphilus* DSM 42118^T and *S. calidiresistens* DSM 2108^T, their closest phylogenomic neighbours, grow from pH 8 to pH 10, indicating that they are alkaliphilic streptomycetes, and from 20 to 40 °C. Table 3 shows that while the isolates and the type strains of their two closest relatives have many phenotypic features (unit characters) in common they can be distinguished using a few such properties. Hovewer, 96 and 79.2% of the unit characters were shared by the isolates and by them and the *S. alkaliphilus* strain, respectively, values above the threshold used to assign closely related strains to *Streptomyces* species in numerical taxonomic studies based on the same experimental procedures and the simple matching coefficient, which includes both positive and negative similarities [83,84]; the corresponding value between the isolates and and *S. calidiresistens* strain was 65% hence well below the species threshold, thereby indicating that they belong to different taxospecies. In contrast, the close phylogenomic relationship between isolate IF17, *S. alkaliphilus* DSM 42118^T and *S.*

Table 2
Phenotypic properties that distinguish isolates OF1^T, OF3 and OF8 from from their closest phylogenomic relatives.

Characteristics	Isolates OF1^T, OF3 and OF8	*S. durbertensis* DSM 104538^T	*S. palmae* CMU-AB204^T	*S. xinghaiensis* S187^T
API-ZYM tests:				
Acid phosphatase, α-galactosidase	–	–	+	–
α-Chymotripsin	+	+	+	–
Esterase (C4)	+	–	+	+
Esterase lipase (C8)	+	–	+	+
Lipase (C14)	+	–	–	–
Trypsin	+	+	+	–
Hydrolysis of:				
Aesculin	+	+	+	–
Arbutin	–	–	+	–
Reduction of nitrate	+	–	+	+
Degradation of:				
Starch	–	+	+	–
Tween 20	–	–	+	+
Tween 80	+	–	+	+
Sole carbon sources:				
Adonitol, L-arabinose, β-*methyl*-D-glucoside, D-glucosamine, D-mannitol, sodium benzoate, sodium hippurate, sodium propionate	–	+	–	–
L-Arabitol, *meso*-erythritol, α-*methyl*-D-glucoside, *meso*-inositol, D-melezitose, D-sucrose, xylitol, D-xylose	+	+	–	–
D-Cellobiose, D-fructose, D-galactose, D-maltose, D-melibiose	+	+	+	–
D-glucose, glycerol, glycogen	–	+	+	–
Inulin	–	–	+	–
D-Lactose, sodium butyrate	–	+	–	+
D-Raffinose	+	–	–	–
D-Ribose, sodium acetate, sodium citrate, sodium fumarate	+	+	–	+
D-Salicin, sodium oxalate	–	–	–	+
Sole nitrogen sources:				
L-Arginine, L-hydroxyproline, L-*iso*leucine, L-phenylalanine, L-serine, L-valine	–	+	+	–
Ethanolamine	+	+	–	–
L-Threonine	–	+	+	+
Tolerance tests:				
pH range	6-11*	6–12	5–9	7–11
Temperature range (°C)	15–45*	15–42	25–40	15–44
Optimal temperature (°C) for growth	28–30	30–35	25	25

Key: +, positive; -, negative; *,Isolates OF1^T and OF8 showed weak growth at pH 12 and 50 °C, respectively.
The strains were positive for alkaline phosphatase, cystine, leucine and valine arylamidases, and naphthol-AS-BI-phosphohydrolase (API-ZYM tests), degradation of Tweens 40 and 60, utilisation of L-asparagine and L-histidine as a sole nitrogen sources and dextrin, sodium malonate, sodium pyruvate, sodium succinate, D-sorbitol and D-trehalose as a sole carbon sources, but were negative for α-fucosidase, β-galactosidase and β-glucuronidase (API-ZYM tests), allantion and urea hydrolysis, adenine, carboxymethylcellulose, casein, chitin, DNA, elastin, guanine, hypoxanthine, pectin, L-tyrosine, uric acid, xanthine and xylan degradation, use of acetamide, L-aspartic acid, L-cysteine and L-glutamic acid as a sole nitrogen sources and D-glucuronic acid as a sole of carbon source.

calidiresistens DSM 42108^T is supported by morphological data as they all produce long chains of smooth-surfaced spores [1,25], a result which provides further evidence of a correlation between phylogeny of well separated streptomycete clades and associated phenotypic data [43].

It can be concluded from the extensive phenotypic data that isolates OF1^T, OF3 and OF8 belong to the same taxospecies and can be distinguished from the type strain of *S. durbertensis*, their closest phylogenomic neighbour, by a wealth of genomic and phenotypic data, notably by low ANI and dDDH similarities. Consequently, it is proposed that the isolates OF1^T, OF3 and OF8 be given species status as *Streptomyces alkaliterrae* sp. nov., with isolate OF1^T as the type strain. The description of the new species is given in Table 4.

It is also apparent that isolates IF11, IF17 and IF19 form a well supported lineage in the 16S rRNA gene tree together with the type strains of *S. alkaliphilus* and *S. calidiresistens*. The isolates are most closely related to the *S. alkaliphilus* strain and together with the latter form a well defined taxospecies. In addition, isolate IF17 and *S. alkaliphilus* DSM 42118^T form a well supported subclade in the phylogenomic tree, produce straight chains of smooth-surfaced spores on aerial hyphae, share almost identical dG + C similarity values and ANI and dDDH similarities that are well above the thresholds used to assign closely related strains to the same species. All of these datasets show that these isolates are authentic strains of *S. alkaliphilus* [1].

Further, in light of the data acquired in this study emended descriptions are given for *S. alkaliphilus* [1], *S. calidirsistens* [25] and *S. durbertensis* [86], notably on genomic sizes and dDNA G + C values, properties considered to be phylogenetically conserved [55].

The genomes of isolates IF17, OF1^T, OF3 and OF8 and their closest phylogenomic neighbours contained between 24 (isolate OF8) and 41 (*S. alkaliphilus* DSM 42118^T) BGCs (Fig. 3) thereby falling within the range expected for *Streptomyces* strains [10,18]. In general, the genomes of these strains contain a similar range of bioclusters, notably ones associated with the production of siderophores, terpenes, non-ribosomal peptide and type III polyketide synthases. **Table S5** shows that many of the bioclusters detected in the genomes of the isolates and their closest phylogenomic neighbours are discontinously distributed. Examples include bioclusters predicted to encode for novel specialised metabolites, as exemplified by a lassopeptide associated with the isolates and the *S. calidiresistens* and *S. durbertensis* strains, a linaridin associated with the *S. alkaliphilus* and *S. calidiresistens* strains, a NPRS-like biocluster considered to encode for rhizomide A/B/C compounds associated with *S. alkaliphilus* DSM 42118^T strain and NRPS bioclusters expressing for thiocoraline and virginiamycin associated with isolates OF1, OF3 and OF8, and the *S. durbertensis* strain. The discontinous distribution of bioclusters in the three *S. alkaliterrae* isolates provide further evidence that members of the some *Streptomyces* species can display different specialised metabolites [46,58]. These

Table 3
Phenotypic properties that distinguish isolates IF11, IF17 and IF19 from their closest phylogenomic relatives.

Characteristics	Isolates IF11, IF17 and IF19	*S. alkaliphilus* DSM 42118^T	*S. calidiresistens* DSM 42108^T
Hydrolysis of:			
Arbutin	+	–	+
Degradation of:			
Starch	+	–	–
Tween 80	–	+	+
Sole carbon sources:			
Adonitol, D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, D-lactose, D-ribose, sucrose, sodium benzoate, sodium citrate, sodium hippurate, sodium oxalate, sodium propionate	+	+	–
Amygdalin, *meso*-erythritol, α- and *β-methyl*-D-glucoside, *meso*-inositol, inulin, D-melezitose, D-melibiose, D-raffinose, D-salicin, sodium butyrate, sodium succinate	+	–	–
D-Glucosamine	–	–	+
Xylitol	+	–	+
Sole nitrogen sources:			
L-Aspartic acid, L-cysteine	–	+	+
Ethanolamine, L-hydroxyproline	+	–	–
Tolerance tests:			
Growth at pH 12	–	+	–
NaCl % w/v tolerance	0-5	0-5	0-1

Key: +; positive, -; negative.
The strains were positive for alkaline phosphatase, cystine, leucine and valine arylamidases, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase and trypsin (API-ZYM tests), used L-alanine, L-arginine, L-asparagine, L-histidine, L-*iso*leucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine and L-valine as sole nitrogen sources, L-arabinose, D- and L-arabitol, glycogen, D-maltose, D-mannitol, L-rhamnose, sodium acetate, sodium fumarate, sodium malonate, sodium pyruvate, D-sorbitol and D-xylose as sole carbon sources, hydrolysed aesculin and degraded carboxymethylcellulose and Tweens 40 or 60, but were negative for acid phosphatase, α-fucosidase, α- and β-galactosidase, β-glucuronidase, N-*acetyl*-β-glucosaminidase and β-glucosidase (API-ZYM tests), nitrate reduction, allantoin and urea hydrolysis, degradation of adenine, casein, chitin, DNA, elastin, guanine, hypoxanthine, pectin, Tween 20, L-tyrosine, uric acid, xanthine and xylan, and for use of acetamide and L-glutamic acid as a sole nitrogen sources, and *p-hydroxy*-benzoic acid, D-glucuronic acid and sodium adipate as sole carbon sources.

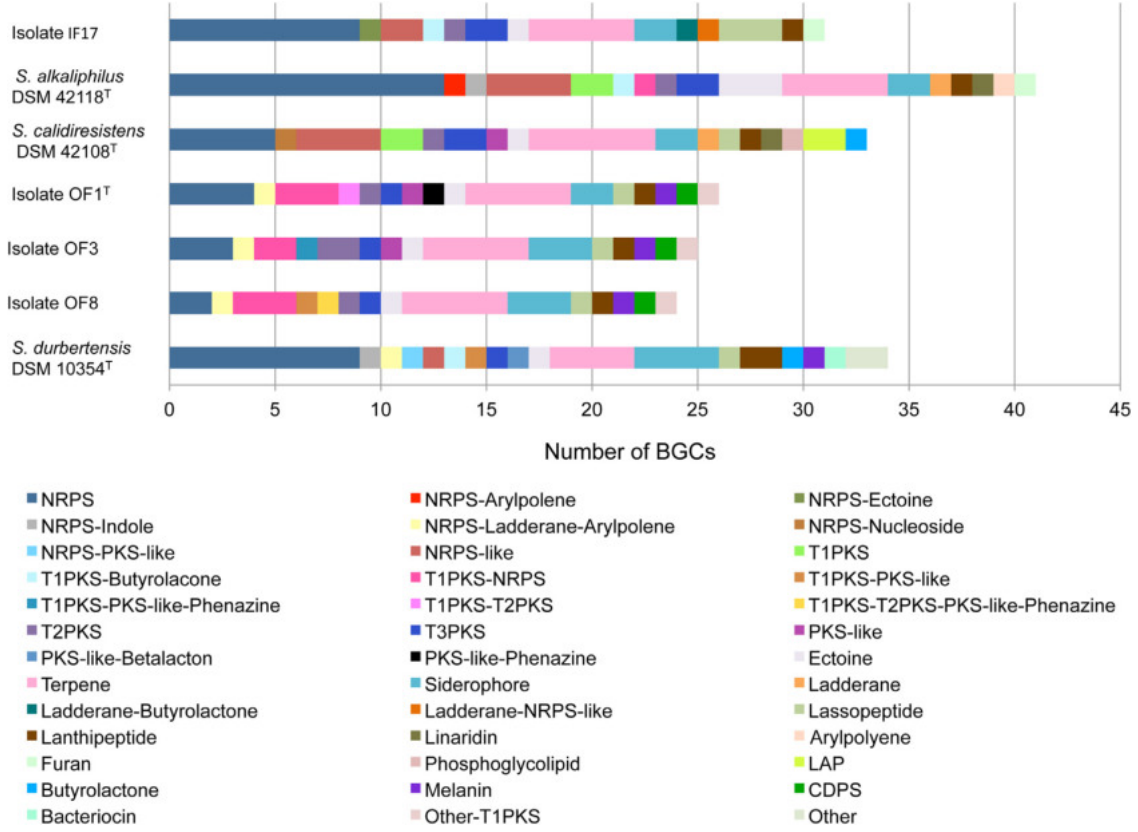

Fig. 3. Biosynthetic gene clusters found in the genomes of representative isolates and those of their closest phylogenetic neighbours using AntiSMASH 4.0 software.

Table 4
Description of *Streptomyces alkaliterrae* sp. nov.

Species name	*Streptomyces alkaliterrae*
Genus name	*Streptomyces*
Specific epithet	*alkaliterrae*
Species status	sp. nov.
Species etymology	*Streptomyces alkaliterrae* (al.ka.li.ter'rae. N.L. n. *alkali* soda ash; L. fem. n. *terra* soil, earth; N.L. gen. n. *alkaliterrae* of alkaline soil)
Description of the new taxon and diagnostic traits	Aerobic, Gram-positive, non-acid alcohol fast, alkalophilic actinobacteria which form an extensively branched substrate mycelium that carries aerial hyphae that differentiate into long straight chains of smooth, cylindrical spores (0.7 × 1.1 μm). Grows optimally around 30 °C, at pH 9.0 and in the presence of 4% NaCl (w/v). Positive for alkaline phosphatase, α-chymotrypsin, cystine and valine arylamidases, esterase (C4), esterase lipase (C8), lipase (C14), naphthol-AS-BI-phosphohydrolase and trypsin, but not for acid phosphatase, α-fucosidase, α- or β-galactosidases or β-glucuronidase (API-ZYM tests). Hydrolyses aesculin, but not allantoin, arbutin or urea. Nitrate is reduced. Degrades Tweens 40, 60 and 80, but not adenine, carboxymethylcellulose, casein, chitin, DNA, elastin, gelatin, guanine, hypoxanthine, pectin, L-tyrosine, Tween 20, uric acid, xanthine or xylan. L-arabitol, D-cellobiose, dextrin, *meso*-erythritol, D-fructose, D-galactose, α-*methyl*-D-glucoside, *meso*-inositol, D-maltose, D-melezitose, D-melibiose, D-sorbitol, D-raffinose, D-ribose, sucrose, D-trehalose, xylitol and D-xylose are used as sole carbon sources for energy and growth, but not adonitol, L-arabinose, β-*methyl*-D-glucosamide, D-glucosamine, D-glucose, glucuronic acid, glycerol, glycogen, inulin, D-lactose, D-mannitol or D-salicin (all at 1%, w/v). Acetate, citrate, fumarate, malonate, pyruvate and succinate are metabolised as sole carbon sources, but not adipate, benzoate, butyrate, hippurate, oxalate, propionate (sodium salts) or *para*-hydroxybenzoic acid (all at 0.1 %, w/v). L-asparagine, ethanolamine, L-histidine and L-proline are used as sole nitrogen sources, but not acetamide, L-aspartic acid, L-arginine, L-cysteine, L-glutamic acid, L-hydroxyproline, L-*iso*leucine, L-phenylalanine, L-serine, L-threonine or L-valine (all at 0.1%, w/v). The cell wall peptidoglycan contains *LL*-diaminopimelic acid; either hexa- or octa-hydrogenated menaquinones with nine isoprenoid units are produced. The predominant fatty acids are *anteiso*-$C_{15:0}$, *iso*-$C_{16:0}$, *anteiso*-$C_{17:0}$, the major polar lipids diphosphatidylglycerol, phosphatidylethanolamine (diagnostic marker), phosphatidylinositol and the whole cell sugars are glucose, ribose and xylose.
Country of origin	India
Region of origin	Buldhana district of Maharashtra, India
Date of isolation	1st August 2014
Source of isolation	Alkaline soil adjacent to Lonar lake
Sampling date	31st May 2014
Geographic location	Buldhana district of Maharashtra, India
Latitude	19°58′21.4″N
Longitude	76°30′17.9E
16S rRNA gene accession nr.	MH430523
Genome accession number [RefSeq]	VJYK00000000
Genome status	Draft genome sequence
Genome size	5,999,109 bp
GC mol%	72
Number of strain in study	3
Source of isolation of non-type strain	Alkaline soil adjacent to lake Lonar
Designation of the type strain	OF1^T
Strain collection numbers	NCIMB 15195^T =PCM 3001^T =MCC 3841^T =MTCC 12906^T

results provide further evidence that alkliphilic streptomycetes are a potentially rich source of drug-like molecules [63,71].

Emended description of Streptomyces alkaliphilus *Akhwale et al. 2015*

This description is based on data taken from the current study and from the earlier description of the species [1].

Grows from pH 8 to 10, between 20-40 °C and in the presence 0-5%, w/v NaCl. Positive for alkaline phosphatase, cystine and leucine arylamidases, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase, but not for acid phosphatase, α-fucosidase, α- or β-galactosidases, β-glucuronidase, N-*acetyl*-β-glucosaminidase or β-glucosidase (API-ZYM tests). Degrades carboxymethylcellulose, Tweens 40 and 60, but not adenine, casein, chitin, DNA, elastin, guanine, hypoxanthine, pectin, Tween 20, L-tyrosine, uric acid, xanthine and xylan. Aesculin is hydrolysed but not allantoin or urea. Nitrate is not reduced. Adonitol, L-arabinose, D- and L-arabitol, D-cellobiose, dextrin, D-fructose, D-galactose, D-glucose, glycerol, glycogen, D-lactose, D-maltose, D-mannitol, D-ribose, L-rhamnose, D-sorbitol, sucrose, D-xylose, and acetate, benzoate, citrate, fumarate, hippurate, malonate, oxalate, propionate and pyruvate (sodium salts) are used as sole carbon sources, but not D-glucosamine, D-glucuronic acid, *meso*-inositol, *p-hydroxy*-benzoic acid or sodium adipate. L-alanine, L-arginine, L-asparagine, L-histidine, L-*iso*leucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine and L-valine are used as sole nitrogen sources, but not acetamide and glutamic acid.

The dG + C content of the genome of the type-strain is 72.5%, its approximate size 5.76 Mbp and its GenBank accession number is VKHT00000000.

Emended description of Streptomyces calidiresistens *Duan et al. 2014*

This description is based on data taken from the current study and from the earlier description of the species [25].

Grows from pH 8 to 10, between 20-40 °C and in the presence 0-1%, w/v NaCl. Positive for alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase, but negative for acid phosphatase, α-fucosidase, α- and β-galactosidases, β-glucuronidase, N-*acetyl*-β-glucosaminidase and β-glucosidase (API-ZYM tests). Degrades carboxymethylcellulose, Tweens 40, 60 and 80, but not adenine, casein, chitin,

DNA, elastin, guanine, hypoxanthine, pectin, starch, Tween 20, L-tyrosine, uric acid, xanthine or xylan. Aesculin and arbutin are hydrolysed, but not allantoin or urea. Nitrate is not reduced. L-arabinose, D- and L-arabitol, D-glucosamine, glycogen, D-maltose, D-mannitol, L-rhamnose, D-sorbitol, xylitol, D-xylose, and acetate, fumarate, malonate and pyruvate (sodium salts) are used as sole carbon sources, but not adonitol, amygdalin, D-cellobiose, dextrin, *meso*-erythritol, D-fructose, D-galactose, D-glucose, α- or β-*methyl*-D-glucoside, D-glucuronic acid, glycerol, *meso*-inositol, inulin, D-lactose, D-melezitose, D-melibiose, D-raffinose, D-ribose, D-salicin, sucrose, *p-hydroxy*-benzoic acid, adipate, benzoate, butyrate, citrate, hippurate, oxalate, propionate or succinate (sodium salts). L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-histidine, L-*iso*leucine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine and L-valine as sole nitrogen sources, but not acetamide, ethanolamine, glutamic acid or L-hydroxyproline.

The dG + C of the genome of the type strain is 73%, its approximate size 6.19 Mbp and its GenBank accession number is VKHS00000000.

Emended description of Streptomyces durbertensis *Yu et al. 2018*

The description is based on data taken from this study and the earlier description of the species [86].

Grows from pH 6 to12, between 15-42 °C and in the presence 0-7%, w/v NaCl. Positive for alkaline phosphatase, α-chymotripsin, cystine, leucine and valine arylamidases, naphthol-AS-BI-phosphohydrolase and trypsin, but negative for acid phosphatase, esterase (C4), esterase lipase (C8), α-fucosidase, α- and β-galactosidases, N-*acetyl*-β-glucosaminidase, β-glucosidase, β-glucuronidase, lipase (C14) or α-mannosidase (API-ZYM tests). Degrades starch and Tweens 40 and 60, but not adenine, carboxymethylcellulose, casein, chitin, DNA, elastin, guanine, hypoxanthine, pectin, Tweens 20 and 80, L-tyrosine, uric acid, xanthine or xylan. Aesculin is hydrolysed, but not allantion, arbutin or urea. Nitrate is not reduced. Adonitol, amygdalin, L-arabinose, D- and L-arabitol, D-cellobiose, dextrin, *meso*-erythritol, D-fructose, D-galactose, D-glucose, α- and β-*methyl*-D-glucoside, D-glucosamine, glycerol, glycogen, *meso*-inositol, D-lactose, D-maltose, D-mannitol, D-melezitose, D-melibiose, D-ribose, sorbitol, D-sucrose, D-trehalose, xylitol, D-xylose, *p-hydroxy*-benzoic acid, acetate, adipate, citrate, benzoate, butyrate, fumarate, hippurate, malonate, propionate, pyruvate and succinate (sodium salts), but not D-glucuronic acid, inulin, L-rhamnose, D-salicin or sodium oxalate. L-Alanine, L-arginine, L-asparagine, ethanolamine, L-histidine L-hydroxyproline, L-*iso*leucine, L-methionine, L-phenylalanine, L-proline L-serine, L-treonine and L-valine are used as a sole nitrogen sources, but not acetamide, L-aspartic acid, L-cysteine or L-glutamic acid.

The dG + C content of the genome of the type strain is 72.4%, its approximate size 5.92 Mbp and its GenBank accession number is WMLF00000000.

Uncited references

[61].

Funding

This work was supported by Grants No. 2017/01/X/NZ8/00140 and 2016/23/N/NZ9/00247 from the National Science Centre (NCN) of Poland and by Grant No. 1207-B from Nicolaus Copernicus University.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

Ethical statement

This article does not contain any studies with human participants or animals.

Acknowledgements

We are grateful to Aharon Oren (The Hebrew University of Jerusalem) for checking the new species epithet and for helpful discussions with Martha Trujillo (Salamanca University). IN is grateful for a postdoctoral scholarship from Newcastle University.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.syapm.2020.126153.

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Supplementary material

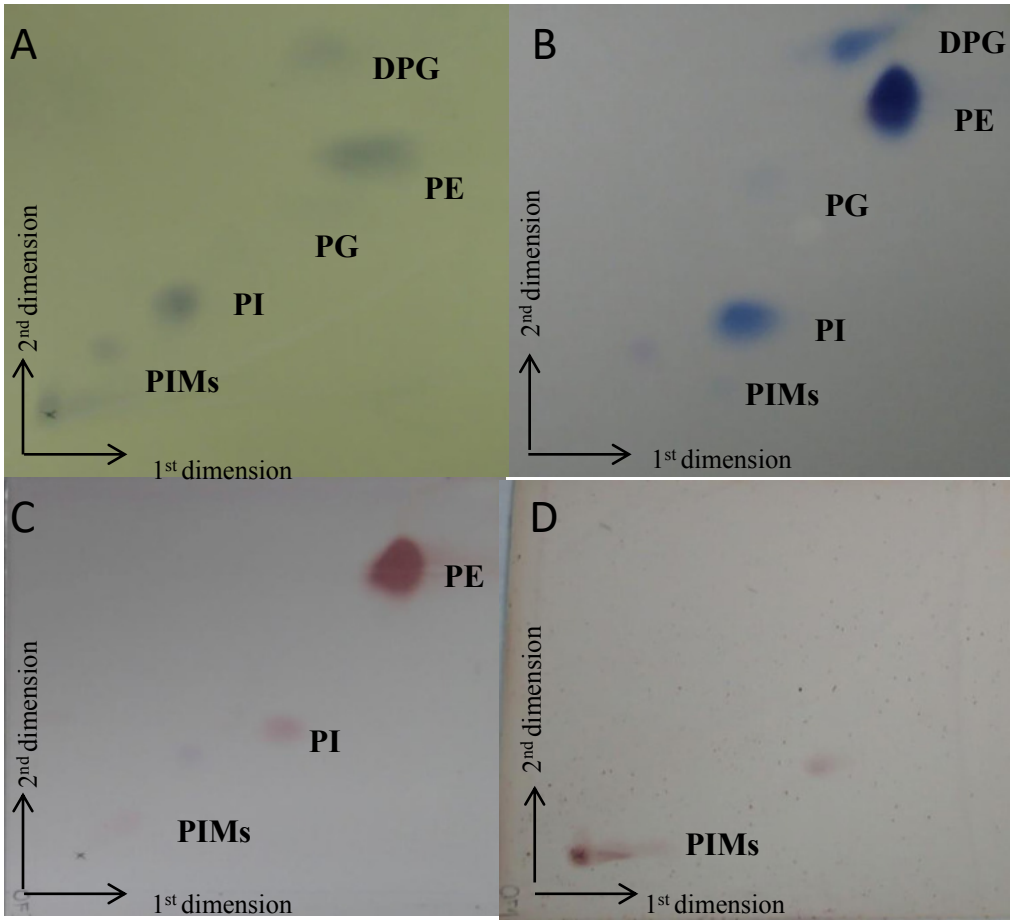

Fig. S1. Two dimensional thin-layer chromatography of polar lipids of isolate OF1^T stained with molybdophosphoric acid (A), molybdenum blue (Sigma) (B), ninhidrin (C) and α-naphtosulfuric acid (D) for the detection of total polar lipids. Chloroform:methanol:water (32.5:12.5:2.0 v/v) was used in the first direction and chloroform:acetic acid:methanol: water (40:7.5:6:2 v/v) in the second direction. DPG diphosphatidylglycerol, PE phosphatidylethanolamine, PG phosphatidylglycerol, PI phosphatidylinositol, PIMs phosphatidylinositol mannosides.

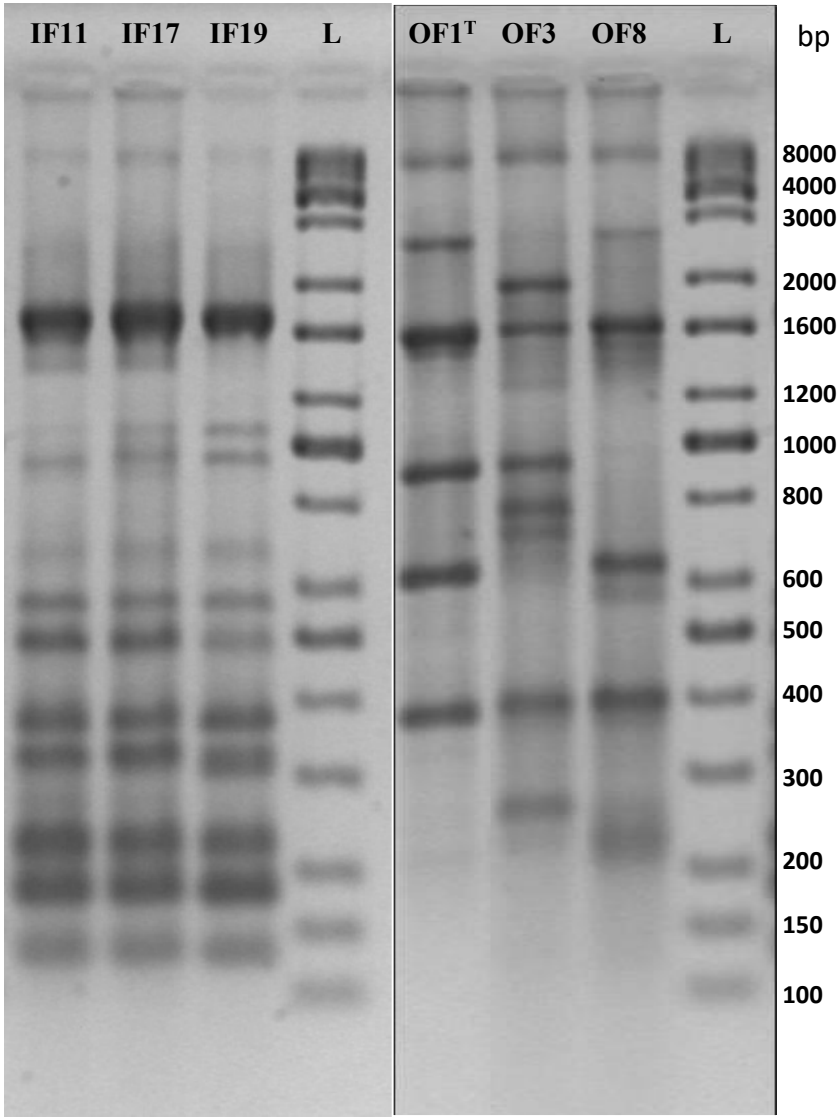

Fig. S2. BOX PCR fingerprint patterns of genomic DNA extracted from the isolates.

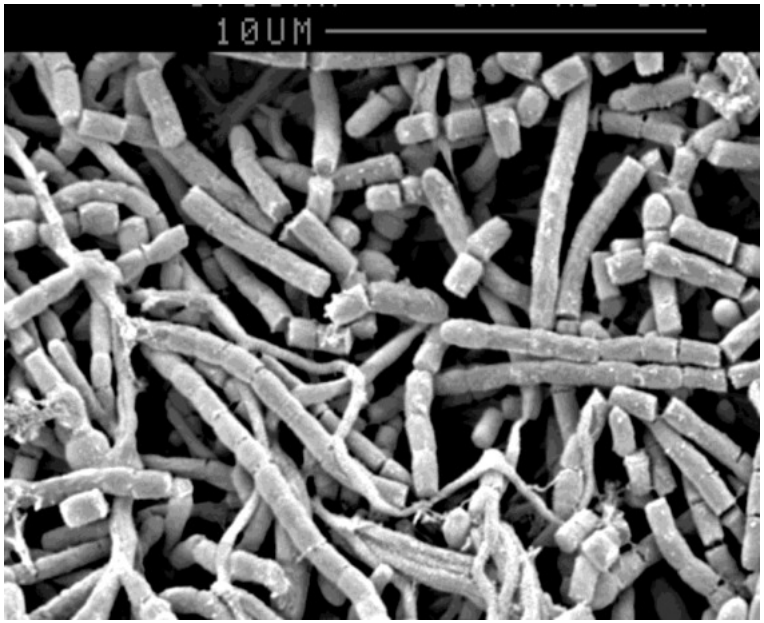

Fig. S3. Scanning electron micrograph of isolate OF1^{T} showing straight chains of smooth surfaced, cylindrical spores following growth on halophilic nutrient agar for 2 weeks at 28 ^{o}C. Bar, 10 µm

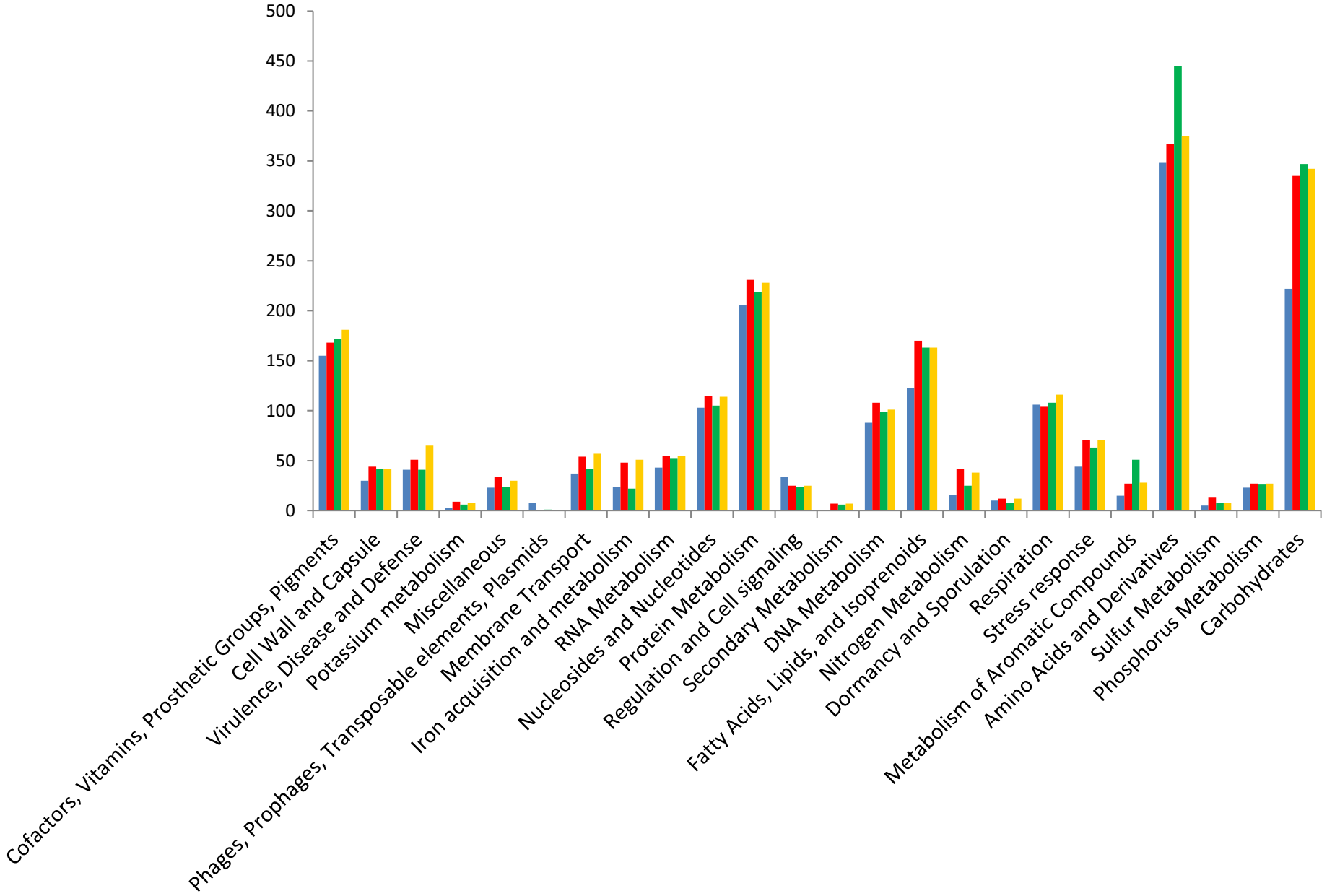

Fig. S4. Distribution of functional gene classes found in the genome of isolate OF1^{T} (blue), *Streptomyces coelicolor* A2(3) (red), *Streptomyces leuwenhoekii* DSM 42122^{T} (green) and *Streptomyces lividans* 66 (yellow) generated using the RAST-SEED webserver at https://rast.nmpdr.org/;

Table S1. Growth and cultural properties of isolates grown on nutrient media for 3 weeks at 28 °C.

Media	Isolates											
	IF11				IF17				IF19			
	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
ISP1	+++	Light gray	Dark reddish brown	Deep brown	+++	Light gray	Dark reddish brown	Deep brown	+++	Light gray	Dark reddish brown	Deep brown
ISP2	++	White	Light orange yellow	None	++	White	Light orange yellow	None	++	White	Orange yellow	None
ISP3	++	Light gray	Moderate olive	None	++	Light gray	Moderate olive	None	++	Light gray	Moderate olive	None
ISP4	+++	Light gray	Dark brown	Moderate yellowish brown	+++	Light gray	Dark brown	Moderate yellowish brown	+++	Light gray	Dark brown	Moderate yellowish brown
ISP5	+++	Light gray	Grayish reddish brown	None	+++	Light gray	Grayish reddish brown	None	+++	Light gray	Grayish reddish brown	None
ISP6	+++	Pale purple	Dark reddish brown	None	+++	Pale purple	Dark reddish brown	None	+++	Pale purple	Dark reddish brown	None
ISP7	+++	Light gray	Deep reddish brown	None	+++	Light gray	Deep reddish brown	None	+++	Light gray	Deep reddish brown	None
HNA	+++	Yellowish white	Dark orange yellow	None	+++	Yellowish white	Dark orange yellow	None	+++	Greenish white	Dark orange yellow	None
MBA	+++	Light gray	Dark brown	None	+++	Light gray	Dark brown	None	+++	Light gray	Dark brown	None

Media	Isolates											
	OF1^T				OF3				OF8			
	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigments
ISP1	+++	White	Moderate yellow	None	+++	White	Moderate yellow	Moderate yellow	+++	White	Moderate yellow	Moderate yellow
ISP2	+++	White	Strong brown	None	+++	White	Strong brown	None	+++	White	Strong brown	None
ISP3	+++	None	Moderate yellow	Pale purple	+++	None	Moderate yellow	Pale purple	+++	None	Moderate yellow	Pale purple
ISP4	+++	White	Light grayish yellowish brown	None	+++	White	Strong yellow	None	+++	White	Light grayish yellowish brown	None
ISP5	+++	Light gray	Light brownish gray	None	+++	None	Strong reddish orange	None	+++	None	Strong reddish orange	None
ISP6	+++	Light gray	Grayish brown	Grayish yellowish brown	+++	None	Grayish brown	Grayish yellowish brown	+++	Light gray	Grayish brown	Grayish yellowish brown
ISP7	++	White	Grayish yellow		++	None	Pale yellow		++	Light gray	Light grayish brown	None
HNA	+++	White	Light greenish yellow	None	+++	White	Light greenish yellow	None	+++	White	Light greenish yellow	None
MBA	++	None	Strong reddish orange	None	++	None	Strong reddish orange	None	++	None	Strong reddish orange	None

Key: ISP1; tryptone-yeast extract agar, ISP2; yeast extract-malt extract agar, ISP3; oatmeal agar, ISP4; inorganic salts-starch agar, ISP5; glucose-asparagine agar, ISP6; peptone-yeast extract-iron, ISP7; tyrosine agar, HNA; halophilic nutrient agar, MBA; modified Bennett's agar, +++; abundant growth, ++; good growth.

Table S2. Cellular fatty acid profiles of the representative isolates.

Fatty acids	**OF1^T**	**IF17**
$C_{14:0}$	0.4	0.1
$C_{15:0}$	2.6	0.4
$C_{16:0}$	4.2	0.8
$C_{17:0}$	0.7	-
$C_{15:1}$ ω6c	0.3	-
$C_{15:1}$ B	-	0.7
$C_{16:1}$ cis 9	-	1.6
$C_{17:1}$ ω6c	0.2	-
$C_{17:1}$ ω8c	0.7	-
$C_{16:0}$ 9-*methyl*	-	0.2
$C_{17:0}$ 10-*methyl*	0.5	-
anteiso-$C_{13:0}$	0.1	-
anteiso- $C_{15:0}$	22.1	38.2
anteiso- $C_{17:0}$	18.1	5.7
anteiso- $C_{17:1}$ C	-	0.2
anteiso-$C_{17:1}$ ω9c	4.4	-
iso-$C_{13:0}$	0.1	0.2
iso-$C_{14:0}$	1.7	7.4
iso-$C_{15:0}$	7.2	18.9
iso-$C_{16:0}$	23.1	20.6
iso-$C_{16:1}$ H	2.6	2.8
iso-$C_{17:0}$	4.9	2.1
iso-$C_{17:1}$ ω9c	3.2	-
iso-$C_{18:0}$	0.4	-
iso-$C_{18:1}$ H	0.6	-
Sum in feature 3	2.0	-

Summed feature 3 comprises *iso*-$C_{15:0}$ 2OH and $C_{16:1}$ ω7c.

Table S3. General features of the genomes of the representative isolates and their closest phylogenetic neighbours

Properties	**Isolate IF17**	***S. alkaliphilus* DSM 42118^T**	***S. calidiresistens* DSM 42108^T**	**Isolate OF1^T**	**Isolate OF3**	**Isolate OF8**	***S. durbertensis* DSM 104538^T**
Size	5870448	5761373	6196881	5999109	5977613	6036367	5923645
Digital DNA G+C content (%)	72.4	72.5	73.0	72.0	72.0	72.0	72.4
Completeness (%)	94.98	94.80	95.81	97.24	97.18	97.10	97.18
Contamination (%)	3.80	0.27	1.96	2.43	1.84	0.89	1.42
Contig number	1861	1801	1643	769	748	473	1002
Longest contig (bp)	48487	37953	60872	62299	65191	103400	39349
Mean contig length (bp)	3154.46	3198.98	3771.69	7801.20	7991.46	12761.87	5911.82
L50	308	328	280	120	111	67	165
N50	5488	5021	6206	15903	15600	26709	10866
Mean coverage	249.0	70.2	121.7	170.0	342.0	229.0	30.0
Number of ambigious bases	2	0	0	0	0	0	0
Number of reads	4478765	1021815	2270261	2349987	4148352	2850634	406599
Coding density	0.85	0.86	0.86	0.88	0.88	0.88	0.88
Genes (total)	5087	5184	5572	5616	5683	5559	5612
CDSs (total)	5019	5116	5502	5539	5601	5472	5528
CDSs (with protein)	4762	4940	5271	5334	5463	5342	5395
CDSs (without protein)	257	176	231	205	138	130	133
RNA genes	68	68	70	77	82	87	84
rRNAs (5S, 16S, 23S)	5, 1, 3	4, 1, 3	5, 1, 3	6, 1, 6	6, 3, 9	6, 3, 9	7, 1, 12
Complete rRNAs (5S, 16S, 23S)	5, 1, 1	4, 1, 1	5, 1, 1	6, 1, 1	4, 1, 0	4, 1, 0	4, 1, 0
Partial rRNAs (5S, 16S, 23S)	0, 0, 2	0, 0, 2	0, 0, 2	0, 0, 5	2, 2, 9	2, 2, 9	3, 0, 12
tRNAs	56	57	58	61	61	66	61
GenBank accession number	VJYJ01000000	VKHT00000000	VKHS00000000	VJYK00000000	JABJWZ000000000	JABJXA000000000	WMLF00000000

Table S4. Phenotypic properties that distinguish between each set of isolates.

	Isolates		
Characteristics	**IF11**	**IF17**	**IF19**
API-ZYM tests:			
α-Chymotrypsin	-	+	+
α-Glucosidase	-	-	+
Lipase (C14)	-	-	+
α-Mannosidase	-	-	+
Growth on sole carbon source (1%, w/v):			
D-Trehalose	-	+	-
Tolerance tests:			
pH growth range	8-10	8-10	8-11

	Isolates		
Characteristics	**OF1^T**	**OF3**	**OF8**
API-ZYM tests:			
N-*acetyl*-β-Glucosaminidase	+	-	-
α-Glucosidase	-	+	+
β-Glucosidase	+	+	-
α-Mannosidase	+	-	-
Growth on sole carbon sources (at 1%, w/v):			
Amygdalin	+	+	-
D-Arabitol	+	-	-
L-Rhamnose	+	-	-
Growth on sole nitrogen sources (at 0.1%, w/v):			
L-Alanine	-	+	-
L-Methionine	-	+	-
Tolerance tests:			
pH growth range	6-12	6-11	6-11
Temperature growth range (°C)	15-45	15-45	15-50
NaCl tolerance (%, w/v)	11	8	10

Key: +, positive; -, negative.

Table S5. Predicted biosynthetic gene clusters

No.	Type	Most similar known cluster	Biosynthetic classes	Number of strains	Isolate IF17	*S. alkaliphilus* DSM 42118^{T}	*S. calidiresistens* DSM 42108^{T}	Isolate OF1^{T}	Isolate OF3	Isolate OF8	*S. durbertensis* DSM 104538^{T}
1	arylpolyene	WS9326	NRP	1		P (17)					
2	bacteriocin			1							U
3	butyrolactone			1			U				
4	butyrolactone	griseoviridin/ fijimycin A	NRP: Cyclic depsipeptide + Polyketide: Trans-AT type I	1							P (11)
5	CDPS			2					U	U	
6	CDPS	lobosamide A/B/C	Polyketide	1				P (4)			
7	ectoine	ectoine	Other	7	P (50)	P (50)	P (100)	P (75)	P (75)	P (100)	P (100)
8	ectoine	kosinostatin	NRP + Polyketide	1		P (13)					
9	ectoine	showdomycin	Other	1		P (17)					
10	furan			1		U					
11	furan	colabomycin E	Poliketype: Type II	1	P (11)						
12	ladderane	WS9326	NRP	2		P (7)	P (7)				
13	ladderane/butyrolactone	colabomycin E	Polyketide: Type II	1	P (18)						
14	ladderane/NRPS-like	WS9326	NRP	1	P (27)						
15	lanthipeptide			4	U	U	U				U
16	lanthipeptide	AmfS	RiPP: Lanthipeptide	1							P (100)
17	lanthipeptide	SBI-06990 A1 / SBI-06989 A2	RiPP: Lanthipeptide	3				P (50)	P (50)	P (50)	
18	LAP	chromomycin A3	Poliketyde: Type II + Saccharide:Oligosaccharide	1			P (11)				
19	LAP	granaticin	Poliketyde: Type II	1			P (5)				
20	lassopeptide			2	3U		U				
21	lassopeptide	citrulassin D	RiPP	4				P (40)	P (40)	P (40)	P (100)
22	linaridin	pentostatine/vidarabine	Other	2		P (6)	P (6)				
23	melanin			1							U
24	melanin	melanin	Other	3				P (28)	P (28)	P (28)	
25	NRPS			6	3U	4U	2U	U	U		6U
26	NRPS	A54145	NRP	1	P (3)						
27	NRPS	clifednamide A	NRP + Polyketide	4				P (30)	P (30)	P (30)	P (30)
28	NRPS	griseoviridin/ fijimycin A	NRP: Cyclic depsipeptide + Polyketide: Trans-AT type I	1							P (16)
29	NRPS	icosalide A/B	NRP: Lipopeptide	1		P (100)					
30	NRPS	maduropeptin	Polyketide: Iterative type I + Polyketide:Enediyne type I	2	P (13)	P (13)					
31	NRPS	mirubactin	NRP	3	P (50)	P (50)	P (35)				
32	NRPS	ML-449	Polyketide: Modular type I	1		P (8)					
33	NRPS	naphthyridinomycin	NRP	1		P (50)					
34	NRPS	sibiromycin	NRP	2	P (7)		P (11)				
35	NRPS	thanamycin	NRP: Beta-lactam	1	P (18)						
36	NRPS	thiazostatin/watasemycin A/B /2-hydroxyphenylthiazoline enantiopyochelin/isopyochelin	NRP	2		P (26)		P (13)			
37	NRPS	thiocoraline	NRP: Cyclic depsipeptide	3				P (15)	P (15)	P (15)	
38	NRPS	virginiamycin S1	NRP + Polyketide	1							P (33)
39	NRPS	S56-p1	NRP	1		P (13)					
40	NRPS	WS9326	NRP	3	P (25)	P (15) P (10)	P (20)				
41	NRPS/arylpolyene	WS9327	NRP	1		P (17)					
42	NRPS/ectoine	kosinostatin	NRP + Polyketide	1	P (13)						
43	NRPS/indole	S56-p1	NRP	1		P (27)					
44	NRPS/indole	teleocidin B1	NRP + Terpene	1							P (100)
45	NRPS/ladderane/arylpolyene	atratumycin	NRP	4				P (42)	P (39)	P (39)	P (28)
46	NRPS-like			3	U	U	U				
47	NRPS-like	(2S, 6R)-diamino-(5R,7)-dihydroxy-heptanoic acid	NRP	1			P (6)				
48	NRPS-like	nocuolin A	Other	1			P (9)				
49	NRPS-like	rhizomide A/B/C	NRP	2		P (100)					P (100)
50	NRPS-like	s56-p1	NRP	1		P (5)					
51	NRPS-like	sibiromycin	NRP	1	P (7)						
52	NRPS-like	WS9326	NRP	2		P (20)	P (20)				
53	NRPS/Nucleoside	pseudouridimycin	Other: Nucleoside	1			P (18)				
54	NRPS/PKS-like	virginiamycin S1	NRP + Polyketide	1							P (55)
55	NRPS/T1PKS	crochelin A	NRP + Polyketide	2				P (7)		P (7)	
56	NRPS/T1PKS	pyridomycin	NRP + Polyketide	3				P (7)	P (7)	P (7)	
57	NRPS/T1PKS	thiolactomycin	Po+C40lyketide	3				P (90)	P (90)	P (90)	

58	other	α,β-epoxyketone	NRP + Polyketide	1								P (15)
59	other	undecylpirogrosin	NRP + Polyketide	1								P (13)
60	T1PKS/other	α,β-epoxyketone	NRP + Polyketide	2				P (15)	P (15)			
61	phosphoglycolipid	teichomycin	Other	1			P (55)					
62	PKS-like	caboxamycin	NRP + Polyketide	1			P (80)					
63	PKS-like	cadaside A/B	NRP	2				P (9)	P (9)			
64	PKS-like/betalactone	LL-D49194α1 (LLD)	Polyketide	1								P (33)
65	PKS-like/phenazine	streptophenazine B/C/F/G/H	NRP + Polyketide	1				P (17)				
66	PKS-like/T1PKS	quartromicin A1	Polyketide	2						P (11)		P (13)
67	siderophore			7	U	U	2U	U	3U	2U		4U
68	siderophore	ficellomycin	NRP	3	P (3)	P (3)		P (3)				
69	siderophore	macrotetrolide	Polyketide	1						P (33)		
70	T1PKS			2		U	2U					
71	T1PKS	piericidin A1	Polyketide: Modular type I	1		P (33)						
72	T1PKS/butyrolactone	lactonamycin	Polyketide	1								P (3)
73	T1PKS/butyrolactone	neocarzinostatin	Polyketide: Iterative type I + Polyketide:Enediyne type I	2	P (21)	P (21)						
74	T1PKS/NRPS			1		U						
75	T1PKS/other	tetronasin	Polyketide	1						P (3)		
76	T1PKS/PKS-like/phenazine	streptophenazine B/C/F/G/H	NRP + Polyketide	1					P (89)			
77	T1PKS/T2PKS	streptophenazine B/C/F/G/H	NRP + Polyketide	1				P (75)				
78	T2PKS	A-74528	Polyketide	3	P (17)	P (34)	P (34)					
79	T2PKS	arixanthomycin A/B/C	Polyketide: Type II + Saccharide: Hybrid/tailoring	3				P (14)	P (14)	P (14)		
80	T2PKS	mayamycin	Polyketide	1					P (50)			
81	T2PKS/T1PKS/PKS-like/phenazine	streptophenazine B/C/F/G/H	NRP + Polyketide	1						P (92)		
82	T3PKS	alkyl-O-dihydrogeranyl-methoxyhydroquinones	Terpene + Polyketide	2	P (28)	P (28)						
83	T3PKS	herboxidiene	Poliketyde	3	P (2)	P (2)	P (2)					
84	T3PKS	lasalocid	Poliketyde	1			P (3)					
85	T3PKS	naringenin	Terpene	4				P (100)	P (100)	P (100)		P (100)
86	terpene			7	3U	U	U	4U	4U	3U		3U
87	terpene	BD-12	NRP	2		P (10)	P (7)					
88	terpene	carotenoid	Terpene	1		P (18)						
89	terpene	geosmin	Terpene	7	P (100)	P (100)	P (100)	P (100)	P (100)	P (100)		P (100)
90	terpene	hopene	Terpene	1			P (15)					
91	terpene	isorenieratene	Terpene	3	P (37)	P (37)	P (37)					
92	terpene	paulomycin	Other	1						P (3)		
93	terpene	phosphonoglycans	Saccharide	1			P (6)					
Total:					31	41	33	26	25	24		34

Key:
1."P"; indicates presence of predicted bioclusters.
2. Percentage of genes in common between bioclusters of the tested strains to the closest known BGCs showing significant BLAST hits [13]
3. "U" uncharacterised clusters.

Systematic and Applied Microbiology 44 (2021) 126164

Contents lists available at ScienceDirect

Systematic and Applied Microbiology

journal homepage: http://www.elsevier.com/locate/syapm

Genomic-based classification of *Catenulispora pinisilvae* sp. nov., novel actinobacteria isolated from a pine forest soil in Poland and emended description of *Catenulispora rubra*

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ARTICLE INFO

Article history:
Received 19 September 2020
Received in revised form 7 November 2020
Accepted 12 November 2020

Keywords:
Acidotolerant actinobacteria
Catenulispora pinisilvae
Genomes
Polyphasic taxonomy
Phylogeny
Anti-microbial activity

ABSTRACT

Two actinobacteria, strains NF3 and NH11^T, isolated from a pine forest soil, near Torun, Poland were examined for diverse chemotaxonomic and morphological properties that placed them in the genus *Catenulispora*. They produced an extensively branched stable mycelium, contained *LL*-diaminopimelic acid as the diamino acid of the peptidoglycan, arabinose as the diagnostic whole-organism sugar, tetra-, hexa- and octa-hydrogenated menaquinones with nine isoprenoid units as the predominant isoprenologues, *iso*-$C_{16:0}$ and *anteiso*-$C_{17:0}$ as major fatty acids, and formed a well supported clade within the *Catenulispora* 16S rRNA gene tree together with *Catenulispora acidiphila* DSM 44928^T and *Catenulispora rubra* DSM 44948^T sharing sequence similarities with the latter of 98.8 and 99.0%, respectively. The sizes of whole genome sequences generated for the isolates and the *C. rubra* strain ranged from 11.20 to 12.80 Mbp with corresponding *in silico* DNA G + C values of 69.9–70.0%. The isolates and the *C. acidiphila* and *C. rubra* strains formed a well supported branch in the actinobacterial phylogenomic tree. Isolates NF3 and NH11^T belong to the same species as they have identical 16S rRNA gene sequences, share many chemotaxonomic, cultural and phenotypic features and show very high average nucleotide identity (ANI) and digital DNA:DNA relatedness (dDDH) similarities. They can be distinguished from their closest phylogenomic neighbours by using a combination of chemotaxonomic and phenotypic properties and by ANI and dDDH values well below the thresholds of these metrics used to assign closely related strains to different species. Consequently, we propose that the isolates be classified as a new *Catenulispora* species, *Catenulispora pinisilvae* sp. nov., the type strain is NH11^T (=DSM 111109^T =PCM 3046^T). An emended description is given for *C. rubra* based on data acquired in the present study. Analyses of the draft genomes of the isolates and the *C. acidiphila* and *C. rubra* strains revealed the presence of many biosynthetic gene clusters with the potential to synthesize novel drug-like metabolites. *In vitro* screens showed that the isolates inhibited the growth of Gram-positive bacteria and wheat pathogens belonging to the genus *Fusarium*.

Introduction

Novel neutrophilic sporoactinomycetes, notably streptomycetes, from extreme habitats which have large genomes *sensu* Baltz [4] are a prolific source of chemically unique natural products which show pronounced anti-microbial and anti-tumour activity [9,69,76]. In contrast, their acidotolerant (pH range 4.5–7.5, optimal pH 5.5) and acidophilic (pH range 3.5–6.5, optimal pH 4.5) counterparts [52,95] have rarely been included in bioprospecting campaigns even though they are common in coniferous soils [29,37,95] and are a prospective source of antifungal compounds [98] and acid stable enzymes [96,99]. The discovery that these and other acid-loving sporoactinomycetes have large genomes, as

Abbreviations: A2pm, diaminopimelic acid; ANI, average nucleotide identity; BGCs, biosynthetic gene clusters; BLAST, Basic Local Alignment Search Tool; dDDH, digital DNA–DNA hybridization; DNA, deoxyribonucleic acid; GGDC, genome to genome distance calculator; ISP, International Streptomyces Project; MIDI, Microbial Identification System; MK, menaquinones; ML, maximum likelihood; MP, maximum parsimony; MRE, maximal-relative-error; RAST, Rapid Annotation using Subsystem Technology; rRNA, ribosomal RNA; C, *Catenulispora*; TNT, Tree Analysis New Technology.

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https://doi.org/10.1016/j.syapm.2020.126164
0723-2020/

exemplified by *Actinospica* [17], *Catenulispora* [63], *Streptacidiphilus* [1,42] and *Streptomyces* [63] strains, underline their potential value as a source of specialised metabolites.

The genus *Catenulispora*, which belongs to the family *Catenulisporaceae* [10] of the order *Catenulisporales* [15], encompasses six validly published species, namely *Catenulispora acidiphila* [10,63], the type species, *Catenulispora fulva* [46], *Catenulispora graminis* [47], *Catenulispora rubra* [86], *Catenulispora subtropica* [85] and *Catenulispora yoronensis* [85]. Representatives of four of these taxa were isolated from acid forest soils; the exceptions, the type strains of *C. graminis* and *C. subtropica* were recovered from bamboo rhizosphere and paddy-field soil, respectively [47,85]. Catenulisporae are aerobic, Gram-positive, non-acid-fast, acidotolerant to acidophilic actinobacteria which form an extensively branched substrate mycelium, aerial hyphae that differentiate into straight to flexuous chains of non-motile spores; the diagnostic diamino acid is *LL*-diaminopimelic acid, the major fatty acids *iso*-$C_{16:0}$ and *anteiso*-$C_{17:0}$, the predominant respiratory quinones tetra-, hexa- and octa-hydrogenated menaquinones with nine isoprene units, polar lipid profiles contain diphospatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides, the major whole-cell sugar is arabinose and the DNA G+C content ranges from 69 to 72 mol% [15,46]. Genome mining of *C. acidiphila* DSM 44928^T led to the detection and biosynthesis of catenulipeptin, a class III lantipeptide [92] and two novel aminocoumarins, cacibiocins A and B, which, unlike known aminocoumarin-antibiotics do not act as gyrase inhibitors or exhibited antibacterial activity [103].

The present study, a continuation of our previous work on the diversity of cultivable sporoactinobacteria from coniferous soils [23–27], was designed to establish the taxonomic status and potential biotechnological value of two presumptive acidotolerant *Catenulispora* strains isolated from a pine forest soil. The isolates, strains NF3 and NH11^T, were compared with closely related type strains of the genus *Catenulispora* using genomic, genotypic and phenotypic procedures. The isolates were found to belong to a novel species of *Catenulispora*; the name proposed for this taxon is *Catenulispora pinisilvae* with isolate NH11^T as the type strain.

Materials and methods

Isolation of strains

Isolates NF3 and NH11^T were recovered from a sample of *Pinus sylvestris* L. forest soil collected from the northern slope of an inland sand dune system located near Toruń, Poland (52°55′ 37″N, 18°42′11″E) in October 2013. Strain NF3 was isolated from partially decomposed pine needles (F horizon) and isolate NH11^T from decomposed needles (H horizon) using a standard dilution plating technique and acidified starch-casein agar (pH 4.5), as described previously [24]; details of the sampling site, including the physicochemical properties of the F and H layers, were also described by these authors.

Maintenance and cultural conditions

The isolates, *C. acidiphila* DSM 44928^T and *C. rubra* DSM 44948^T were maintained on starch-casein agar [44] slopes (pH 5.5) at room temperature and as suspensions of mycelial fragments in 20% (v/v) glycerol at −80 °C. The type strains were purchased from the Leibniz Institute, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany. Biomass for the molecular systematic and most of the chemotaxonomic studies was grown in shake flasks (150 revolutions per minute) of yeast extract-malt extract broth (ISP2; International Streptomyces Project medium 2) [78] at pH 5.5 for three weeks at 28 °C, harvested by centrifugation (6000 x *g* for 10 min) and washed three times with sterile distilled water. Biomass for the chemotaxonomic analyses was freeze dried and that for the molecular systematic studies stored at −20 °C. Biomass for the fatty acid analyses was scrapped from ISP2 agar plates (pH 5.5) following incubation for 14 days at 28 °C. The pH of all media were adjusted with 1 N HCl.

Phylogenetic analyses

Genomic DNA was extracted from isolates NF3 and NH11^T using a GenElute^{TM} Bacterial Genomic Kit (Sigma-Aldrich, Germany) and 16S rRNA genes amplified by PCR following procedures described by Golinska et al. [23,26]. The PCR products were purified using a purification kit (Qiagen, Germany), their quality checked using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and the purified PCR products sequenced at the Institute of Biochemistry and Biophysics (IBB) of the Polish Academy of Sciences, Warsaw, Poland, using an ABI 3730xl Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, USA). The complete 16S rRNA gene sequences of the isolates were compared with corresponding sequences of the type strains of *Catenulispora* species using the EzBiocloud server [102], as well as with the type strains of *Actinospica* and *Actinocrinis* species which are known to be phylogenetically close to the genus *Catenulispora* [10,11,38,46,85,86]. Maximum likelihood (ML) and maximum parsimony (MP) phylogenetic trees based on the 16S rRNA gene sequences were inferred using the Genome-to-Genome Distance Calculator (GGDC) web server [54] adapted for single genes; the web server (http://ggdc.dsmz.de) was also used to calculate pairwise sequence similarities [56,57] Multiple sequence alignments were generated using MUSCLE software [16] and a ML tree inferred from alignments with RAxML [80] using rapid bootstrapping together with the auto maximal-relative-error (MRE) bootstrapping criterion [66]. Similarly, a MP tree was generated from alignments with the Tree Analysis New Technology (TNT) program [28] using 1000 bootstraps together with tree bisection and reconnection branch swapping and ten random sequence replicates. The sequences were checked for compositional bias using the X^2 test as implemented in PAUP* [84]. In addition, the MEGA7 software package [43] and the neighbour joining algorithm [72] were used to generate a phylogenetic tree and evolutionary distances calculated using the Kimura-2-parameter model [39] with 1000 bootstrap repetitions [21]. The root position of the trees were determined using a 16S rRNA gene sequence taken from the genome of *Streptomyces thermoautotrophicus* UBT1^T (GenBank accession number: JYIK01000000) using the SEED viewer [3].

BOX typing

BOX-PCR fingerprinting profiles were generated from genomic DNA extracted from the isolates using the BOXA1R primer [91] and previously described experimental conditions [88].

Whole genome sequencing and genome analyses

Genomic DNA was extracted from wet biomass of single colonies of isolates NF3 and NH11^T and *C. rubra* DSM 44948^T grown on ISP2 agar for 7 days at 28 °C using the protocol provided by MicrobesNG (Birmingham, UK; http://www.microbesng.uk) and sequenced on a MiSeq instrument (Illumina, San Diego, USA). Genomic DNA libraries of each sequence were prepared at Microbes NG using Nextera XT library preparation kits. The purity and concentration of the extracted genomic DNA was measured using the Microlab STAR handling system (Hamilton, Birmingham, UK) and libraries generated using Kapa Biosystems library quantifica-

tion kits designed for Illumina instruments on a Lightcycler 96 real time PCR instrument (Roche, West Sussex, UK). The resultant libraries were sequenced following the 2 × 250 bp paired end protocol (MicrobesNG). Reads were trimmed using Trimmomatic software, version 0.30 [8] and their quality assessed using in-house scripts from MicrobesNG. Reads under 250 bp were discarded, contigs of each strain assembled using Spades 3.7 software [5], annotated using RAST-SEED server [2] with default options, and sequence based comparisons achieved using the SEED Viewer [3]. The draft genome sequences of isolates NF3 and NH11^T and *C. rubra* DSM 44948^T were deposited in the GenBank database under accession numbers: JACEGI000000000, JAAFZA000000000 and JAACYW000000000, respectively.

The genome sequences of the isolates and the type strains of *Actinospica durhamensis*, *Actinocrinis puniceicyclus* and *C. rubra* were uploaded onto the Type (Strain) Genome Server (TYGS) [55] and compared against the type strain genomes available in the TYGS database using the MASH algorithm which allows a fast approximation of intergenomic relatedness between strains [65]. A phylogenomic tree was inferred with FastME 2.1.4 [48] from GBDP distances calculated from the genome sequences and branch lengths scaled using the GBDP distance formula d5 [56]; numbers above the branches are GBDP pseudo-bootstrap support values based on 100 replications. The genome sequences of the isolates and the type strains of *C. acidiphila* and *C. rubra* were screened for the presence of biosynthetic gene clusters (BGCs) using anti-SMASH 5.0 software with "strict" detection criteria and extra features including KnownClusterBlast, ClusterBlast, SubClusterBlast, ActiveSiteFinder and Cluster Pfam analyses [6,7]. The genome sequences of isolates NF3 and NH11^T were compared with one another and with those of *C. acidiphila* DSM 44928^T (GenBank accession number: NC013131) and *C. rubra* DSM 44948^T, their closest phylogenomic neighbours, and average nucleotide identity (ANI) [70] and digital DNA:DNA hybridization (dDDH) [57] values determined using the online resource from the Rodriguez and Konstantinidis group (http://enve-omics.gatach.edu/) and the GGDC web server (http://ggdc.dsmz.de/ggdc).

Chemotaxonomy

The isolates were examined for the presence of chemical markers characteristic of members of the family *Catenulisporaceae* using standard chromatographic methods. To this end, they were examined for isomers of diaminopimelic acid [81], isoprenoid quinones [13], polar lipids [60] and whole organism sugars [32], using appropriate controls. Cellular fatty acids extracted from biomass of the isolates were methylated after Miller [59] with minor modifications from Kuykendall et al. [45], analysed using the protocol of the Sherlock Microbial Identification (MIDI) system, version 5 [75] and the resultant acids identified using the TSBA40 database.

Cultural and phenotypic properties

The growth and cultural properties of the isolates and the *C. acidiphila* and *C. rubra* strains were recorded from tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic-salts starch, glucose-asparagine, peptone-yeast extract-iron and tyrosine agars (International *Streptomyces* Project media 1–7) [78] and from Bennett's, and modified Bennett's agar [34], HSA5 agar [10], nutrient and 100-fold diluted nutrient agar (Becto Dickinson, USA) and tap water agar [31] after 4 weeks at 28 °C; aerial spore mass, substrate mycelial and diffusible pigments were determined by comparison against NBS/IBCC Colour Charts [36]. The strains were also examined for their ability to grow over a range of pH values (pH 5–12 at single unit intervals) and temperatures (4, 10, 15, 20, 25, 30, 33, 35, 37, 40 °C) and in the presence of various NaCl concentrations (1–10 at single unit intervals) using ISP2 agar [78] as the basal medium; the pH values were achieved using KH_2PO_4/HCl, KH_2PO_4/K_2HPO_4) and K_2HPO_4/NaOH buffer systems. The strains were examined for standard biochemical, degradative and physiological properties using media and methods described by Williams et al. [97], albeit with media adjusted to pH 5.5. All of these tests were carried out, in triplicate, using 12-welled plates that were inoculated using a standard inoculum corresponding to 5 on the McFarland scale [61] and a multipoint inoculator (Mast Uri®Dot, Mast Group Ltd., Merseyside, UK); the inoculated plates were incubated for 3 weeks at 28 °C. The enzymic activities of the isolates and the *C. acidiphila* and *C. rubra* strains were determined, in duplicate, using API-ZYM kits (BioMerieux, France), according to the manufacturer's instructions.

Antimicrobial screens

Isolates NF3 ns NH11^T were tested against wild type strains of *Bacillus subtilis, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* (sourced from diagnostic microbiology laboratory then maintained by one of us, namely MG) using a standard well-diffusion method [53,89]. Aliquots of 100 µl of microbial suspensions (5×10^5 c.f.u ml^{-1}) in saline were spread aseptically onto Tryptic Soy Agar (TSB, Becton Dickinson, USA) in Petri plates, wells (ø = 5 mm) prepared using a sterile cork borer were then filled with 50 µl of 14-day old ISP2 broth cultures of each of the isolates, incubated for 24 h at 28 °C prior to the measurement of inhibition zones in milimeters. A co-culture method was used to determine whether the isolates inhibited the growth of strains of *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium oxysporum,* all of which are pathogens of wheat. Growth from each of the isolates prepared on ISP2 agar was streaked as a line across one side of three Potato Dextrose Agar (PDA, Becton Dickinson) plates which were incubated for 14 days at 28 °C. The fungal pathogens were grown on PDA plates for 7–14 days at 28 °C; discs (ø = 10 mm), prepared using sterile cork borers, of each of the strains were placed, one per plate, on the opposite sides of plates inoculated with actinobacteria and the preparations incubated for 7 (*F. culmorum*) and 21 days (*F. graminearum* and *F. oxysporum*) at 28 °C. The positive controls were the cultures of the fungi grown under the same incubation conditions. Inhibition (I) of fungal growth was calculated using the formula: I (%)= (C-T/C) x 100, where C is the diameter of fungal growth in the control sample and T the diameter of the fungal growth in each of the co-culture samples.

Results and discussion

Phylogeny

Almost complete 16S rRNA gene sequences of isolates NF3 and NH11^T (1525 nucleotides [nt]) were deposited in GeneBank under accession numbers MT785435 and MT785436, respectively. The isolates, which had identical 16S rRNA gene sequences, formed a well supported branch in the *Catenulispora* 16S rRNA gene tree with *C. acidiphila* DSM 44928^T and *C. rubra* DSM 44928^T, as shown in Fig. 1. The isolates showed sequence similarities with the *C. acidiphila* and *C. rubra* strains of 98.8 and 99.0%, values equivalent to 18 and 15 nt differences at 1525 and 1523 locations, respectively; the corresponding sequence similarities with the remaining *Catenulispora* type strains fell within the range of 97.2–98.4%. All of these similarity values are below the 98.7% threshold that triggers the need for DNA:DNA relatedness studies in actinobacteria [12] thereby indicating that the isolates do not belong to the validly published *Catenulispora* species. The isolates also formed a well

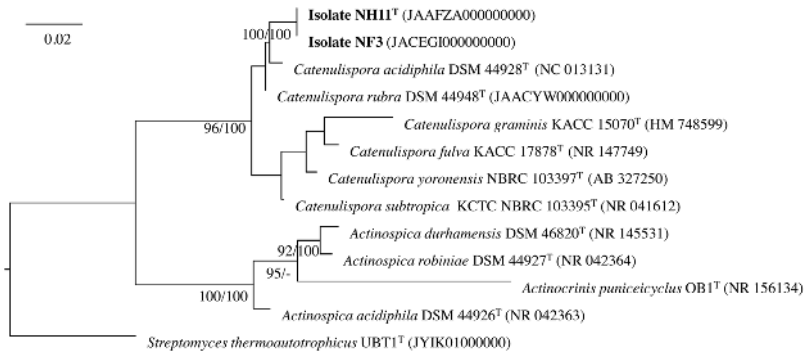

Fig. 1. Maximum-likelihood and maximum-parsimony trees based on nearly complete 16S rRNA gene sequences showing relationships between isolates NH11^T and NF3 and between them and the type strains of *Catenulispora* species. The numbers above the branches are bootstrap support values when over 70% from ML (left) and MP (right). Bar; 0.02 substitutions per nucleotide position. The root position of the tree was determined using *Streptomyces thermoautotrophicus* UBT1^T.

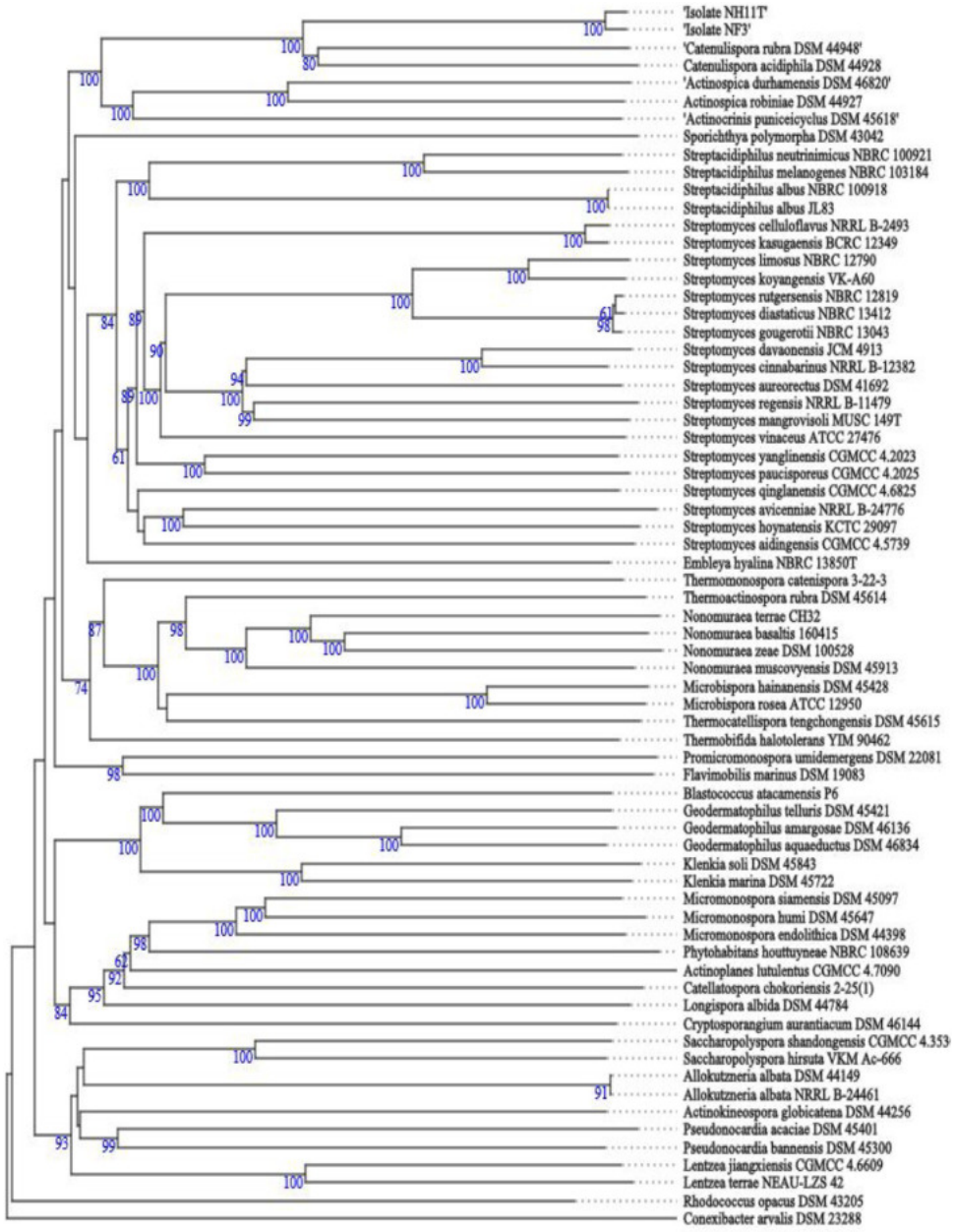

Fig. 2. Phylogenomic tree showing relationships between isolates NF3 and NH11^T and between the isolates and the type strains of closely related actinobacterial species deposited in TYGS server. The numbers above the branches are GBDP pseudo-bootstrap support values >60% from 100 replications with an average branch support of 83.2%. The tree was rooted at the midpoint [20].

Table 1
Growth and cultural characteristics of the isolates and their closest phylogenomic relatives.

Medium	1	2	3	4
Bennett's agar				
Growth	+++	+++	++	+++
Substrate mycelial colour	Vivid purplish red	Pale yellow	Strong yellowish brown	Deep purplish red
Glycerol-asparagine agar (ISP5)				
Growth	+++	+++	+++	+++
Substrate mycelial colour	Vivid purplish red	Strong purplish red	Strong orange yellow	Dark purplish pink
HSA5 agar				
Growth	+	+	+	+
Substrate mycelial colour	Strong brown	Light brown	Light grayish brown	Grayish brown
Modified Bennett's agar				
Growth	+	+++	+	++
Substrate mycelial colour	Vivid purplish red	Pale yellow	Pale yellow	Deep purplish red
Nutrient agar				
Growth	+	+	+	+
Substrate mycelial colour	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Nutrient agar 100-fold diluted				
Growth	+	+	None	+
Substrate mycelial colour	Yellowish white	Yellowish white	ND	Yellowish white
Oatmeal agar (ISP 3)				
Growth	+++	+++	+++	+++
Aerial mycelial colour	ND	ND	White	Brilliant purplish pink
Substrate mycelial colour	Vivid purplish red	Strong purplish red	Dark reddish orange	Vivid red
Peptone – yeast extract iron agar (ISP 6)				
Growth	++	+	++	+
Substrate mycelial colour	Pinkish white	Pinkish white	Yellowish white	Yellowish white
Tap water agar				
Growth	+	+	None	+
Substrate mycelial colour	Yellowish white	Yellowish white	ND	Deep purplish pink
Tryptone-yeast extract agar (ISP 1)				
Growth	++	++	++	+
Substrate mycelial colour	Pinkish white	Pinkish white	Yellowish white	Yellowish white
Tyrosine agar (ISP 7)				
Growth	+++	+++	+++	+++
Aerial mycelial colour	ND	ND	White (scant)	Very light violet
Substrate mycelial colour	Vivid purplish red	Strong purplish red	Grayish reddish brown	Strong purplish red
Yeast extract - malt extract agar (ISP 2)				
Growth	+++	+++	+++	+++
Aerial mycelial colour	ND	ND	ND	White (scant)
Substrate mycelial colour	Yellowish gray	Yellowish gray	Dark grayish yellow	Grayish yellow

Strains: 1, isolate NF3; 2 isolate NH11^T; 3, *Catenulispora acidiphila* DSM 44928^T; 4, *Catenulispora rubra* DSM 44948^T. +++, good growth; ++, moderate growth; +, weak growth; ND, not determined, as the isolates did not form aerial hyphae. None of the strains grew on inorganic – salt - starch agar (ISP4).

supported branch in the neighbour-joining tree together with *C. acidiphila* and *C. rubra* strains (**Fig. S1**). The isolates and the *C. acidiphila* and *C. rubra* strains were recovered as a well supported clade in the phylogenomic tree (Fig. 2) that was closely related to a sister clade composed of *A. durhamensis* [27], *Actinospica robiniae* [11], the type species of the genus, and *Actinocrinis puniceicyclus* [38], which also encompass acid-loving actinobacteria. This aggregate taxon is sharply separated from corresponding clades containing diverse actinobacteria, including one corresponding to the genus *Streptacidiphilus* [35,79].

Phenotypic properties

Isolates NF3 and NH11^T were found to share a broad range of properties consistent with their classification in the genus *Catenulispora* [15,46,47]. They are aerobic, Gram-stain positive, non-acid-alcohol-fast actinobacteria which form stable, extensively branched substrate mycelia; LL-A_2pm is the diamino acid of the peptidoglycan, the major whole-organism sugar is arabinose, the predominant isoprenologues tetra-, hexa and octahydrogenated menaquinones with nine isoprene units and the major fatty acids *iso*-$C_{16:0}$ (55.9 and 43.0%) and *anteiso*-$C_{17:0}$ (13.3 and 18.7%) in isolates NF3 and NH11^T, respectively. Catenulisporae rarely produce aerial hyphae on standard nutrical media, notably on ISP media [46,47]. It can be seen from Table 1 that *C. acidiphila* DSM 44928^T and *C. rubra* DSM 44928^T produced aerial hyphae on oatmeal and tyrosine agar, the *C. rubra* strain also formed scant white aerial hyphae on yeast extract - malt extract agar. In contrast, neither of the isolates were induced to produce aerial hyphae either on the standard nutrient media or diluted nutrient agar or on tap water agar (Table 1), as exemplified in **Figure S2**. It can be seen from the distinctive BOX-PCR fingerprint patterns (**Fig. S3**) that the isolates are not clones.

Identical results were recorded for the duplicated and triplicated phenotypic tests that were carried out in duplicate. The isolates were found to have many phenotypic features in common (Tables 1 and 2) though several phenotypic properties can be weighted to distinguish them (Table 3) thereby providing further evidence that they are not clones. Thus, isolate NH11^T, unlike isolate NF3, was positive for alkaline phosphatase, β-galactosidase and β-glucosidase and used L-hydroxyproline as a sole nitrogen source whereas only the latter strain showed esterase lipase (C8) and trypsin activity, degraded L-tyrosine and used *meso*-erythritol as a sole carbon source. Similarly, it is evident from Table 1 that the isolates and the *C. acidiphila* and *C. rubra* strains, their closest phylogenomic neighbours, can be distinguished using combinations of phenotypic properties; only the isolates, for instance, used D-melezitose and sodium butyrate as sole carbon sources. The isolates, unlike *C. rubra* DSM 44948^T, their closest phylogenomic relative, used L-rhamnose and D-salicin as sole carbon sources and L-histidine, L-*iso*leucine and L-valine as sole nitrogen sources. In contrast, only the *C. rubra* strain produced α-fucosidase, degraded casein and hypoxanthine, used adonitol as a sole carbon source and acetamide as a sole nitrogen source. Other combinations of pheno-

Table 2
Phenotypic properties that distinguish the isolates from their closest phylogenomic relatives.

Characteristics	1 and 2	3	4
API-ZYM tests:			
Acid phosphatase, α –glucosidase and lipase (C14)	+	–	+
α-Chymotrypsin and cystine, leucine and valine arylamidases	–	+	–
α -Fucosidase	–	–	+
Degradation of:			
Casein	–	+	+
Hypoxanthine	–	–	+
Tween 60	+	–	+
Growth on sole carbon sources (at 1%):			
Adonitol	–	+	+
Amygdalin, D-ribose and sodium acetate	+	–	+
D-Melezitose and sodium butyrate	+	–	–
L-Rhamnose and D-salicin	+	+	–
Sodium oxalate	–	+	–
Growth on sole nitrogen sources (at 0.1%):			
Acetamide	–	+	+
L-Glutamic acid	+	–	+
L-Histidine, L-*iso*leucine and L-valine	+	+	–
L-Phenylalanine	–	+	–
Tolerance tests:			
NaCl % w/v tolerance	0–1	0–1	0–2
pH range for growth	5–7.5	5–7.0	5–7.5
Temperature profile	10–33	10–35	10–30

Strains: 1, isolate NF3; 2, isolate NH11^T; 3, *Catenulispora acidiphila* DSM 44928^T; 4, *Catenulispora rubra* DSM 44948^T.+, positive; -, negative.
All strains were positive for α-galactosidase and α-mannosidase but negative for esterase (C4), N-acetyl-β-glucosaminidase, β-glucuronidase and naphthol-AS-BI-phosphohydrolase (API-ZYM tests), degraded Tween 40, but not adenine, chitin, DNA, elastin, gelatin, guanine, Tweens 20 or 80, uric acid, xanthine or xylan, and hydrolysed aesculin and arbutin, but not allantoin or urea. They were positive for the utilisation of L-arabinose, D-arabitol, D-cellobiose, dextran, D-fructose, D-galactose, D-glucosamine, D-glucose, glycerol, glycogen, *meso*-inositol, inulin, D-lactose, D-maltose, D-mannitol, D-melibiose, *α- and β-methyl*-D-glucosides, D-raffinose, sucrose, D-trehalose, D-xylose and citrate, fumarate, hippurate, propionate, pyruvate and succinate (sodium salts) as sole carbon sources, but negative for L-arabitol, D-glucuronic acid, *para*-aminobenzoic acid, xylitol and adipate and benzoate (sodium salts), used L-alanine, L-arginine, L-asparagine, L-cysteine, L-methionine, L-serine, L-threonine as sole nitrogen sources, but not ethanolamine.

Table 3
Properties that distinguish between the isolates.

Characteristics	1	2
API-ZYM tests:		
Alkaline phosphatase	–	+
Esterase lipase (C8)	+	–
β-Galactosidase	–	+
β-Glucosidase	–	+
Trypsin	+	–
Degradation of:		
L-Tyrosine	+	–
Growth on sole carbon source:		
Meso-erythritol	+	–
Growth on sole nitrogen source:		
L-Hydroxyproline	–	+
Isoprenoid quinones proportions (%)		
MK-9(H_4)	32.8	11.8
MK-9(H_6)	38.4	36.2
MK-9(H_8)	28.8	52.0

Strains: 1, NF3; 2, NH11^T. +, positive; -, negative.

typic features can be weighted to distinguish the isolates from *C. acidiphila* DSM 44928^T. The isolates can also be distinguished by quantitative differences in their fatty acid and menaquinone components profiles (Tables 3 and 4) though these data need to be interpreted with care as they can be sensitive to small differences in growth conditions and culture age [18,71,101].

Table 4
Fatty acid profiles of isolates NF3 and NH11^T and their closest phylogenomic neighbours. Values are percentages of total fatty acids.

Fatty acids	1	2	3	4
$C_{12:0}$	ND	tr	tr	tr
$C_{14:0}$	tr	tr	tr	tr
$C_{15:0}$	1.1	tr	tr	tr
$C_{16:0}$	3.5	3.5	5.6	3.1
$C_{17:0}$	1.0	tr	tr	tr
$C_{15:1}$ ω6c	ND	tr	tr	tr
$C_{16:0}$ 9 methyl	ND	ND	tr	4.7
$C_{16:1}$ ω9c	ND	ND	tr	1.9
$C_{17:0}$ cyclo	1.1	1.0	1.2	1.5
$C_{17:1}$ ω8c	1.2	1.6	1.1	tr
$C_{17:0}$ 10 methyl	tr	tr	tr	1.2
$C_{18:1}$ ω7c	ND	tr	tr	tr
$C_{18:1}$ ω9c	tr	tr	1.4	ND
anteiso- $C_{13:0}$	tr	tr	tr	tr
anteiso-$C_{15:0}$	2.6	4.9	2.2	3.2
anteiso- $C_{17:0}$	13.3	18.7	12.7	22.2
anteiso-$C_{17:1}$	ND	ND	tr	4.4
anteiso-$C_{17:1}$ ω9c	3.2	5.5	2.8	ND
iso-$C_{12:0}$	tr	tr	tr	tr
iso-$C_{14:0}$	tr	1.1	tr	ND
iso-$C_{15:0}$	3.9	3.5	4.3	3.8
iso-$C_{16:0}$	55.9	43.0	47.0	47.9
iso-$C_{16:1}$ H	2.4	4.6	3.4	2.0
iso-$C_{17:0}$	3.8	2.9	5.7	4.7
iso-$C_{17:1}$ ω9c	2.6	3.6	4.7	tr
iso-$C_{18:0}$	tr	tr	tr	tr
Sum in feature 3[a]	1.0	2.5	3.2	tr
Sum in feature 6[a]	ND	tr	tr	tr

Strains: 1, isolate NF3; 2, isolate NH11^T; 3, *C. acidiphila* DSM 44928^T; 4, *C. rubra* DSM 44948^T.
The data for the *C. acidiphila* and *C. rubra* strains were taken from from Busti et al. [10] and Tamura et al. [86], respectively.
[a] Summed features 3 and 6 were composed of $C_{16:1}$ ω7c/ *iso*-$C_{15:0}$ 2OH and $C_{19:1}$ ω9c/ $C_{19:1}$ ω11c, respectively; tr, trace amounts; ND, not detected.

Catenulispora strains have similar fatty acid and menaquinone profiles but exhibit heterogeneity with respect to polar lipid and whole-cell sugar patterns [15,46,47]. In this context, the isolates were found to produce whole-cell hydrolysates that included major amounts of arabinose, mannose, rhamnose and xylose plus a trace of ribose and a polar lipid pattern characterized by diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside and several unknown lipids (**Fig. S4**), properties which distinguish them from all of the *Catenulispora* species. The polar lipid profile of the *C. rubra* strain, unlike the isolates, is characterized by presence of only the phosphatidylglycerol and phosphatidylinositol [80]. The detection of phosphatidylcholine in the polar lipid profiles of the *C. fulva* SA-246^T [46], for instance, distinguishes it from the isolates and the type strains of the remaining *Catenulispora* species [10,47,85]. Similarly, the presence of xylose in whole-cell hydrolysates of the isolates separated them from the type strains of *C. fulva* [46], *C. rubra* [86], *C. subtropica* and *C. yoronensis* [85].

Genomic characterization

The draft genome sizes of *C. rubra* DSM 44948^T and isolates NF3 and NH11^T were 12.80, 11.2 and 11.47 Mb, respectively and the corresponding average *in silico* DNA G + C values are 70.0, 69.9, 69.9%. Additional genomic features of the isolates and corresponding characteristics of *C. acidiphila* DSM 44,928^T are shown in Table 5. The functional gene clusters found in the genomes of the isolates and the *C. acidiphila* and *C. rubra* strains were similar, as shown in **Figure S5**. In all cases the largest classes were associated with amino acids and derivatives (382–476 genes), carbohydrates (378–478 genes),

Table 5
General features of the genomes of the isolates and those of its closest phylogenomic relatives.

Features	1	2	3	4
Assembly size (bp)	11,217,370	11,469,319	10,467,782	12,797,989
Number of contigs	843	676	1	871
GC content (%)	69.9	69.9	69.8	70.0
N50 value	27,042	38,215	n/a	33,266
L50 value	117	91	1	110
Protein coding sequences	10,298	10,543	9376	11,849
Number of RNAs	61	65	66	63
Mean coverage	32	58	n/a	38
Accession numbers	JACEGI000000000	JAAFZA000000000	NC 013131	JAACYW000000000

Strains: 1, isolate NF3; 2, isolate NH11^T; 3, *Catenulispora acidiphila* DSM 44928^T; 4, *Catenulispora rubra* DSM 44948^T. n/a, not applicable.

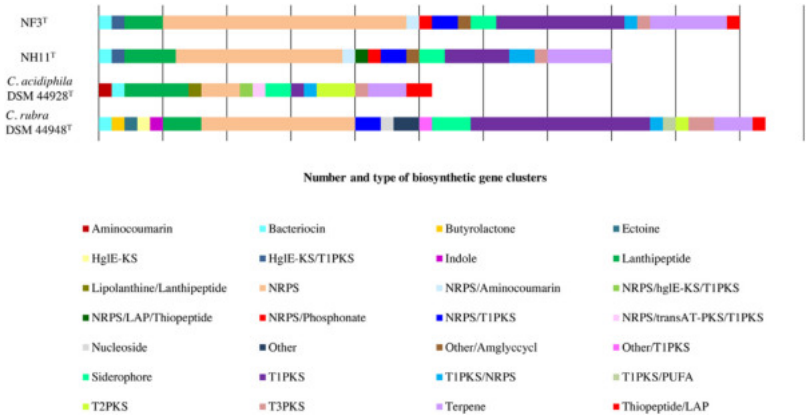

Fig. 3. Biosynthetic gene clusters detected in the genomes of isolates NF3 and NH11^T and *C. acidiphila* DSM 44928^T and *C. rubra* DSM 44948^T using AntiSMASH 5.0 software.

proteins (228–245 genes), cofactors, vitamins, prosthetic groups and pigments (211–238 genes) and fatty acids, lipids and quinones (182–242 genes).

The ANI and dDDH values between the isolates were 99.23 and 92.60%, respectively; values that are much higher than the generally recognized threshold of 95–96% and 70%, respectively, used to assign closely related strains to the same species [12,30,94]. In contrast, the ANI and dDDH values between isolates NF3 and NH11^T and *C. acidiphila* DSM 44928^T were below these thresholds given values of 86.28 and 86.25% and 29.6% (both strains); the corresponding results with *C. rubra* DSM 44948^T were 86.91 and 86.81% and 30.8 and 30.6%, respectively. These data show that the isolates belong to a species that can be separated from the *C. acidiphila* and *C. rubra* strains, their closest phylogenomic neighbours.

Biosynthetic gene clusters coding for secondary metabolites

AntiSMASH predicts biosynthetic gene clusters (BGCs) that are based on the percentage of genes from the closest known bioclusters which show significant BLAST hits to the genomes of the tested strains [6]. In this study, the number of bioclusters in the genomes of the isolates and their closest phylogenomic neighbours ranged from 26 to 52, as shown in Fig. 3. The genomes of the isolates and *C. rubra* strain contained the highest number of bioclusters, a result in good agreement with comparable studies on *Amycolatopsis* and *Rhodococcus* strains where the highest numbers of BGCs were associated with genomes generated from smaller contigs [73,87]. In general, the genomes of all of the strains were predicted to encode for non-ribosomal polyketide synthetases (NRPS), polyketide synthases (PKS), lanthipeptides, siderophores and terpenes (Fig. 3 and **Table S1**). Thirty out of the 90 bioclusters were detected in the genomes of more than one of the strains, the remainder were strain specific. Interestingly, the genomes of isolates NF3 and NH11^T contained 15 and 12 bioclusters, respectively, that were predicted to encode for unknown compounds. Other bioclusters found in the genomes of the isolates were associated with the synthesis of arylomycin (66% gene similarity), a lipoglucopeptide antibiotic [49] and stenothricin (9% gene similarity), a cyclic depsipeptide [51]; these compounds have antibacterial properties [49,51].

All of the strains contained genomes with bioclusters that were identical to ones associated with the synthesis of known compounds, as exemplified by those encoding for volatile terpenes, geosmin and 2-methylisoborneol. In contrast, other BGCs detected in these genomes showed low levels of gene similarity with known compounds, as with the terpenoid, hopene (38–53% gene similarity) and lobosamides A/B/C (4–8% gene similarity) which have antiparasitic properties [77]. Similarly, the genomes of the isolates and *C. rubra* DSM 44,948^T have bioclusters predicted to encode for alkylresorcinol (100 % gene similarity), a type III polyketide first isolated from a *Streptomyces griseus* strain, that has multiple biological functions [22,62], the siderophore, ficellomycin (3–5% gene similarity) and the type I PKS compounds, maduropeptin (13–15% gene similarity), which has anti-cancer activity [90] and undecylprodigiosin (36–81% gene similarity) that exhibits anti-cancer [50], anti-malarial [82] and anti-fungal [58] properties. The isolates and *C. acidiphila* strain contained bioclusters that had identical gene similarities to ones encoding for desferrioxamin B, a hydroxamate-type siderophore used to treat acute and chronic iron poisoning [40]. The genome of isolate NF3 is characterized by bioclusters predicted to encode for paenibacterin (40% gene similarity), which shows anti-bacterial activity [33]; rhizomide A–C (100% gene similarity), which have anti-cancer and anti-microbial properties [93] and pepticinnamin E (10% gene similarity) that exhibits anti-cancer and anti-malarial activity [74]. Similarly, the genome of isolate NH11^T has bioclusters predicted to encode for acyldepsipeptide 1 (15% gene similarity) and griseoviridin/fijimycin A (5% gene similarity), which have anti-bacterial properties [19,83,100], caniferolides A–D (11% gene similarity) that have anti-cancer and anti-fungal

Table 6
Description of *Catenulispora pinisilvae* sp. nov.

Species name	*Catenulispora pinisilvae*
Genus name	*Catenulispora*
Specific epithet	*pinisilvae*
Species status	sp. nov.
Species etymology	*Catenulispora pinisilvae* (pi.ni.sil'vae. L. fem. n. *pinus*, a pine tree; L. fem. n. *silva*, a forest; N.L. gen. n. *pinisilvae*, of a pine forest)
Description of the new taxon and diagnostic traits	Aerobic, Gram-stain positive, non-acid alcohol fast, acidotolerant actinobacteria which form an extensively branched substrate mycelium, grow from 10 to 33 °C, optimally around 28 °C, from pH 5 to 7.5, optimally around pH 5.5 and in presence of up to 1% (w/v) NaCl. Aesculin and arbutin are hydrolysed but not allantoin or urea. Tweens 40 and 60 are degraded, but not adenine, casein, chitin, elastin, gelatin, guanine, hypoxanthine, Tweens 20 or 80, uric acid, xanthine or xylan. Nitrate is not reduced. Positive for acid phosphatase, α-galactosidase, α-glucosidase, lipase (C14) and α-mannosidase, but negative for α-chymotrypsin, cystine, leucine and valine arylamidases, esterase (C4), α-fucosidase, N-acetyl-β-glucosaminidase, β-glucuronidase and naphthol-AS-BI-phosphohydrolase. Amygdalin, L-arabinose, D-arabitol, D-cellobiose, dextran, D-fructose, D-galactose, D-glucosamine, D-glucose, glycerol, glycogen, *meso*-inositol, inulin, D-lactose, D-maltose, D-mannitol, D-melezitose, D-melibiose, *α*- and *β*-methyl-D-glucosides, D-raffinose, L-rhamnose, D-ribose, D-salicin, sucrose, D-trehalose, D-xylose and acetate, butyrate, citrate, fumarate, hippurate, propionate and succinate (all sodium salts) are used as sole carbon sources but not L-arabitol, D-glucuronic acid, xylitol, adipate, benzoate or oxalate (sodium salts). L-Alanine, L-arginine, L-asparagine, L-cysteine, L-glutamic acid, L-histidine, L-*iso*leucine, L-methionine, L-serine, L-threonine, L-valine are used as sole nitrogen sources, but not acetamide, ethanolamine or L-phenylalanine. The major fatty acids are *iso*-$C_{16:0}$ and *anteiso*-$C_{17:0}$, and the predominant isoprenologues MK-9(H_4), MK-9(H_6) and MK-9(H_8). The polar lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside and 3 unknown lipids. Whole cell hydrolysates contain arabinose, mannose, rhamnose and xylose and a trace of ribose.
Country of origin	Poland
Region of origin	Kuyavian-Pomeranian voivodeship
Date of isolation	7th October 2013
Source of isolation	Pine forest soil
Sampling date	7th October 2013
Geographic location	Toruń / Poland
Latitude	52°55′ 37″N
Longitude	18°42′11″E
16S rRNA gene accession nr.	MT785436
Genome accession number [RefSeq]	JAAFZA000000000
Genome status	Draft genome sequence
Genome size	11.47Mbp
GC mol%	69.9
Number of strain in study	2
Source of isolation of non-type strain	Pine forest soil
Designation of the type strain	NH11^T
Strain collection numbers	=DSM 111109^T =PCM 3046^T

Table 7
Emended description of *Catenulispora rubra* Tamura et al. 2007.

Species name	*Catenulispora rubra*
Genus name	*Catenulispora*
Specific epithet	*rubra*
Species status	Emended description
Emended description of the taxon and diagnostic traits	The description is as before [86] with the additions given below. Grows from pH 5 to7.5, between 10–30 °C and in the presence 0-2%, w/v NaCl. Positive for acid phosphatase, α-fucosidase, α- and β-galactosidases and lipase (C14), but negative for α-chymotrypsin, esterase (C4), esterase lipase (C8), N-acetyl-β-glucosaminidase, β-glucuronidase, leucine and valine arylamidases, naphthol-AS-BI-phosphohydrolase and trypsin (API-ZYM tests), degrades casein, hypoxanthine, Tweens 40 and 60 and L-tyrosine, but not adenine, chitin, DNA, elastin, gelatin, guanine, Tweens 20 or 80, uric acid, xanthine or xylan. Arbutin is hydrolysed, but not allantoin. Nitrate is not reduced. Adonitol, amygdalin, D-arabitol, D-cellobiose, dextran, *meso*-erythritol, D-galactose, D-glucosamine, glycerol, glycogen, *meso*-inositol, inulin, D-lactose, D-maltose, D-mannitol, D-melibiose, *α*- and *β*-*methyl*-D-glucosides, D-ribose, D-trehalose, and acetate, citrate, fumarate, hippurate, propionate, pyruvate and succinate (sodium salts) are used as sole carbon sources, but not L-arabitol, D-glucuronic acid, D-melezitose, *para*-aminobenzoic acid, D-salicin, xylitol and adipate, benzoate, butyrate or oxalate (sodium salts). Acetamide, L-alanine, L-arginine, L-asparagine, L-cysteine, L-glutamic acid, L-hydroxyproline, L-methionine, L-serine, L-threonine are used as sole nitrogen sources, but not ethanolamine, L-histidine, L-*iso*leucine, L-phenylalanine or L-valine.
Genome accession number [RefSeq]	JAACYW000000000
Genome status	Draft genome sequence
Genome size	12.8 Mbp
GC mol%	70.0
Designation of the type strain	DSM 44948^T
Strain collection numbers	=DSM 44948^T

properties [67] and phenalamide A2 (41% gene similarity) an anti-viral agent [104]. Similarly, the genome of *C. acidiphila* DSM 44,928^T contained bioclusters predicted to encode for cacibiocin B (92% gene similarity) [103] and catenulipeptin (100% gene similarity) [92] whereas that of *C. rubra* DSM 44,948^T contained bioclusters associated with the production of concanamycin A (50% gene similarity), which has anti-viral and anti-protozoal properties [64] and butyrolactol, a γ-lactone containing polyketide, which exhibits

anti-fungal activity [41]. Other discontinuously distributed bioclusters that have low percentage gene similarities with known bioclusters are shown in **Table S1**.

It is clear from the genome mining studies that catenulisporae have a much greater potential to synthesize specialised metabolites, notably antibiotics, than previously realised. Consequently, *Catenulispora* strains should feature in bioprospecting campaigns designed to find novel antibiotics of therapeutic value. However, molecular investigations are needed to determine the functional impact of bioclusters found to predict for unknown products or ones that show low levels of gene similarity with known compounds.

Antimicrobial activity

The isolates, especially strain NH11^T, inhibited the growth of the *B. subtilis, M. luteus* and *S. aureus* strains giving inhibition zones of 10, 10 and 6 mm, respectively, but not that of the remaining wild type strains. Isolates NF3 and NH11^T suppressed the growth of the *F. culmorum* (17 and 19%, respectively), *F. oxysporum* (34 and 25%, respectively) and *F. graminearum* (57 and 53%, respectively) strains thereby providing further evidence that acidotolerant actinobacteria can inhibited the growth of pathogenic fungi [14,68].

It can be concluded from the genomic and polyphasic analyses that the isolates not only belong to the same species but can be distinguished from *C. acidiphila* DSM 44928^T and *C. rubra* DSM 44948^T, their closest phylogenomic neighbours, using a combination of chemotaxonomic, phenotypic and phylogenomic properties, notably by low ANI and dDDH values. Consequently, it is proposed that the isolates be assigned to a new *Catenulispora* species, *Catenulispora pinisilvae* sp. nov., with isolate NH11^T as the type strain. The description of this new species is given in Table 6.

Further, in light of data acquired in this study an emended description is given for *C. rubra* [86], as shown in Table 7.

Funding

This work was supported by Grants No. 2017/01/X/NZ8/00140 and 2016/23/N/NZ9/00247 from the National Science Centre (NCN) of Poland and by Grant No. 1207-B from Nicolaus Copernicus University.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

Ethical statement

This article does not contain any studies with human participants or animals.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.syapm.2020.126164.

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Supplementary material

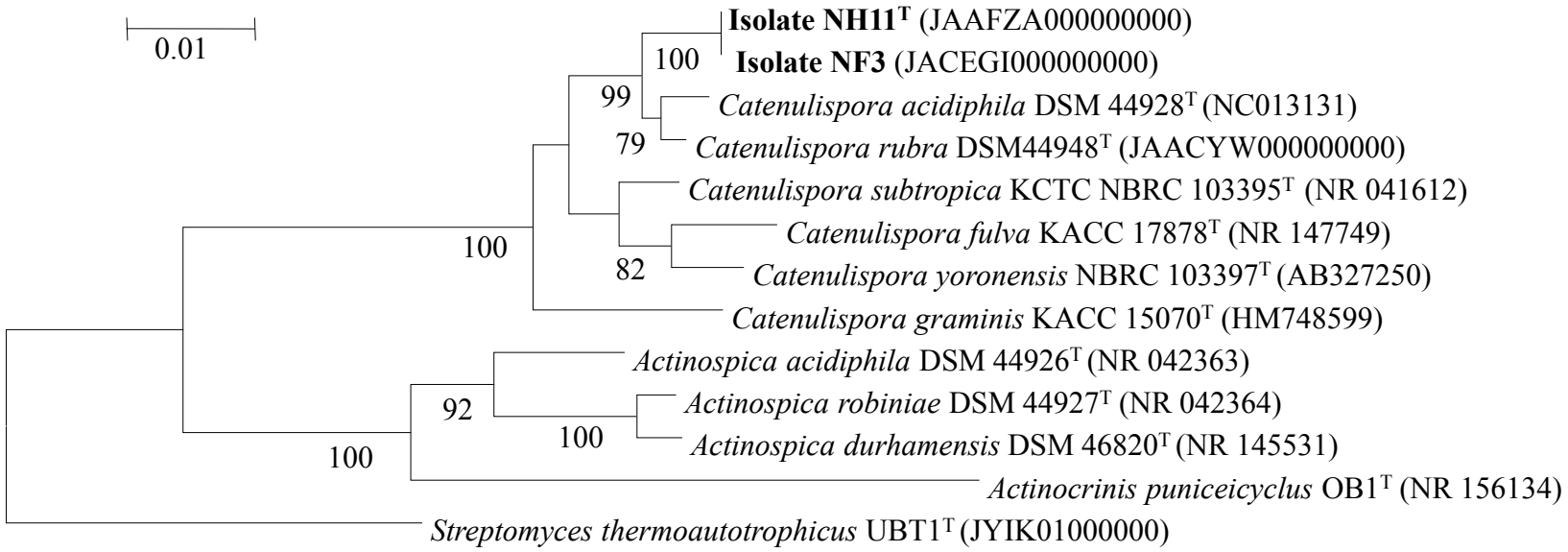

Fig. S1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between isolates NH11^T and NF3 and between them and the type strains of species classified in the order *Catenulisporales* . The numbers at the nodes are bootstrap support values over 70%. Bar; 0.01 substitutions per nucleotide position. The root position of the tree was determined using *Streptomyces thermoautotrophicus* UBT1^T.

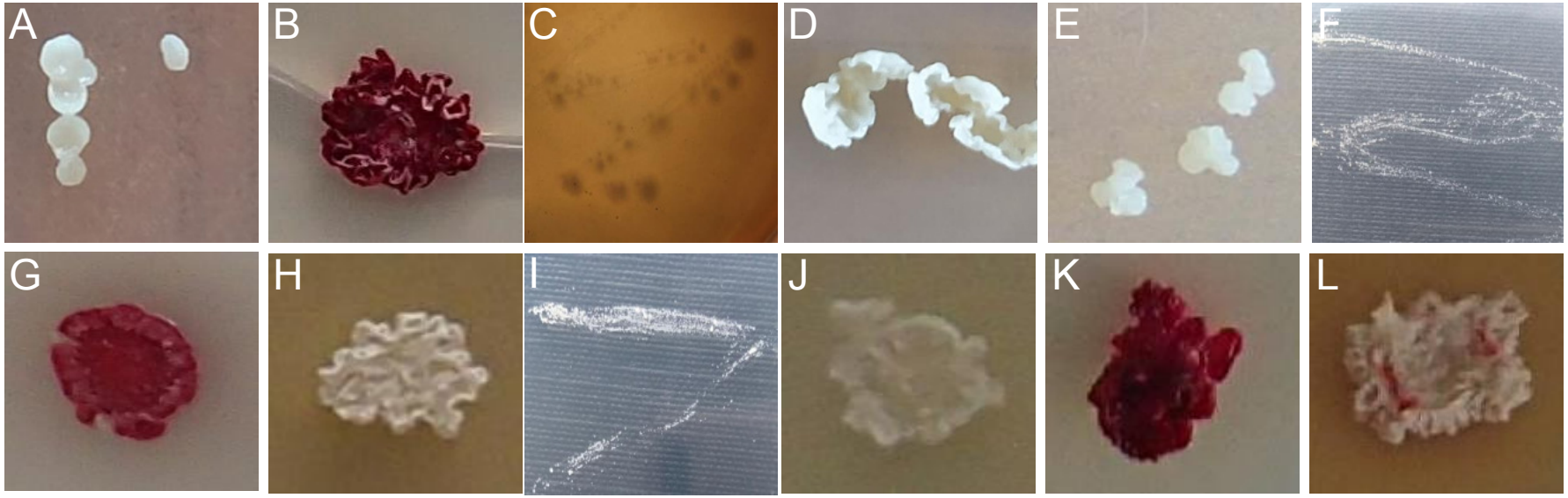

Fig. S2. Growth and cultural characteristics of isolate NH11^{T}

A; Bennett's agar (BA), B; Glycerol-asparagine agar (ISP5), C; HSA5 agar, D; Modified Bennett's agar, E; Nutrient agar (NA), F; Nutrient Agar (NA) 100-fold diluted, G; Oatmeal agar (ISP 3), H; Peptone – yeast extract iron agar (ISP 6), I; Tap water agar (TWA), J; Tryptone-yeast extract agar (ISP 1), K; Tyrosine agar (ISP 7), L; Yeast extract - malt extract agar (ISP 2).

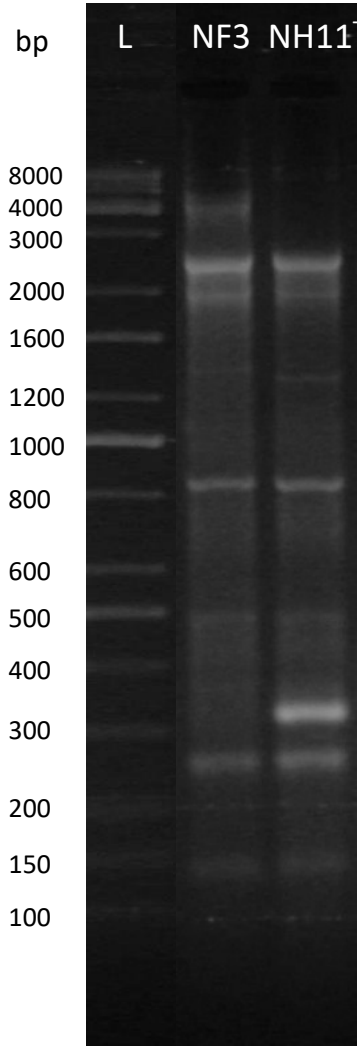

Fig. S3. BOX PCR fingerprint patterns of genomic DNA extracted from isolates NH11^{T} and NF3.
L, ladder.

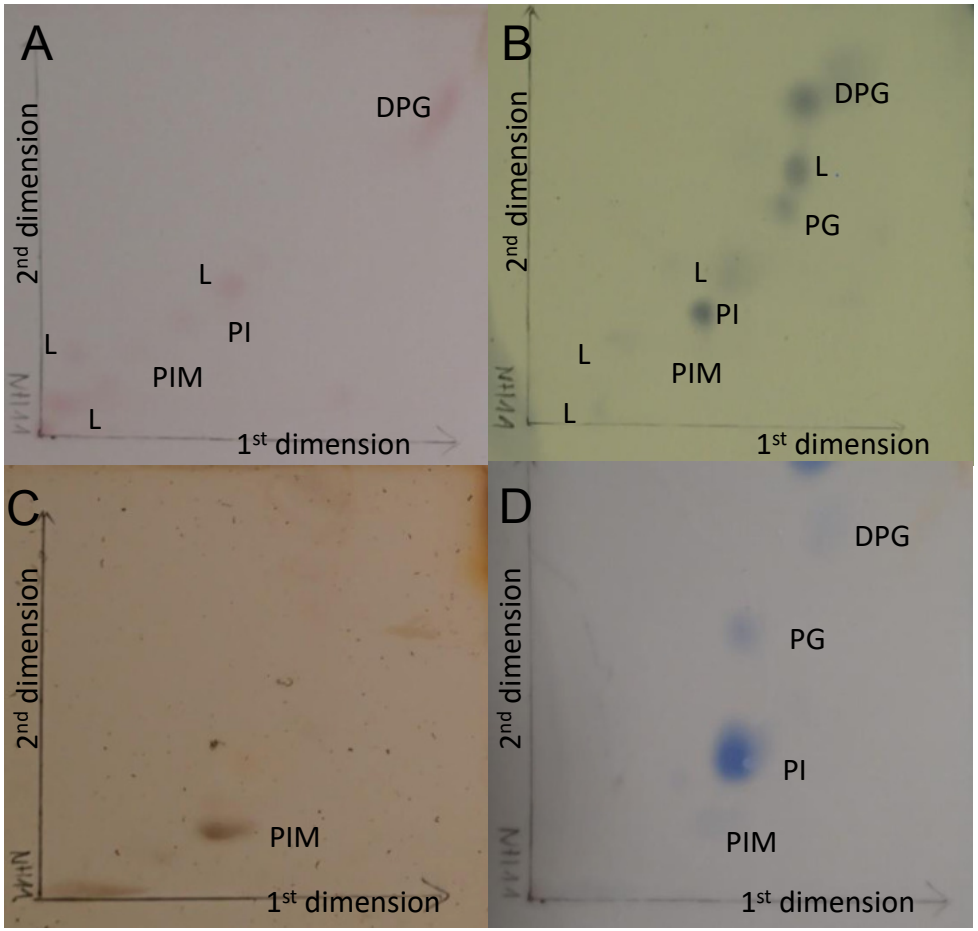

Fig. S4. Polar lipid profile of isolate NH11^T obtained using two dimensional thin-layer chromatography. TLC plates were stained with ninhidrin (A), molybdophosphoric acid (B), α-naphtosulfuric acid (C) and molybdenum blue (Sigma) (D) for the detection of total polar lipids. Chloroform:methanol:water (32.5:12.5:2.0 v/v) was used in the first direction and chloroform:acetic acid:methanol: water (40:7.5:6:2 v/v) in the second direction. DPG diphosphatidylglycerol, PG phosphatidylglycerol, PI phosphatidylinositol, PIM phosphatidylinositol mannoside, L unknown lipid.

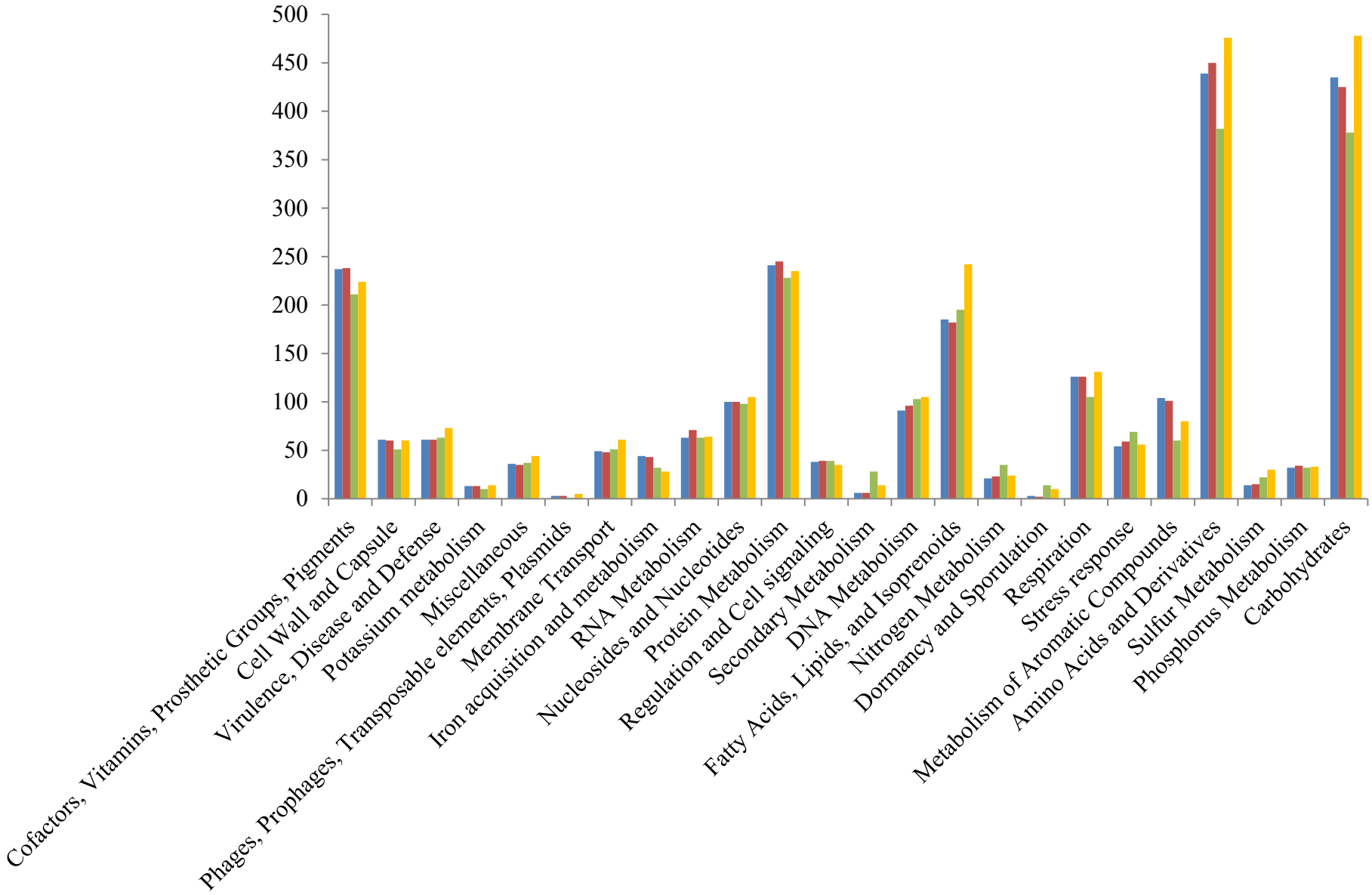

Fig. S5**.** Distribution of functional gene classes detected in the genomes of isolate NF3 (blue), NH11^{T} (red), *C. acidiphila* DSM 44928^{T} (green) and *C. rubra* DSM 44948^{T} (yellow) using the RAST-SEED server (https://rast.nmpdr.org/).

Table S1 Predicted biosynthetic gene clusters

No.	Type	Most similar known cluster	Biosynthetic classes	Number of strains	NF3	NH11^T	*C. acidiphila* DSM 44928^T	*C. rubra* DSM 44948^T
1	aminocoumarin	cacibiocin B	Other	1			P (92)	
2	bacteriocin			3	1 U	1 U	1 U	1 U
3	butyrolactone			1				1 U
4	ectoine	showdomycin	Other	1				P (23)
5	hglE-KS	cinnamycin B	RiPP	1				P (10)
6	hglE-KS/T1PKS			2	1 U	1 U		
7	indole			1				1 U
8	lanthipeptide			4	2 U	3 U	4 U	3 U
9	lanthipeptide	catenulipeptin	RiPP:Lanthipeptide	1			P (100)	
10	lanthipeptide	MS-271	RiPP	2	P (14)	P (14)		
11	LAP/thiopeptide	auroramycin	Polyketide	1	P (2)			
12	lipolanthine/lanthipeptide			1			1 U	
13	NRPS			3	6 U	4 U		1 U
14	NRPS	A54145	NRP	2	P (6)	P (6)		
15	NRPS	vazabitide	NRP	1				P (19)
16	NRPS	acyldepsipeptide 1	NRP	2		P (15) P (15)		
17	NRPS	arylomycin	NRP:Lipopeptide	2	P (66)	P (66)		
18	NRPS	crochelin A	NRP + Polyketide	1			P (11)	
19	NRPS	daptomycin	NRP	1				P (4)
20	NRPS	enduracidin	NRP	1				P (12) P (4)
21	NRPS	ficellomycin	NRP	2	P (3)	P (3)		
22	NRPS	funisamine	Poliketyde	2	P (10)	P (10)		
23	NRPS	GE81112	NRP	1	P (7)			
24	NRPS	glycinocin A	NRP	2	P (6)			P (6)
25	NRPS	griseoviridin/fijimycin A	NRP:Cyclic depsipeptide + Polyketide:Trans-AT type I	1		P (5)		
26	NRPS	JBIR-78/95	NRP	1	P (44)			
27	NRPS	lipopeptide 8D1-1/2	NRP	1	P (11)			
28	NRPS	lobosamide A/B/C	Poliketyde	4	P (8)	P (4)	P (8)	P (8)
29	NRPS	maduropeptin	Polyketide:Iterative type I + Polyketide:Enediyne type I	1				P (7)
30	NRPS	naphthyridinomycin	NRP	1				P (7)
31	NRPS	paenibacterin	NRP	1	P (40)			
32	NRPS	pepticinnamin E	NRP + Polyketide	1	P (10)			
33	NRPS	polyoxypeptin	NRP + Polyketide	1				P (32)
34	NRPS	rhizomide A/B/C	NRP	1	P (100)			
35	NRPS	S56p1	NRP	1			P (39)	
36	NRPS	sarpeptin A/B	NRP	1				P (33)
37	NRPS	stenothricin	NRP:Cyclic depsipeptide	2	P (9)	P (9)		
38	NRPS	taromycin A	NRP:Ca+-dependent lipopeptide	1				P (13)
39	NRPS/aminocoumarin	simocyclinone D8	Saccharide + Polyketide:Modular type I + Polyketide:Type II + Other:Aminocoumarin	2	P (6)	P (6)		
40	NRPS/hglE-KS/T1PKS	simocyclinone D8	Saccharide + Polyketide:Modular type I + Polyketide:Type II + Other:Aminocoumarin	1			P (10)	
41	NRPS/LAP/thiopeptide	GE81112	NRP	1		P (7)		
42	NRPS/phosphonate	aldgamycin J/K/P/E	Poliketyde	2	P (5)	P (5)		
43	NRPS/T1PKS	crochelin A	NRP + Polyketide	1				P (11)
44	NRPS/T1PKS	echinomycin	NRP	2	P (11)	P (11)		
45	NRPS/T1PKS	streptolydigin	NRP + Polyketide:Modular type I + Saccharide:Hybrid/tailoring	1		P (10)		
46	NRPS/T1PKS	stigmatelin	NRP + Polyketide:Modular type I	1	P (20)			
47	NRPS/T1PKS	thiolutin	NRP	1				P (8)
48	NRPS/transAT-PKS/T1PKS	leinamycin	NRP + Polyketide:Modular type I + Polyketide:Trans-AT type I	1			P (11)	
49	nucleoside	toyocamycin	Other	1				P (30)
50	other	ecumicin	NRP	1				P (10)
51	other	natamycin	Poliketyde	1				P (9)
52	other/amglyccycl	cetoniacytone A	Other:Cyclitol	2	P (12)	P (12)		
53	other/T1PKS	apoptolidin	Polyketide	1				P (33)
54	siderophore			1			1 U	2 U
55	siderophore	desferrioxamin B	Other	3	P (100)	P (100)	P (100)	
56	siderophore	ficellomycin	NRP	3	P (3)	P (3)		P (5)
57	T1PKS			3	4 U	1 U		4 U
58	T1PKS	amycolamycin A/B	Poliketyde	1			P (10)	
59	T1PKS	apoptolidin	Poliketyde	2	P (23)	P (23)		
60	T1PKS	butyrolactol A	Poliketyde	1				P (40)
61	T1PKS	caniferolide A /B/C/D	Polyketide:Modular type I	1		P (11)		
62	T1PKS	chlorothricin/deschlorothricin	Polyketide:Modular type I + Polyketide:Iterative type I + Saccharide:Oligosaccharide	1				P (13) P (18)
63	T1PKS	concanamycin A	Poliketyde	1				P (50)
64	T1PKS	maduropeptin	Polyketide:Iterative type I + Polyketide:Enediyne type I	3	P (15)	P (15)		P (13)
65	T1PKS	maklamicin	Poliketyde	1	P (10)			
66	T1PKS	monensin	Poliketyde	1	P (5)			
67	T1PKS	nanchangmycin	Poliketyde	1				P (21)
68	T1PKS	neocarzilin A/B	Poliketyde	1				P (14)
69	T1PKS	niphimycins C-E	Poliketyde	1				P (29)
70	T1PKS	nystatin A1	Polyketide:Modular type I + Saccharide:Hybrid/tailoring	1	P (18)			
71	T1PKS	tetronomycin	Poliketyde	1				P (21)
72	T1PKS/NRPS			1		1 U		
73	T1PKS/NRPS	A23187	Polyketide	1				P (27)
74	T1PKS/NRPS	echinomycin	NRP	1	P (11)			
75	T1PKS/NRPS	nanchangmycin	Polyketide	1			P (9)	
76	T1PKS/NRPS	phenalamide A2	NRP + Polyketide	1		P (41)		
77	T1PKS/NRPS-like			1	1 U			
78	T1PKS	undecylprodigiosin	NRP + Polyketide	3	P (36)	P (50)		P (81)
79	T1PKS/PUFA	nataxazole	Polyketide	1				P (18)
80	T2PKS	actinorhodin	Polyketide:Type II	1			P (45)	
81	T2PKS	prejadomycin/rabelomycin/gaudimycinA/C/D/UWM6	Polyketide:Type II + Saccharide:Hybrid/tailoring	1			P (27)	
82	T2PKS	spore pigment	Polyketide	2			P (66)	P (66)
83	T3PKS	alkylresorcinol	Poliketyde	3	P (100)	P (100)		P (100)
84	T3PKS	furaquinocin B	Terpene + Polyketide	1				P (17)
85	T3PKS	acarviostatin I 3/II 3/III 3/IV 3	Saccharide	1			P (25)	
86	terpene			2	1 U	1 U		
87	terpene	2-methylisoborneol	Terpene	4	P (100)	P (100)	P (100)	P (50)
88	terpene	geosmin	Terpene	4	P (100) P (100) P (100)	P (100) P (100)	P (100)	P (100)
89	terpene	hopene	Terpene	4	P (53)	P (53)	P (38)	P (38)
90	thiopeptide/LAP			2			1 U	1 U
91	thiopeptide/LAP	meridamycin	NRP + Polyketide	1			P (7)	
	Total:				50	40	26	52

1."P"; indicates presence of predicted bioclusters.

2. Percentage of genes in common between bioclusters of the tested strains to the closest known BGCs showing significant BLAST hits [13]

3. "U" uncharacterised clusters.

Publikacja VI

INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

TAXONOMIC DESCRIPTION

Świecimska *et al.*, *Int. J. Syst. Evol. Microbiol.* 2021;71:005063

DOI 10.1099/ijsem.0.005063

MICROBIOLOGY SOCIETY

Catenulispora pinistramenti sp. nov., novel actinobacteria isolated from pine forest soil in Poland

Magdalena Świecimska[1], Patrycja Golińska[1,*], Magdalena Wypij[1] and Michael Goodfellow[2]

Abstract

The taxonomic status of two filamentous actinobacteria, isolates NF23 and NL8^T, recovered from the litter layer of a pine forest soil in Poland was established in a genome-based polyphasic study. The isolates showed a combination of chemotaxonomic, morphological and physiological properties associated with their classification in the genus *Catenulispora*. They formed a well supported lineage within the *Catenulispora* 16S rRNA gene tree and were most closely related to the type strains of *Catenulispora acidiphila* (99.1%), *Catenulispora pinisilvae* (99.9%) and *Catenulispora rubra* (99.1%), and like them, were found to have large genomes (10.8 and 11.5 Mbp, respectively). A phylogenomic tree based on the draft genomes of isolates NF23 and NL8^T and their phylogenetic neighbours showed that they formed a distinct branch in the *Catenulispora* clade that was most closely related to *C. pinisilvae* DSM 111109^T. The isolates shared a combination of genomic, genotypic and phenotypic features, and had high average nucleotide index (ANI) and digital DNA:DNA hybridization (dDDH) similarities consistent with their assignment to the same species. The isolates were distinguished from the *C. acidiphila, C. pinisilvae* and *C. rubra* strains by a wealth of taxonomic data and by low ANI (84.9–93.9%) and dDDH (29.6–54.7%) values. It is proposed that the isolates be classified in the genus *Catenulispora* as *C. pinistramenti* sp. nov. with isolate NL8^T (=DSM 111110^T=PCM 3045^T) as the type strain. The genomes of strains NF23 and NL8^T are rich in natural product-biosynthetic gene clusters hence these strains have the potential to synthesize new specialised metabolites.

INTRODUCTION

Filamentous acidophilic and acidotolerant actinobacteria are an understudied group even though they have a role in the turnover of organic matter in acidic soils, and are a potential source of new specialized metabolites, including compounds that protect and promote plant growth [1–4]. These organisms accommodate members of the order *Catenulisporales* [5] which includes the families *Catenulisporaceae* [6] and *Actinospicaceae* [7] composed of the genera *Catenulispora* [6], and *Actinospica* [7] and *Actinocrinis* [8], respectively. Representatives of these taxa can be considered especially gifted [9, 10] as they have large genomes rich in natural product – biosynthetic gene clusters (NP-BGCs) predicted to encode for drug-like molecules, notably non-ribosomal peptide synthetases (NRPS) and hybrid clusters [11, 12]. The search for such compounds from *Catenulispora* strains is in its infancy though the type strain of *Catenulispora acidiphila* produces catenulipeptin, a class III lantipeptide [13] and two novel aminocoumarins, cacibiocins A and B [14].

The genus *Catenulispora* encompasses aerobic, Gram-stain-positive, non-acid-fast, predominantly acidophilic strains which form extensively branched, non-fragmenting substrate hyphae that may bear aerial hyphae which differentiate into flexuous chains of rod-shaped spores with smooth surfaces,

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Keywords: *Catenulispora pinistramenti*; forest soil; phylogeny; genomics; polyphasic taxonomy.
Abbreviations: ANI, average nucleotide identity; A2pm, diaminopimelic acid; dDDH, digital DNA–DNA hybridization; DDH, DNA:DNA hybridization; DNA, deoxyribonucleic acid; GBDP, Genome blast Distance Phylogeny; GGDC, genome to genome distance calculator; HPLC, high-performance liquid chromatography; IBB, Institute of Biochemistry and Biophysics; ISP, International *Streptomyces* Project; MIDI, Microbial Identification System; MK, menaquinones; ML, maximum-likelihood; MP, maximum-parsimony; MRE, maximal relative error; NCBI, National Centre for Biotechnology Information; NP-BGCs, natural product - biosynthetic gene clusters; NRPS, non-ribosomal peptide synthetases; PCM, Polish Collection of Microorganisms; PKS, polyketide synthases; RAST, Rapid Annotation using Subsystem Technology; rpm, revolutions per minute; rRNA, ribosomal RNA; TLC, thin-layer chromatography; TNT, Tree Analysis using New Technology.
The accession numbers of the 16S rRNA gene sequences of isolates NF23 and NL8^T are MW577119 and MW577118, respectively and the corresponding whole-genome sequence accession numbers are JACEGJ000000000 and JAAFYZ000000000.
Six supplementary figures are available with the online version of this article.

having *LL*-diaminopimelic acid (*LL*-A_2pm) as the diamino acid of the cell wall peptidoglycan, arabinose as a whole-organism sugar, *iso*-$C_{16:0}$ and *anteiso*-$C_{17:0}$ as major fatty acids, tetra-, hexa- and octa-hydrogenated menaquinones with nine isoprene units (MK-9[H_4,H_6,H_8]) as the predominant respiratory quinones, diphospatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides as characteristic polar lipids, DNA G+C values within the range 69–72 mol% and genomes ranging in size from 10.5 to 12.8 Mbp [5, 6, 12].

Catenulispora strains are currently assigned to seven validly published species (https://lpsn.dsmz.de/genus/catenulispora) which can be distinguished using genomic, genotypic and phenotypic features [5, 12]. *C. acidiphila* [6, 15], the type species, was isolated from temperate forest soil while representatives of *Catenulispora fulva* [16], *Catenulispora pinisilvae* [12] and *Catenulispora rubra* [12, 17] were recovered from coniferous forest soils and *Catenulispora graminis* [18], *Catenulispora subtropica* [19] and *Catenulispora yoronensis* [19] from bamboo rhizosphere, paddy-field and forest soils, respectively.

The present study was designed to establish the taxonomic status of two presumptive *Catenulispora* strains, isolates NF23 and NL8^T, recovered from coniferous litter and to determine their biotechnological potential. The strains were subjected to a genome-based polyphasic study which showed that they formed a new species within the genus *Catenulispora*.

ISOLATION, MAINTENANCE AND CULTIVATION

Strains NF23 and NL8^T were isolated from acidic litter from a 145-year-old pine forest. The samples were collected from the northern slope of an inland sand dune of the forest near the Toruń Basin, Poland (52°55′ 37″N, 18°42′11″E) in October 2013. Isolates NF23 and NL8^T were recovered from partially decomposed (F horizon) and intact needles (L horizon), respectively, using a standard dilution plate procedure [20] and starch-casein agar [21] adjusted to pH 4.5 using 1M HCl. Details of the sampling sites, physicochemical properties of the F and L horizons and the selective isolation procedure were described previously by Golińska *et al.* [22]. The isolates and *C. acidiphila* DSM 44928^T, *C. pinisilvae* DSM 111109^T and *C. rubra* DSM 44948^T were maintained on starch-casein agar (SCA) slopes (pH 5.5) at room temperature and as suspensions of mycelial fragments in 20% (v/v) glycerol at −80 °C. The type strains were purchased from the Leibniz Institute, DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany.

Biomass for most of the chemotaxonomic and molecular systematic studies was harvested from isolates and the three reference strains grown in shake flasks (150 r.p.m.) of yeast extract-malt extract broth cultures (ISP2; International *Streptomyces* Project medium 2) [23], pH 5.5, at 28 °C for 3 weeks. The biomass was centrifuged, washed three times with sterile distilled water; the biomass for the molecular systematic analyses was stored at −20 °C and that for the chemotaxonomic analyses was freeze dried.

CULTURAL AND CHEMOTAXONOMIC PROPERTIES

Isolates NF23 and NL8^T were examined for cultural and chemotaxonomic properties known to be of value in *Catenulispora* systematics [5, 12]. Cultural properties of the isolates and the type strains of *C. acidiphila, C. pinisilvae* and *C. rubra* were recorded on tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic-salts-starch, glucose-asparagine, peptone-yeast extract-iron and tyrosine agar (ISP media 1–7) [23], and from Bennett's and modified Bennett's agar [24], HSA5 agar [6], nutrient and 100-fold diluted nutrient agar (Becton Dickinson, USA) and tap water agar [25] following incubation at 28 °C for 4 weeks. Colony pigments were compared against the NBS/IBCC Colour System [26]. The isolates grew well on ISP media 2, 5 and 7 but did not form aerial mycelium or produce diffusible pigments on any of the test media (Table 1, available in the online version of this article).

The isolates were examined for key cell chemical markers using standard chromatographic methods with appropriate controls. Isomers of diaminopimelic acid (A_2pm) were determined according to Staneck and Roberts [27] and whole-cell sugars following Hasegawa *et al.* [28], in each case using thin-layer chromatography (TLC). Isoprenoid quinones and polar lipids were extracted from freeze-dried cells, as described by Minnikin *et al.* [29]. The isolated menaquinones were separated by HPLC [30], and the polar lipids by two-dimensional TLC [29]. Cellular fatty acids of the isolates and *C. pinisilvae* DSM 111109^T were extracted, methylated and analysed using the protocol of the Sherlock Microbial Identification (MIDI) system, version 5 [31] and the resultant peaks identified using the TSBA40 database.

The chemotaxonomic properties of the isolates were consistent with their classification in the genus *Catenulispora* [5]. Whole-organism hydrolysates of the isolates were rich in *LL*-A_2pm, arabinose and xylose though lesser amounts of mannose, rhamnose and ribose were recorded (Table 1), results similar to those recorded for *Catenulispora pinisilvae* DSM 111109^T [12]. The menaquinones of isolates NF23 and NL8^T were composed of hexa- and octa-hydrogenated isoprenologues with nine isoprene units (32.7 and 67.3% and 23.6 and 76.4%, respectively) (Fig. S2) and were in line with those found in the type strains of *C. rubra* [17], *C. subtropica* and *C. yoronensis* [19]. The major polar lipids of the novel isolates were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, glycophospholipids (sometimes misidentified as phosphatidylinositol mannosides) and four unknown lipids (Fig. S3), as previously determined for the type strains of *C. acidiphila* [6], *C. fulva* [16] and *C. pinisilvae* [12]. The predominant fatty acids were *iso*-$C_{16:0}$ and *anteiso*-$C_{17:0}$ (Table 2).

Table 1. Growth, cultural and chemotaxonomic characteristics of the isolates and their closest phylogenetic relatives

Medium	NF23	NL8^T	*C. acidiphila* DSM 44928^T	*C. pinisilvae* DSM 111109^T	*C. rubra* DSM 44948^T
Bennett Agar (BA)					
Growth	++	++	++	+++	+++
Substrate mycelial colour	Vivid purplish red	Moderate red	Strong yellowish brown	Pale yellow	Deep purplish red
Glycerol-asparagine agar (ISP5)					
Growth	+++	+++	+++	+++	+++
Substrate mycelial colour	Vivid purplish red	Moderate purplish red	Strong orange yellow	Strong purplish red	Dark purplish pink
HSA5 agar					
Growth	+	++	+	+	+
Aerial mycelial colour	Pinkish white	Greenish grey	Absent	Absent	Absent
Substrate mycelial colour	Light greyish yellowish brown	Strong brown	Light greyish brown	Light brown	Greyish brown
Modified Bennett's agar (MBA)					
Growth	+	+++	+	+++	++
Substrate mycelial colour	Vivid purplish red	Strong purplish red	Pale yellow	Pale yellow	Deep purplish red
Nutrient agar (NA)					
Growth	–	+	+	+	+
Substrate mycelial colour	–	Pale yellow	Pale yellow	Pale yellow	Pale yellow
Nutrient agar (NA) 100-fold diluted					
Growth	–	+	–	+	+
Substrate mycelial colour	–	Greenish white	–	Yellowish white	Yellowish white
Oatmeal agar (ISP 3)					
Growth	+	+	+++	+++	+++
Aerial mycelial colour	Absent	Absent	White	Absent	Brilliant purplish pink
Substrate mycelial colour	Yellowish white	Greenish white	Dark reddish orange	Strong purplish red	Vivid red
Peptone – yeast extract iron agar (ISP 6)					
Growth	++	++	++	+	+
Substrate mycelial colour	Yellowish white	Yellowish white	Yellowish white	Pinkish white	Yellowish white
Tap water agar (TWA)					
Growth	+	+	–	+	+
Substrate mycelial colour	Yellowish white	Greenish white	–	Yellowish white	Deep purplish pink
Tryptone-yeast extract agar (ISP 1)					
Growth	++	++	++	++	+
Substrate mycelial colour	Yellowish white	Yellowish white	Yellowish white	Pinkish white	Yellowish white
Tyrosine agar (ISP 7)					

Continued

Table 1. Continued

Medium	NF23	NL8^T	*C. acidiphila* DSM 44928^T	*C. pinisilvae* DSM 111109^T	*C. rubra* DSM 44948^T
Growth	+++	+++	+++	+++	+++
Aerial mycelial colour	Absent	Absent	White (scant)	Absent	Very light violet
Substrate mycelial colour	Vivid purplish red	Deep purplish red	Greyish reddish brown	Strong purplish red	Strong purplish red
Yeast extract - malt extract agar (ISP 2)					
Growth	+++	+++	+++	+++	+++
Aerial mycelial colour	Absent	Absent	Absent	Absent	White (scant)
Substrate mycelial colour	Yellowish grey	Strong purplish pink	Dark greyish yellow	Yellowish grey	Greyish yellow
Chemotaxonomy					
Menaquinones (MK)	9 (H_6) 9 (H_8)		9 (H_4) 9 (H_6) 9 (H_8)	9 (H4) 9 (H_6) 9 (H_8)	9 (H_4) 9 (H_6) 9 (H_8)
Polar lipids*	DPG, PG, PI, GPL, 4L		DPG, PG, PI, PIMs, 2PL	DPG, PG, PI, PIMs, 3L	PG, PI
Whole-cell hydrolysate sugars*	Ara, Xyl, Mann, Rhamn, Rib		Ara, Xyl, Rib, Rhamn, Glu	Ara, Mann, Rhamn, Xyl, Rib (trace)	Rib, Mann, Ara, Glu

Key: +++, abundant growth; ++, good growth; +, weak growth; –, no growth.
None of the strains grew on inorganic – salt - starch agar (ISP4) or formed aerial hyphae on BA, ISP 1, 5 or 6, MBA, NA, NA 100-fold diluted or TWA.
*Polar lipids and whole-cell hydrolysate sugars data for *C. acidiphila* DSM 44928^T and *C. rubra* DSM 44948^T are from Busti *et al.* [6] and Tamura *et al.* [17], respectively.

DPG, diphosphatidylglycerol; GPL, glycophospholipids; PG, phosphatidylglycerol; PI, phosphatidylinositol, PIMs, phosphatidylinositol mannosides; L, lipid; PL, phospholipid. Ara, arabinose; Glu, glucose; Mann, mannose; Rhamn, rhamnose; Rib, ribose; Xyl, xylose.

GENERATION OF WHOLE-GENOME SEQUENCES

Genomic DNA was extracted from wet biomass prepared from single colonies of the isolates grown in ISP2 broth [23] for 7 days at 28 °C following the protocol provided by MicrobesNG (http://www.microbesng.uk), and sequenced on a MiSeq instrument (Illumina, San Diego, USA). Genomic DNA libraries were prepared at MicrobesNG (Birmingham, UK) using Nextera XT library preparation kits. The purity and concentration of the extracted genomic DNA preparations were measured using the Microlab STAR handling system (Hamilton, Birmingham, UK) and libraries generated using Kapa Biosystems library quantification kits designed for Illumina instruments on a LightCycler 96 real time PCR instrument (Roche, West Sussex, UK). The libraries were sequenced following the 2×250 bp paired end protocol (MicrobesNG). The reads were trimmed using Trimmomatic software, version 0.30 [32], their quality assessed using in-house scripts from MicrobesNG and those under 250 bp discarded. The resultant reads of each isolate were assembled into contigs using Spades 3.6.2 software [33]. The draft genome assemblies of isolates NF23 and NL8^T were annotated using the RAST server [34] and Prokka 1.11 [35] and analysed using the SEED Viewer [36], and deposited in the GenBank database under accession numbers of JACEGJ000000000 and JAAFYZ000000000, respectively.

PHYLOGENY AND PHYLOGENOMICS

Nearly complete 16S rRNA of isolates NF23 and NL8^T (1519 and 1398 nucleotides, respectively, with GenBank accession numbers: MW577119 and MW577118) taken from the whole-genome sequences were compared with corresponding sequences of the type strains of species classified in the genera *Actinocrinis*, *Actinospica* and *Catenulispora* using the EzBioCloud server (https://www.ezbiocloud.net) [37]. Maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic trees based on 16S rRNA gene sequences were inferred using the Single-Gene Trees Phylogeny online tool (https://www.dsmz.de/services/online-tools/single-gene-phylogenies) [38] adapted for single genes and multiple sequence alignments realised using MUSCLE software [39]. The ML tree was generated from alignments with RAxML [40] with rapid bootstrapping together with the auto maximal-relative-error (MRE) bootstrapping criterion [41]. The MP tree was inferred from alignments with the Tree Analysis New Technology (TNT) programme [42] using 1000 bootstraps

Table 2. Fatty acid profile (%) of isolates NF23 and NL8^T and their closest phylogenetic neighbours. Values are percentages of total fatty acids

Fatty acids	Isolates		*C. acidiphila* DSM 44928^T	*C. pinisilvae* DSM 111109^T	*C. rubra* DSM 44948^T
	NF23	NL8^T			
$C_{16:0}$	2.8	4.3	5.6	3.5	3.1
$C_{16:0}$ 9 methyl	–	–	TR	–	4.7
$C_{16:1}$ ω9c	–	–	TR	–	1.9
$C_{17:0}$ cyclo	TR	1.4	1.2	1.0	1.5
$C_{17:0}$ 10 methyl	TR	TR	TR	TR	1.2
$C_{17:1}$ ω8c	TR	TR	1.1	1.6	TR
$C_{18:1}$ ω9c	–	TR	1.4	TR	–
anteiso-$C_{15:0}$	5.6	4.7	2.2	4.9	3.2
anteiso- $C_{17:0}$	17.9	21.5	12.7	18.7	22.2
anteiso-$C_{17:1}$	–	–	TR	–	4.4
anteiso-$C_{17:1}$ ω9c	2.6	4.2	2.8	5.5	–
iso-$C_{14:0}$	2.3	1.1	TR	1.1	–
iso-$C_{15:0}$	6.8	4.2	4.3	3.5	3.8
iso-$C_{16:0}$	47.7	42.1	47.0	43.0	47.9
iso-$C_{16:1}$ h	1.4	3.7	3.4	4.6	2.0
iso-$C_{17:0}$	5.3	4.1	5.7	2.9	4.7
iso-$C_{17:1}$ ω9c	2.6	3.0	4.7	3.6	TR
Summed feature 3*	TR	1.8	3.2	2.5	TR

The data for the *C. acidiphila* and *C. rubra* strains were from Busti *et al.* [6] and Tamura *et al.* [17], respectively. Key: TR, trace amounts; –, not detected.

*Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 3 is composed of $C_{16:1}$ *ω*7c/*iso*-$C_{15:0}$2OH.

together with tree-bisection-and-reconnection branch swapping and ten random sequence replicates. The sequences were checked for compositional bias using the X^2 test, as implemented in PAUP* [43]. In addition, a 16S rRNA phylogenetic tree was generated using the MEGA 7 software package [44] and the neighbour-joining algorithm [45] from alignments obtained using CLUSTAL W [46]. The topology of the tree was evaluated by bootstrap analyses based on 1000 replicates [47] from the MEGA 7 software package and evolutionary distances calculated using the Kimura-two-parameter model [48]. The root positions of all of the trees were determined using the 16S rRNA gene sequence of *Streptomyces thermoautotrophicus* UBT1^T (GenBank accession number: NZ_JYIK00000000) as outgroup.

The isolates had identical 16S rRNA gene sequences and formed a well supported branch in the *Catenulispora* 16S rRNA gene tree together with *C. pinisilvae* DSM 111109^T (Fig. 1). Similar results were found in the neighbour-joining 16S rRNA gene tree (Fig. S4). The isolates shared high 16S rRNA gene sequence identities with *C. pinisilvae* DSM 111109^T (99.9%), *C. acidiphila* DSM 44928^T (99.1%) and *C. rubra* DSM 44948^T (99.1%), respectively; these identity values are above the threshold of 97% proposed by Stackebrandt and Goebel [49], 98.7–99.0% recommended by Stackebrandt and Ebers [50], 98.2–99.0% proposed by Meier-Kolthoff *et al.* [51] and 98.65% recommended by Kim *et al.* [52] for assigning closely related actinobacteria to the same species. The isolates shared lower sequence identities with the remaining *Catenulispora* type strains (98.4–97.5%), with those of the *Actinospica* species (93.7–93.1%) and with *A. puniceicyclus* OB1^T (92.0%).

The draft genome sequences of isolates NF23 and NL8^T, *Actinospica acidiphila* B-2276 (GenBank accession number: JABZEM010000000), *Actinospica durhamensis* DSM 46820^T, *A. puniceicyclus* OB1^T, *C. pinisilvae* DSM 111109^T (GenBank accession number: JAAFZA000000000) and *C. rubra* DSM 44948^T (GenBank accession number: JAACYW000000000) were uploaded onto the Type (Strain) Genome Server (TYGS) [53] and compared against

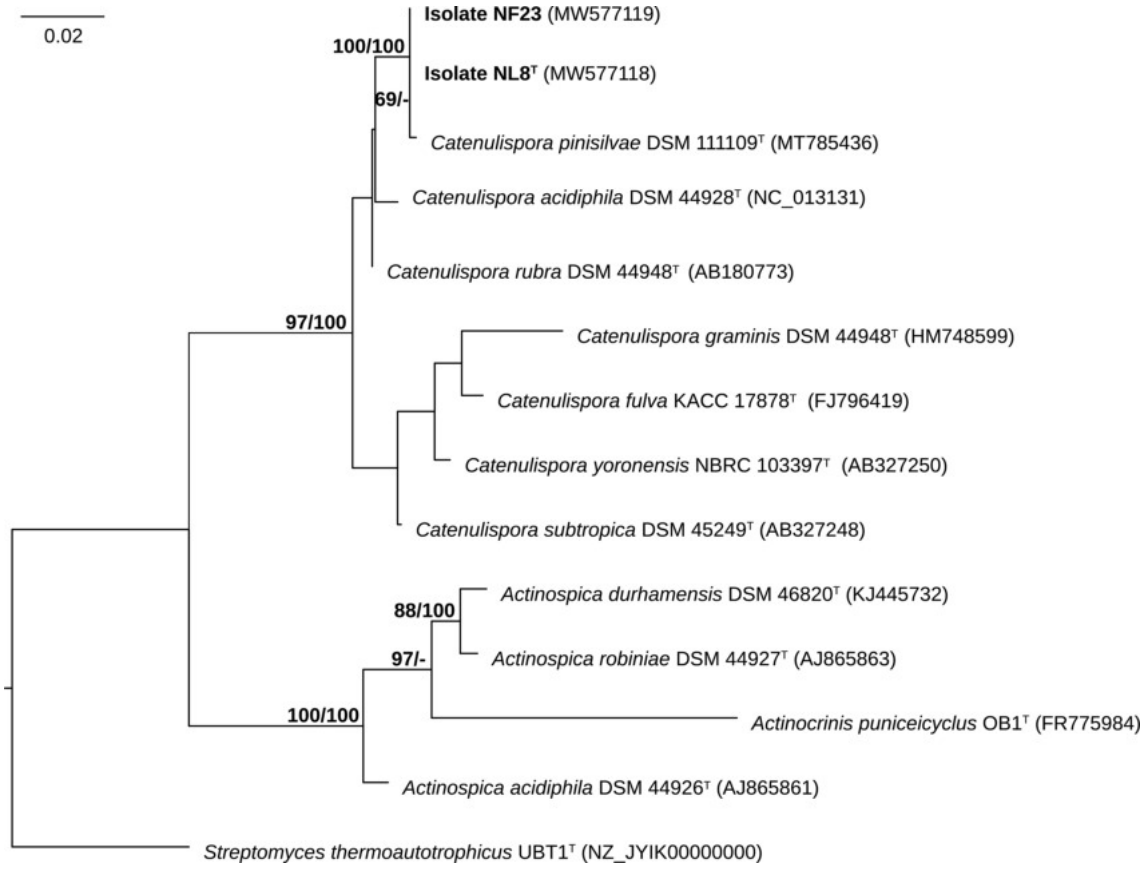

Fig. 1. Maximum-likelihood and maximum-parsimony trees based on nearly complete 16S rRNA gene sequences showing relationships between isolates NF23 and NL8^T and between them and the type strains of Actinocrinis, Actinospica and *Catenulispora* species. The numbers above the branches are bootstrap support values when greater than 60% for ML (left) and MP (right). Bar; 0.02 substitutions per nucleotide position. The root position of the tree was determined using *Streptomyces thermoautotrophicus* UBT1^T.

genomes of their closest phylogenetic neighbours available in the TYGS database using the MASH algorithm for fast approximation of intergenomic relatedness between strains [54]. A phylogenomic tree was inferred with FastME 2.1.6 [55] based on the Genome BLAST Distance Phylogeny (GBDP) and distances calculated from pairwise genome comparisons using formula d_5 from Meier-Kolthoff *et al.* [51]. GBDP pseudo-bootstrap support values were determined based on 100 replications. The tree was rooted at the midpoint [56].

More confidence can be placed in the topology of phylogenomic trees than in corresponding 16S rRNA gene trees as the former are generated from millions, not hundreds, of unit characters [15]. Fig. 2 shows that the isolates and the *C. pinisilvae* strain form a well supported lineage within the well-defined *Catenulispora* clade. This taxon is sharply separated from a clade composed of the type strains of *Actinospica durhamensis* and *A. robiniae*, and from *Actinocrinis puniceicyclus* OB1^T. The phylogenomic data underpin the close relationship between the isolates and the type strain of *C. pinisilvae*.

BOX-PCR TYPING

BOX-PCR fingerprint profiles of genomic DNA extracted from the isolates were generated using the BOXA1R primer [57] and experimental conditions described by Trujillo *et al.* [58]. Isolates NF23 and NL8^T have different fingerprint patterns indicating that they are not clones (Fig. S5).

GENOMIC CHARACTERIZATION AND POTENTIAL TO PRODUCE SPECIALISED METABOLITES

Key genomic features of the isolates and closely related *Catenulispora* strains are shown in Table 3. It is particularly interesting that all of the strains have large genomes and that the corresponding digital G+C contents fall within a narrow band ranging from 69.3–70.2%. More variation is seen in the corresponding genome sizes which range from 10.8 Mbp in isolate NF23 to 12.8 Mbp in that of *C. rubra* DSM 44948^T. The orthologous average nucleotide identities (orthoANIs) and digital DNA:DNA hybridization (dDDH) values between isolates NF23 and NL8^T and

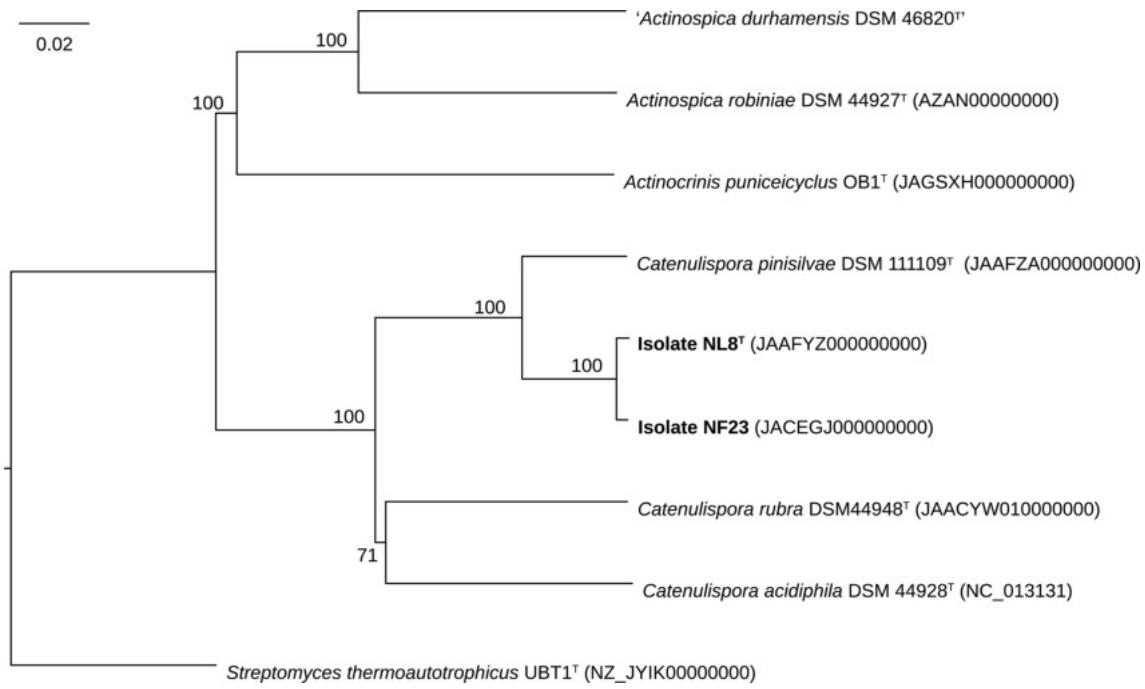

Fig. 2. Phylogenomic tree showing relationships between isolates NF23 and NL8^T and between them and type strains of Actinocrinis, Actinospica and *Catenulispora* species held in the TYGS server. The numbers above the branches are GBDP pseudo-bootstrap support values >60% from 100 replications with an average branch support of 94.0%. The tree was rooted at the midpoint [53].

between them and *C. acidiphila* DSM 44928^T (GenBank accession number: NC_013131), *C. pinisilvae* DSM 111109^T and *C. rubra* DSM 44948^T, their closest phylogenomic relatives, were determined using the ANI calculator tool from EzBioCloud (https://www.ezbiocloud.net/tools/ani) [59] and the GGDC web server (http://ggdc.dsmz.de/ggdc), respectively. The recommended ANI and dDDH values used to assigned closely related strains to different species are 95–96 and 70%, respectively [60, 61]. Table 4 shows that on this basis the isolates belong to a novel *Catenulispora*

Table 3. General features of the genomes of the isolates and those of their closest phylogenetic relatives

	NF23	**NL8^T**	***C. acidiphila* DSM 44928^T**	***C. pinisilvae* DSM 111109^T**	***C. rubra* DSM 44948^T**
Size (bp)	10824067	11465056	10467782	11469319	12797989
No. of contigs	885	672	1	676	871
G+C content (%)	70.2	70.0	69.8	69.9	70.0
N50 value	23923	38876	10467782	38215	33266
L50 value	138	87	1	91	110
Protein coding sequences	9171	9588	8816	9469	10544
Mean coverage	31	67	ND	58	38
RNAs	74	74	72	75	91
rRNAs	14	15	9	14	25
tRNAs	57	56	60	58	53
Accession number	JACEGJ000000000	JAAFYZ000000000	NC_013131	JAAFZA000000000	JAACYW000000000

Key: N50; the length of the shortest contig for which longer and equal length contigs cover at least 50 % of the assembly, L50; count of smallest number of contigs whose length sum makes up half of genome size.
ND; not determined.

Table 4. Orthologous average nucleotide identity (OrthoANI) and digital DNA:DNA hybridization (dDDH) values found between the isolates and between them and their closest phylogenetic neighbours

Strains		OrthoANI (%)	dDDH (%)
NL8^T	NF23	99.4	94.8
NL8^T	*C. acidiphila* DSM 44928^T	84.9	29.6
NL8^T	*C. pinisilvae* DSM 111109^T	93.9	54.6
NL8^T	*C. rubra* DSM 44948^T	85.2	30.5
NF23	*C. acidiphila* DSM 44928^T	85.1	29.8
NF23	*C. pinisilvae* DSM 111109^T	93.9	54.7
NF23	*C. rubra* DSM 44948^T	85.4	30.7

species that is most closely related to *C. pinisilvae* DSM 111109^T.

The genomes of the isolates and *C. acidiphila*, *C. pinisilvae* and *C. rubra* strains were annotated and assigned to the different functional gene classes using the RAST web server (http://rast.nmpdr.org). The relative distributions of the different functional gene classes in the genomes of the isolates were not only very similar but also in the same range as those found in the genomes of their closest related phylogenomic neighbours, as shown in Fig. S6. In all cases the largest classes were associated with amino acids and derivatives (382-476), carbohydrates (380-479), protein metabolism (228-245), cofactors, vitamins, prosthetic groups and pigments (211-238), as well as with fatty acids, lipids and quinones (178-242).

The draft genomes of the isolates and *C. acidiphila* DSM 44928^T, *C. pinisilvae* DSM 111109^T and *C. rubra* DSM 44948^T were analysed for the presence of NP-BGCs using anti-SMASH 5.0 software, 'strict' detection criteria and extra features, including KnownClusterBlast, ClusterBlast, SubClusterBlast, ActiveSiteFinder and Cluster Pfam analyses [62].

AntiSMASH predicts BGCs and potential natural products based on the percentage of genes from the closest known bioclusters which show significant BLAST hits to the genomes under consideration. The genomes of isolates NL8^T and NF23 contained 34 and 40 BGCs within a range that ran from 26 bioclusters in the genomes of *C. acidiphila* DSM 44928^T to 52 in that of *C. rubra* DSM 44948^T. These results show that the isolates are a potentially rich sources of new specialised metabolites.

PHENOTYPIC PROPERTIES

Isolates NF23 and NL8^T, *C. acidiphila* DSM 44928^T, *C. pinisilvae* DSM 111109^T and *C. rubra* DSM 44948^T were examined for a broad range of phenotypic properties. The enzymatic profiles of the strains were determined using API-ZYM strips (BioMérieux), according to the manufacturer's instruction. The biochemical, degradative and physiological properties were determined using media and methods from Williams *et al.* [63], albeit with acidified media (pH 5.5). The ability of the strains to grow over a range of pH (4, 5, 6, 7, 7.5, 8, 9, 10, 11 and 12) and temperatures (4, 10, 15, 20, 25, 30, 33, 35, 37, 40 and 45 °C) and in the presence of various NaCl concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9 and 10%, w/v) were determined using acidified ISP2 agar. The tolerance tests were carried out in 12-well plates (Nest Scientific Inc. NJ, USA) which were inoculated using a standard actinobacterial inoculum in sterile distilled water, corresponding to 5 on the McFarland scale [64] and a multipoint inoculator (Mast UriDot, Mast Group Ltd., Merseyside, UK). All of the tests were carried out in duplicate and inoculated plates were incubated for 3 weeks at 28 °C, apart from the temperature tests.

Table 5. Phenotypic properties that distinguish between the isolates

Characteristics	NF23	NL8^T
API-ZYM tests:		
Acid phosphatase	−	+
β-Galactosidase	−	+
Lipase (C14)	+	−
Degradation of:		
L-Tyrosine	−	+
Growth on sole carbon sources:		
Adonitol	−	+
Amygdalin	−	+
L-Rhamnose	−	+
Tolerance tests:		
Temperature growth range (°C)	15–30	15–33
NaCl tolerance (%, w/v)	0–1	0–2

Key: +; positive, −; negative.

Identical results were obtained for all of the duplicated cultures. Isolates NF23 and NL8^T had many phenotypic properties in common, but were distinguished from each other by several such properties, as shown in Table 5 thereby providing further evidence that they are not clones. Table 6 shows that a broad range of phenotypic properties can be weighted to separate isolates NF23 and NL8^T from their closest phylogenetic neighbours though all of the strains shared many such features. The isolates, unlike *C. pinisilvae* DSM 111109^T, their closest evolutionary neighbour, produced α-fucosidase and used *meso*-erythritol as a sole of carbon source. In contrast, *C. pinisilvae* DSM 111109^T produced alkaline phosphatase, degraded Tween 60, used sodium butyrate as a sole carbon

Table 6. Phenotypic properties that distinguish the isolates from the type strains of their closest phylogenetic relatives

All strains produced α-galactosidase, *β*-glucosidase and α-mannosidase (API-ZYM tests), degraded Tween 40, hydrolysed aesculin and arbutin and utilized L-arabinose, D-arabitol, cellobiose, dextran, D-fructose, D-galactose, D-glucosamine, D-glucose, glycerol, glycogen, *meso*-inositol, inulin, lactose, maltose, D-mannitol, melibiose, α-*methyl*-D-glucoside, *β*-*methyl*-D-glucoside, raffinose, sucrose, trehalose, D-xylose and citrate, fumarate, hippurate, propionate, pyruvate, succinate (sodium salts) as sole carbon sources, and L-alanine, L-arginine, L-asparagine, L-cysteine, L-methionine, L-serine, L-threonine as sole nitrogen sources. They did not produce esterase (C4), esterase lipase (C8), N-*acetyl*-*β*-glucosaminidase, *β*-glucuronidase, naphthol-AS-BI-phosphohydrolase or trypsin (API-ZYM tests), degrade adenine, chitin, DNA, elastin, gelatine, guanine, Tweens 20 or 80, uric acid, xanthine or xylan, hydrolyse allantoin or urea, use *para*-amino benzoic acid, L-arabitol, D-glucuronic acid, xylitol or adipate and benzoate (sodium salts) as sole carbon sources, or ethanolamine as a sole nitrogen source.

Characteristics	**NF23, NL8^T**	***C. acidiphila* DSM 44928^T**	***C. pinisilvae* DSM 111109^T**	***C. rubra* DSM 44948^T**
API-ZYM tests:				
Alkaline phosphate	–	+	+	+
α-Chymotrypsin	–	+	–	–
Cystine arylamidase	–	+	–	–
α-Glucosidase	+	–	+	+
α-Fucosidase	+	–	–	+
Leucine and valine arylamidases	–	+	–	–
Degradation of:				
Casein	–	+	–	+
Hypoxanthine	–	–	–	+
Tween 60	–	–	+	+
Growth on sole carbon sources:				
Meso-Erythritol	+	+	–	+
Melezitose	+	–	+	–
D-Ribose, sodium acetate	+	–	+	+
D-Salicin	+	+	+	–
Sodium butyrate	–	–	+	–
Sodium oxalate	–	+	–	–
Growth on sole nitrogen sources:				
Acetamide	–	+	–	+
L-Glutamic acid	+	–	+	+
L-Histidine	+	+	+	–
L-Hydroxyproline	–	+	+	+
L-*Iso*leucine	+	+	+	–
L-Phenylalanine	–	+	–	–
L-Valine	+	+	+	–
Tolerance tests:				
pH range for growth	5–7.5	5–7.0	5–7.5	5–7.5

Key: +; positive, –; negative.

source, and L-hydroxyproline as a sole nitrogen source. Similarly, combinations of phenotypic features can be used to distinguish the isolates from the *C. acidiphila* and *C. rubra* strains (Table 6).

It can be concluded from the genomic, genotypic and phenotypic data that isolates NF23 and NL8^T belong to a novel *Catenulispora* species that is most closely related to *C. pinisilvae*. Consequently, it is proposed that they be assigned to the genus *Catenulispora* as *Catenulispora pinistramenti* sp. nov. with isolate NL8^T as the type strain.

DESCRIPTION OF *CATENULISPORA PINISTRAMENTI* SP. NOV.

Catenulispora pinistramenti (pi.ni.stra.men'ti. L. fem. n. *pinus*, a pine tree; L. neut. n. *stramentum* litter; N. L. gen. n. *pinistramenti* of pine litter)

Aerobic, Gram-stain-positive, non-acid-alcohol fast, acidotolerant actinobacteria which form an extensively branched substrate mycelium, but not aerial hyphae. Grows from 15–33 °C, optimally around 28 °C, from pH 5 to 7.5, optimally around pH 5.5, and in the presence of 0–2% NaCl (w/v). Aesculin and arbutin are hydrolysed, but not allantoin or urea. Tween 40 is degraded, but not adenine, casein, chitin, DNA, elastin, gelatin, guanine, hypoxanthine, Tweens 20, 60 or 80, uric acid, xanthine or xylan. Positive for α-galactosidase, α- and *β*-glucosidases, α-fucosidase, α-mannosidase, but negative for alkaline phosphate, α-chymotrypsin, cystine, leucine and valine arylamidases, esterase (C4), esterase lipase (C8), N-*acetyl*-*β*-glucosaminidase, *β*-glucuronidase, naphthol-AS-BI-phosphohydrolase and trypsin. Adonitol, L-arabinose, D-arabitol, cellobiose, dextrin, *meso*-erythritol, D-fructose, D-galactose, D-glucosamine, D-glucose, glycerol, glycogen, *meso*-inositol, inulin, lactose, maltose, D-mannitol, melezitose, melibiose, α- and *β*-*methyl*-D-glucosides, raffinose, D-ribose, D-salicin, sucrose, trehalose and D-xylose are used as sole carbon sources for energy and growth, but not L-arabitol, D-glucuronic acid or xylitol (all at 1% w/v). Acetate, citrate, fumarate, hippurate, propionate, pyruvate, succinate are used as sole carbon sources for energy and growth, but not adipate, benzoate, butyrate, oxalate (all sodium salts) or *para*-aminobenzoic acid (all at 0.1% w/v). L-Alanine, L-arginine, L-asparagine, L-cysteine, L-glutamic acid, L-histidine, L-*iso*leucine, L-methionine, L-serine, L-threonine and L-valine are used as sole nitrogen sources, but not acetamide, ethanolamine, L-hydroxyproline or L-phenylalanine (all at 0.1% w/v). Whole-organism hydrolysates are rich in LL-A_2pm, arabinose and xylose, the predominant fatty acids are *iso*-$C_{16:0}$ and *anteiso*-$C_{17:0}$, the major menaquinones are MK-9(H_6) and MK-9(H_8) and polar lipid pattern consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, glycophospholipids and four unknown lipids. Genome sizes range from 10.8 to 11.5 Mbp and genomic DNA G+C content from 70.0–70.2%.

The type strain, NL8^T (=DSM 111110^T=PCM 3045^T), was isolated from the litter layer of a pine forest soil near the Toruń Basin, Poland. The GenBank 16S rRNA gene sequence accession number of the type strain is MW577118 and the corresponding genome sequence accession number JAAFYZ000000000.

Funding information
This work was supported by Grants No. 2017/01/X/NZ8/00140 and 2016/23 /N/NZ9/00247 from the National Science Centre (NCN) of Poland.

Acknowledgements
We are extremely grateful to Professor Aharon Oren (The Hebrew University of Jerusalem, Israel) for suggesting the species name.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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Supplementary material

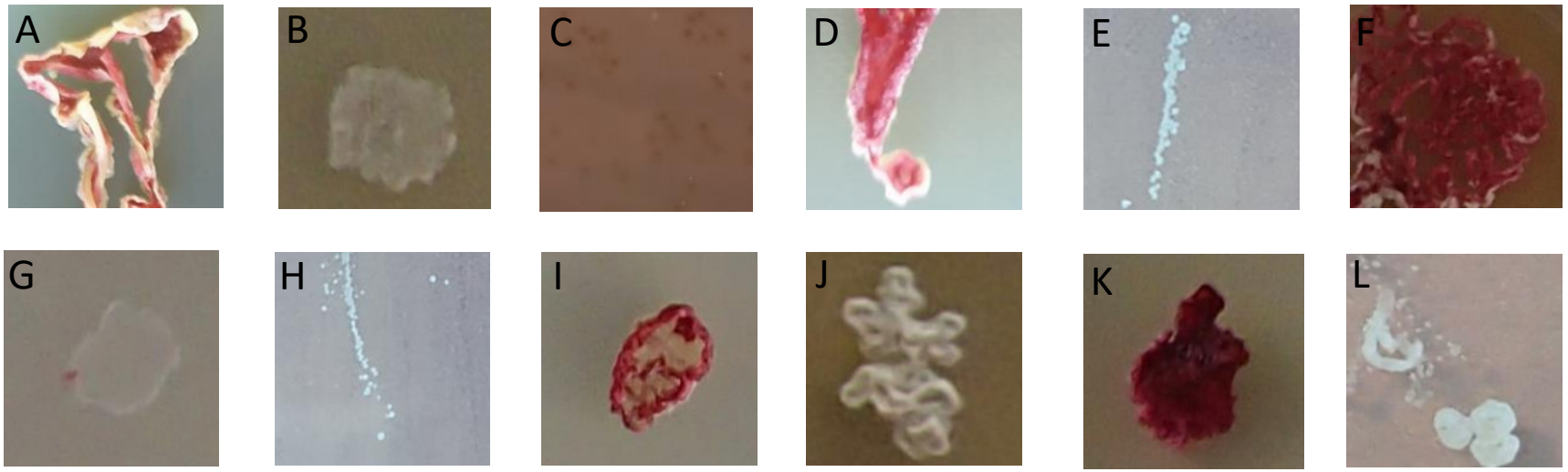

Fig. S1. Growth and cultural characteristics of isolate NL8^T on: A; Bennett's agar (BA), B; glycerol-asparagine agar (ISP5), C; HSA5 agar, D; modified Bennett's agar, E; nutrient agar (NA), F; nutrient agar (NA) 100-fold diluted, G; oatmeal agar (ISP 3), H; peptone – yeast extract- iron agar (ISP 6), I; tap water agar (TWA), J; tryptone yeast extract agar (ISP 1), K; tyrosine agar (ISP 7), L; yeast extract - malt extract agar (ISP 2).

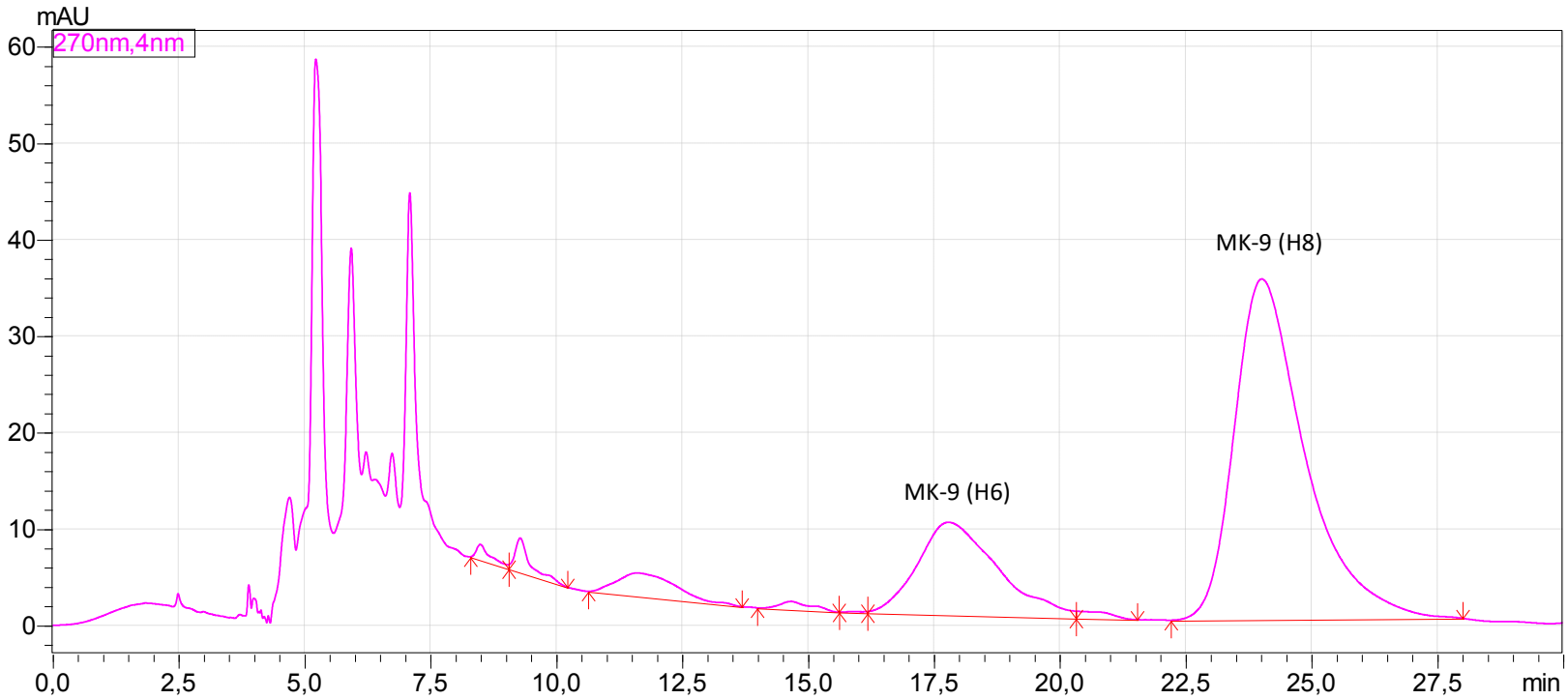

Fig. S2. Menaquinone profile of isolate NL8^{T} obtained using high performance liquid chromatography.

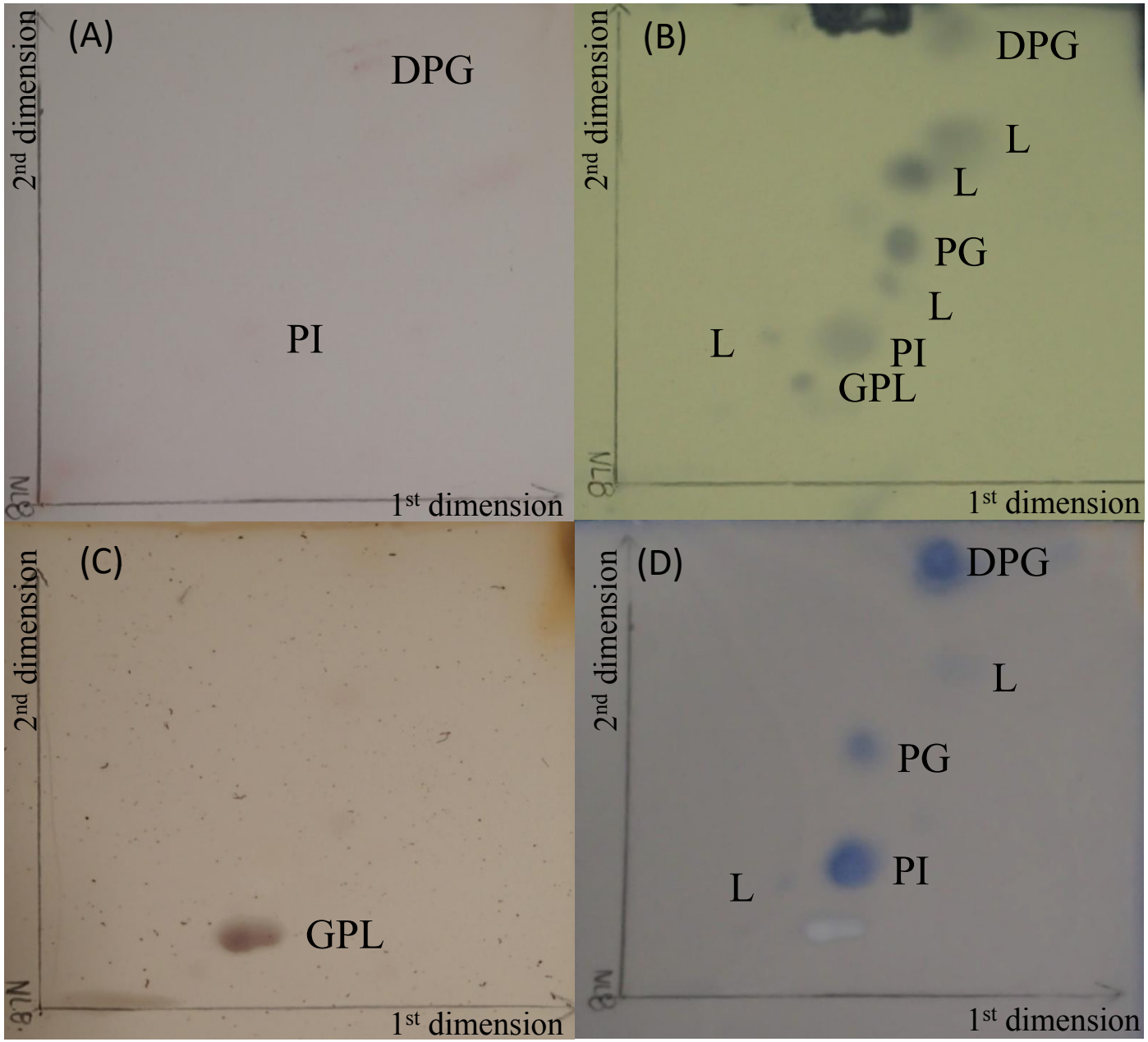

Fig. S3. Polar lipid profile of isolate NL8^T obtained using two dimensional thin-layer chromatography. TLC plates were stained with ninhydrin (A), molybdatophosphoric acid (B), α-naphthosulfuric acid (C) and molybdenum blue (Sigma) (D), and for the detection of total polar lipids. Chloroform:methanol:water (32.5:12.5:2.0 v/v) was used in the first direction and chloroform:acetic acid:methanol: water (40:7.5:6:2 v/v) in the second direction. DPG diphosphatidylglycerol, GPL glycophospholipids, PG phosphatidylglycerol, PI phosphatidylinositol, L unknown lipid.

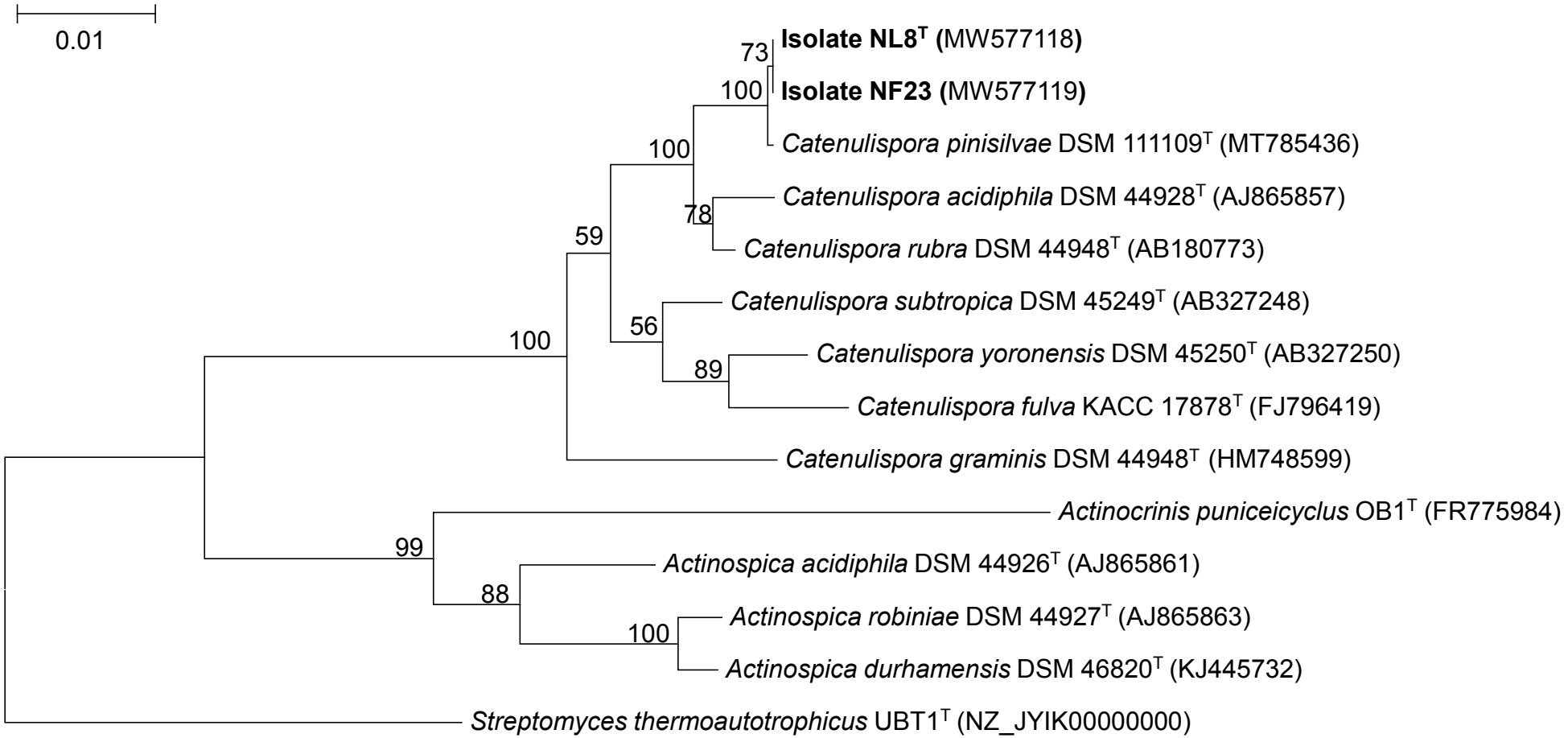

Fig. S4. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between isolates NF23 and NL8^T and between them and the type strains of species classified in the order *Catenulisporales*. The numbers at the nodes are bootstrap support values over 50%. Bar; 0.01 substitutions per nucleotide position. The root position of the tree was determined using *Streptomyces thermoautotrophicus* UBT1^T.

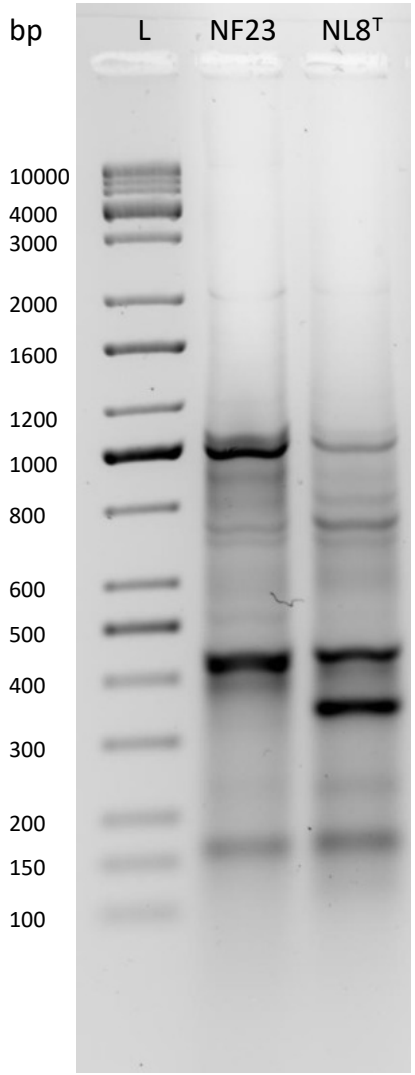

Fig. S5. BOX PCR fingerprint patterns of genomic DNA extracted from isolates NF23 and NL8^T. L, ladder.

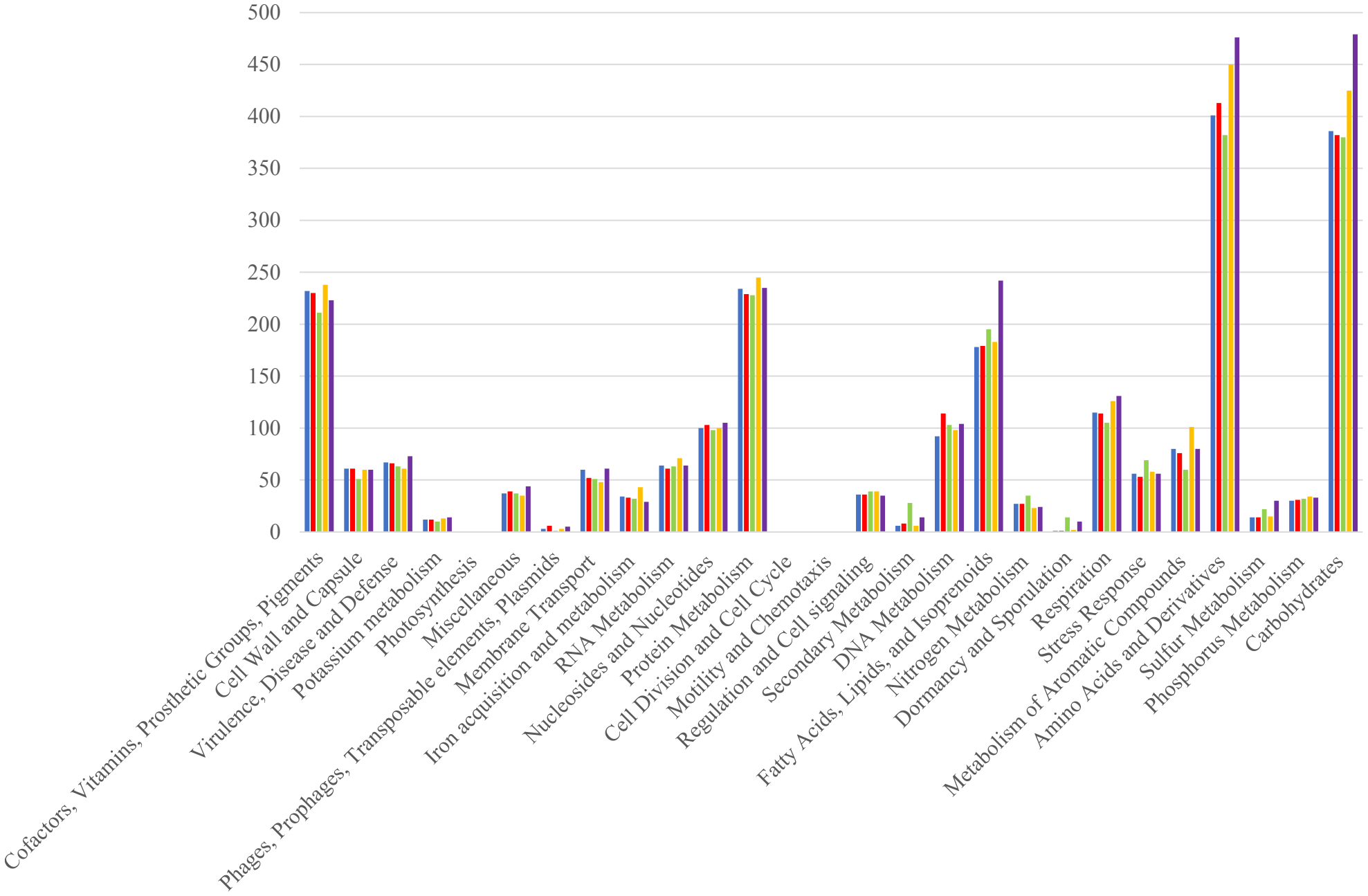

Fig. S6. Distribution of functional gene classes detected in the genomes of isolate NF23 (blue), NL8^{T} (red), *C. acidiphila* DSM 44928^{T} (green), *C. pinisilvae* DSM 111109^{T} (yellow) and *C. rubra* DSM 44948^{T} (purple) using the RAST-SEED server (https://rast.nmpdr.org).

Publikacja VII

Antonie van Leeuwenhoek
https://doi.org/10.1007/s10482-022-01734-8

ORIGINAL PAPER

Genome-based classification of *Streptomyces pinistramenti* sp. nov., a novel actinomycete isolated from a pine forest soil in Poland with a focus on its biotechnological and ecological properties

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Received: 15 December 2021 / Accepted: 19 March 2022

Abstract A genomic-based polyphasic study was undertaken to establish the taxonomic status and biotechnological and ecological potential of a *Streptomyces* strain, isolate SF28^{T}, that was recovered from the litter layer in a Polish *Pinus sylvestris* forest. The isolate had morphological characteristics and chemotaxonomic properties consistent with its classification in the genus *Streptomyces*. It formed long straight chains of spores with smooth surfaces, contained *LL*-diaminopimelic acid, glucose and ribose in whole-organism hydrolysates, produced major proportions of straight, *iso*- and *anteiso*- fatty acids, hexa- and octa-hydrogenated menaquinones with nine isoprene units and had a polar lipid pattern composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmethylethanolamine, glycophospholipids and three uncharacterized components. Phylogenetic trees prepared using 16S rRNA gene and multilocus gene sequences of conserved housekeeping genes showed that the isolate formed a branch that was loosely associated with the type strains of several validly published *Streptomyces* species. A draft genome generated for the isolate was rich in natural product-biosynthetic gene clusters with the potential to produce new specialised metabolites, notably antibiotics, and stress related genes which provide an insight into how it may have become adapted to the harsh conditions that prevail in acidic forest soils. A phylogenomic tree based on the genomes of the isolate and its phylogenetic neighbours confirmed that it formed a distinct lineage well separated from its closest evolutionary relatives. The isolate shared low average nucleotide identity and digital DNA:DNA hybridization values with its phylogenomic neighbours and was also distinguished from them using a combination of cultural and micromorphological properties. Given this wealth of taxonomic data it is proposed that isolate SF28^{T} (=DSM 113360^{T}=PCM 3163^{T}) be classified in the genus *Streptomyces* as *Streptomyces pinistramenti* sp. nov. The isolate showed pronounced antimicrobial activity, especially against fungal plant pathogens.

Keywords *Streptomyces pinistramenti* · Genome · Polyphasic taxonomy · Phylogeny · Biosynthetic Gene Clusters · Antimicrobial activity

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10482-022-01734-8.

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Published online: 11 April 2022

Abbreviations

A2pm	Diaminopimelic acid
ANI	Average nucleotide identity
BGCs	Biosynthetic gene clusters
BLAST	Basic Local Alignment Search Tool
dDDH	Digital DNA–DNA hybridization
DSMZ	German Collection of Microorganisms and Cell Cultures
GGDC	Genome to genome distance calculator
HPLC	High-performance liquid chromatography
IBB	Institute of Biochemistry and Biophysics
ISP	International *Streptomyces* Project
MIDI	Microbial Identification System
ML	Maximum-likelihood
MP	Maximum-parsimony
MRE	Maximal-relative-error
PCM	Polish Collection of Microorganisms
PUAP	Phylogenetic Analysis Using Parsimony
RAST	Rapid Annotation using Subsystem Technology
TLC	Thin-layer chromatography
TNT	Tree Analysis New Technology
TYGS	Type Strain Genome Server

Introduction

The genus *Streptomyces* was proposed by Waksman and Henrici (1943) for aerobic, filamentous, spore-forming actinomycetes and its formal description subsequently emended by Kämpfer (2012). The genus currently includes 685 validly published species (http://www.bacterio.net/streptomyces.html) but remains underspeciated (Sivalingam et al. 2019). Multilocus sequence analyses of concatenated, protein coding, conserved house-keeping genes and associated phenotypic properties provide a more reliable way of recognizing novel *Streptomyces* species than corresponding studies based on 16S rRNA gene sequences (Labeda et al. 2012, 2017; Zhuang et al. 2020). It is also clear that genomic-based classifications are accelerating progress in streptomycete systematics as they provide greater resolution between closely related *Streptomyces* species than corresponding trees based on single and concatenated sequences of conserved genes (Carro et al. 2018; Nouioui et al. 2018; Kusuma et al. 2021). In addition, improved metrics, such as pairwise average nucleotide identity (ANI) and in silico DNA:DNA hybridization (DDH) values, facilitate the recognition of species boundaries (Chun et al. 2018).

Streptomycetes are a unique source of antibiotics which include many used in agriculture, medicine and veterinary practice (Chater, 2016; Qi et al. 2021). Members of the genus are considered to be gifted (Baltz 2017) as they have large genomes ($\geq$ 8.0 Mbp) rich in natural product-biosynthetic gene clusters (NP-BGCs) with the potential to encode for novel and uncharacterized antibiotics of potential therapeutic value, as exemplified by *Streptomyces leeuwenhoekii* strains isolated from an extreme hyper-arid Atacama desert soil (Busarakam et al. 2014; Castro et al., 2018). Novel streptomycetes from extreme biomes are proving to be a potential rich source of new bioactive molecules (Rateb et al. 2018; Sivalingam et al. 2019; Sivakala et al., 2021) thereby underpinning the premise that abiotic conditions in extreme biomes select for strains with the capacity to synthesize novel specialised metabolites (Bull 2011). However, little attention has been focused on the delineation of *Streptomyces* species isolated from coniferous forest soils (Golińska et al. 2022), exceptions include the recognition of *Streptomyces abietis* (Fujii et al. 2013), *Streptomyces pini* (Madhaiyan et al. 2016) and *Streptomyces piniterrae* (Zhuang et al. 2020). Streptomycetes from pine forest soils are also known to be antagonistic towards fungal pathogens of pine seedlings (Golińska and Dahm, 2013). It is becoming increasingly apparent that whole-genomes of actinomycetes isolated from extreme habitats contain stress-related genes that can provide an insight into how they adapt to harsh abiotic conditions that prevail therein (Abdel-Mageed et al. 2020; Golińska et al. 2022).

The present study, a continuation of earlier work on the diversity of filamentous actinomycetes in coniferous litter and soil (Golińska et al. 2022), was designed to establish the taxonomic provenance of a *Streptomyces* strain, isolate SF28^T, recovered from pine forest litter and to determine its ability to inhibit the growth of fungal pathogens. The isolate and its closest phylogenomic neighbours were the subject of a polyphasic study that included information drawn from whole-genome sequences. The resultant data show that the strain inhibits the growth of diverse fungal phytopathogens and belongs to a new *Streptomyces* species, designated *Streptomyces pinistramenti* sp. nov.

Materials and methods

Isolation, maintenance and cultural conditions

Strain SF28^T was isolated from partially decomposed needles (F-horizon) under *Pinus sylvestris* trees growing on the southern slope of an inland sand dune in the Torun Basin, Poland (52°55′37′′N, 18°42′11′′E) in October 2013, using a standard dilution plating procedure (Goodfellow et al. 1967) and starch-casein agar (Küster and Williams 1964) adjusted to pH 4.5 using 1 M HCl. Details of the sampling site and the selective isolation procedure have been described previously (Golińska et al. 2016). The isolate was maintained on starch-casein agar slopes (pH 5.5) at room temperature and as suspensions of mycelial fragments and spores in 20% (v/v) glycerol at − 80 °C. Biomass for most of the chemotaxonomic and molecular systematic studies was prepared by growing the strain in flasks of yeast extract-malt extract broth (ISP 2; International *Streptomyces* Project medium 2; Shirling and Gottlieb 1966), adjusted to pH 5.5, and shaken at 150 rpm for 3 weeks at 28 °C. Cells were harvested by centrifugation and washed three times in sterile distilled water; biomass for the chemotaxonomic analyses was freeze-dried and that for the molecular systematic studies stored at − 20 °C.

Phylogenetic analyses

Genomic DNA was extracted from the isolate using a GenElute™ Bacterial Genomic Kit (Sigma-Aldrich, Germany) and a 16S rRNA gene amplified by PCR following procedures described by Golińska et al. (2013). The PCR product was purified using a purification kit (Qiagen, Germany), according to the manufacturer's instructions, and a quality check made using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). The purified PCR product was sequenced at the Institute of Biochemistry and Biophysics (IBB) of the Polish Academy of Sciences in Warsaw, Poland, using an ABI 3730xl Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, USA).

The almost complete 16S rRNA gene sequence of the isolate was compared with corresponding sequences of type strains of closely related species using the EzBioCloud server (https://www.ezbiocloud.net; Yoon et al. 2017). A maximum-likelihood (ML) and maximum-parsimony (MP) phylogenetic tree was generated using the Single-Gene Trees Phylogeny online tool (https://www.dsmz.de/services/online-tools/single-gene-phylogenies; Meier-Kolthoff et al. 2013a) adapted for single genes. Multiple sequence alignments were generated using MUSCLE software (Edgar 2004) and a ML tree was inferred from alignments with RAxML (Stamatakis 2014) using rapid bootstrapping together with the auto maximal-relative-error (MRE) bootstrapping criterion (Pattengale et al. 2010). Similarly, a MP tree was constructed from alignments with the Tree Analysis New Technology (TNT) program (Goloboff et al. 2008) using 1000 bootstraps together with tree bisection and reconnection branch swapping and ten random sequence replicates. The sequences were checked for compositional bias using the X^2 test, as implemented in PAUP* (Swofford 2002). The neighbour-joining algorithm (Saitou and Nei 1987) and the MEGA7 software package (Kumar et al. 2016) were used to generate a phylogenetic tree and evolutionary distances calculated using the Kimura-2-parameter model (Kimura 1980) with 1000 bootstrap repetitions (Felsenstein 1985). The root position of the trees were determined using a 16S rRNA gene sequence taken from the genome of *Kitasatospora setae* DSM 43861 ^{T} (NC_016109.1) using the SEED viewer (Aziz et al. 2012). A multilocus genome analysis based on 16S rRNA, *atpD*, *gyrB*, *recA* and *rpoB* gene sequences was carried out using an established procedure (Carro et al. 2012) and a MLSA tree generated from nearly 4000 nt using the ML algorithm. Sequence data from all of these genes for each of the tested strains have been deposited in GenBank with the accession numbers shown in Table S1.

Cultural, morphological and phenotypic properties

The growth and cultural properties of the isolate were recorded from tryptone-yeast extract, yeast extract-malt extract, oatmeal, inorganic salts-starch, glucose-asparagine, peptone-yeast extract-iron and tyrosine agars (ISP media 1–7; Shirling and Gottlieb 1966) and from Bennett's and modified Bennett's agar (Jones 1949), HSA5 agar (Busti et al. 2014), nutrient (Becton Dickinson, USA) and 100-fold diluted nutrient agar (Becton Dickinson, USA) and tap water (Harris 1986) agar after 4 weeks incubation at 28 °C. The colour of aerial and substrate mycelia and diffusible

pigments were determined by comparison against NBS/IBCC Colour Charts (Kelly 1958). Hyphal and spore chain features of the isolate were recorded on acidified ISP 2 agar plates (pH 5.5), after 4 weeks at 28 °C, using the coverslip technique of Kawato and Shinobu (1959). Spore arrangement and spore surface ornamentation were established by examining gold-coated dehydrated preparations with growth taken from ISP 2 agar plates (pH 5.5), using the procedure described by O'Donnell et al. (1993) and a scanning electron microscope (Model 1430 V P, LEO Electron Microscopy Ltd, Cambridge, England).

The isolate was also examined for a combination of phenotypic properties. Its ability to grow over a range of pH values (pH 4–13 at single unit intervals), temperatures (4, 10, 15, 20, 25, 30, 35, 40 °C) and in the presence of various NaCl concentrations (1–15 at single unit intervals) were determined using acidified ISP 2 agar (Shirling and Gottlieb 1966) as the basal medium; the pH levels were achieved using KH_2PO_4/HCl (pH range 4–5), KH_2PO_4/K_2HPO_4 (pH range 6–8) and K_2HPO_4/NaOH (pH range 9–13) buffer systems. Standard biochemical, degradative and physiological properties were examined using media and methods described by Williams et al. (1983), albeit with media adjusted to pH 5.5. All of the tests were carried out, in triplicate, using 12-well plates that were inoculated using a standard inoculum corresponding to 5 on the McFarland scale (Murray et al. 1999) and a multipoint inoculator (Mast Uri®Dot, Mast Group Ltd., Merseyside, UK); the inoculated plates were incubated for 3 weeks at 28 °C (apart from the temperature tests). The enzymic activities of the isolate were determined, in duplicate, using API-ZYM kits (BioMerieux, France), according to the manufacturer's instructions.

Chemotaxonomy

Isolate SF28^T was examined for the presence of chemical markers using standard chromatographic methods with appropriate controls. Thin-layer chromatography (TLC) was used to determine isomers of diaminopimelic acid following Staneck and Roberts (1974) and whole-organism sugars according to Hasegawa et al. (1983). Isoprenoid quinones and polar lipids were extracted from freeze-dried cells, as described by Minnikin et al. (1984) and separated using high performed liquid chromatography (HPLC; Kroppenstedt 1985) and two-dimensional TLC (Minnikin et al. 1984), respectively. Cellular fatty acids were extracted, methylated after Miller (1982) with minor modifications from Kuykendall et al. (1988), analysed using the protocol of the Sherlock Microbial Identification (MIDI) system, version 5 (Sasser 1990) and the resultant peaks identified using the ACTIN1 3.80 database.

Whole genome sequencing and phylogenomic analyses

Genomic DNA was extracted from biomass of isolate SF28^T following growth in ISP 2 broth for 7 days at 28 °C using the protocol provided by MicrobesNG (Birmingham, UK; http://www.microbesng.uk) and sequenced on a MiSeq instrument (Illumina, San Diego, USA). Genomic DNA libraries were prepared at MicrobesNG using Nextera XT library preparation kits. The purity and concentration of the extracted genomic DNA was measured using the Microlab STAR handling system (Hamilton, Birmingham, UK) and libraries generated using Kapa Biosystems library quantification kits designed for Illumina instruments on a LightCycler 96 real time PCR instrument (Roche, West Sussex, UK). The libraries were sequenced following the 2×250 bp paired-end protocol (MicrobesNG). Reads were trimmed using Trimmomatic software version 0.30 (Bolger et al. 2014) and their quality assessed using in-house scripts from MicrobesNG; those under 1000 bp were discarded. The resultant reads were assembled into contigs using Spades 3.7 software (Bankevich et al. 2012), annotated with Prokka 1.11 (Seemann 2014) and analysed using the SEED Viewer (Aziz et al. 2012). The genome sequence of isolate SF28^T was deposited in the GenBank database under accession number JAJCXB000000000.

The genome sequences of isolate SF28^T, the type strains of closely related *Streptomyces* species and *Kitasatospora setae* DSM 43861 ^{T} were uploaded onto the Type (Strain) Genome Server (TYGS; Meier-Kolthoff and Göker 2019) and compared using the MASH algorithm which allows a fast approximation of intergenomic relatedness between strains (Ondov et al. 2016). A phylogenomic tree was inferred with FastME 2.1.4 (Lefort et al. 2015) from GBDP distances calculated from the genome sequences and branch lengths scaled using the GBDP distance

formula d_5 (Meier-Kolthoff et al. 2013a); GBDP pseudo-bootstrap support values above branches on the tree were based on 100 replications. The tree was rooted at the midpoint (Farris 1972). Average nucleotide identity (ANI; Rodriguez and Konstantinidis 2016) and digital DNA:DNA hybridization (dDDH; Meier-Kolthoff et al. 2013b) values between genomes of the isolate and its closest phylogenomic neighbours were determined using the online resource from the Rodriguez and Konstantinidis group (http://enveomics.gatach.edu/) and the GGDC web server (http://ggdc.dsmz.de/ggdc), respectively.

Genomic analysis

The presence of BGCs in the genomes of the isolate and its phylogenomic neighbours was investigated using anti-SMASH 6.0 software with "strict" detection criteria and extra features, including KnownClusterBlast, ClusterBlast, SubClusterBlast, MIBiG cluster comparison, ActiveSiteFinder, RREFinder, Cluster Pfam analyses, Pfam-based GO term annotation and TIGRFam analysis (Blin et al. 2021). The distribution of functional gene classes and the presence of stress response genes in the genome of isolate SF28^T were analyzed using the RAST-SEED webserver at https://rast.nmpdr.org/

Antimicrobial screening

The isolate was tested for its ability to inhibit the growth of *Bacillus subtilis* PCM 2021, *Escherichia coli* ATCC 25,922, *Klebsiella pneumoniae* ATCC 700,603, *Micrococcus luteus* ATCC 10,240, *Pseudomonas aeruginosa* ATCC 10,145 and *Staphylococcus aureus* ATCC 25,923 using a standard agar plug assay (Fiedler 2004). It was grown on ISP 2 agar (Shirling and Gottlieb 1966) for 3 weeks at 28 °C when agar plugs (ø = 5 mm) were taken from the plates and placed in square Petri dishes (Sterilin, UK). Overnight cultures of the reference strains (50 µL) grown at 37 °C were used to seed 25 mL of Luria Bertani broth (LB, Becton Dickinson, USA) to an optical density (OD) of 0.6 prior to diluting them to an OD of 0.0125 with 100 mL of LB broth and the same volume of nutrient agar (Becton Dickinson, USA). The final concentration of bacterial cells in the preparations was $1.5–2 \times 10^6$ CFU mL^{-1}. The resultant preparations were thoroughly mixed and poured into the square Petri dishes containing the plugs and incubated for 24 h at 37 °C; inhibition zones around the agar plugs were recorded in millimetres. All of the tests were carried out in triplicate.

A co-culture method described by Świecimska et al. (2021) was used to determine the ability of the isolate to inhibit the growth of fungal and fungal-like plant and human pathogens. Briefly, the isolate was streaked as lines across one side of Potato Dextrose Agar (PDA, Becton Dickinson, USA) plates which were incubated for 14 days at 28 °C. The discs (ø = 8 mm) of pathogens grown on PDA Petri plates for 7–14 days were placed on the opposite side of the plates inoculated with the isolate and the preparations incubated for 7 days in the case of *Alternaria alternata* IOR 1783, *Fusarium culmorum* and *Fusarium oxysporum* (isolated from pine roots), *Fusarium culmorum* D and *Fusarium graminearum* D (isolated from wheat), *Phytophthora plurivora* (isolated from the rhizosphere of oak), *Rhizoctonia solani* (isolated from a pine root) and *Sclerotina sclerotiorum* IOR 2242, for 14 days for *Fusarium poae* A and *Fusarium tricinctum* A (isolated from wheat), *Botritis cinerea* IOR 1873 (isolated from tomato), *Colletotrichum acutatum* IOR 2153 (isolated from blueberry), *Fusarium culmorum* IOR 2333 (isolated from pine), *Fusarium oxysporum* IOR 342 (isolated from pine), *Phytophtora cactorum* IOR 1925 (isolated from strawberry), and for 21 days for *Fusarium graminearum* A and *Fusarium oxysporum* D (isolated from wheat), *Fusarium solani* IOR 825 (isolated from parsley), *Phytophthora cryptogea* IOR 2080 (isolated from Lawson cypress), *Phytophthora megasperma* IOR 404 (isolated from raspberry) and *Phoma lingam* IOR 2284 (isolated from rape). The human pathogens, *Trichophyton erinacei* DSM 25374 ^{T} and *Trichophyton thuringense* DSM 25373 ^{T} were incubated for 14 days. All tests were carried out in triplicate at 28 °C; the negative controls were cultures of the pathogens grown under the same incubation conditions. Inhibition (I) of pathogen growth was calculated using the formula: $I\ (\%) = (C\text{-}T/C) \times 100$, where C is the diameter of pathogen growth in the control sample and T the diameter of the pathogen growth in each of the co-culture samples.

Results and discussion

Isolate SF28^T showed chemotaxonomic, cultural and morphological properties consistent with its classification in the genus *Streptomyces* (Kämpfer 2012; Nouioui et al. 2018). The organism was found to be aerobic, Gram-stain positive, formed an extensively branched substrate mycelium and aerial hyphae that differentiated into long chains of smooth surfaced spores (Fig. S1), and grew from pH 4 to 12, from 4 to 30 °C and in the presence of up to 11% (w/v) NaCl. Whole-organism hydrolysates of the isolate contained *LL*-A_{2pm}, glucose and ribose, the predominant isoprenologues were hexa- and octa-hydrogenated menaquinones with nine isoprene units (74.1 and 23.1%, respectively) and the polar lipid pattern consisted of diphosphatidylglycerol, glycophospholipids, phosphatidylethanolamine (diagnostic component), phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylinositol, and 3 unknown lipids, as shown in Figure S2. The fatty acid profile contained major proportions of *anteiso*-$C_{15:0}$ (26.2%), $C_{16:0}$ (20.0%), *iso*-$C_{16:0}$ (11.0%) and *anteiso*-$C_{17:0}$ (11.5%), smaller proportions (< 11%) of $C_{14:0}$ (1.4%), $C_{15:0}$ (2.4%), 9 methyl-$C_{16:0}$ (1.0%), $C_{16:1}$ cis 9 (5.3%), *anteiso*-$C_{15:0}$ 2OH (3.0%), *anteiso*-$C_{17:1}$ ω9c (1.6%), *iso*-$C_{14:0}$ (1.7%), *iso*-$C_{15:0}$ (8.1%) and *iso*-$C_{17:0}$ (3.1%), and traces of cyclo $C_{17:0}$ and *iso*-$C_{16:1}$ H, and is in line with fatty acid patterns found in related *Streptomyces* strains (Table S2).

The isolate grew well on ISP 2, ISP 6, Bennett's and modified Bennett's agar, moderately well on ISP 1 and 3 and nutrient agar but poorly or not at all on the remaining media, as shown in Table S3.

Phylogeny

The almost complete 16S rRNA gene sequence generated for isolate SF28^T (1414 nt) was deposited in Genbank (accession number: OK576049). The isolate formed a branch in a well-supported subclade in the ML/MP tree which included the type strains of 22 *Streptomyces* species few of which were closely related based on low bootstrap values. Isolate SF28^T was most closely related to the type strains of *Streptomyces kronopolitis* (Liu et al. 2016), *Streptomyces chattanoogensis* (Burns and Holtman 1959) and *Streptomyces lydicus* (De Boer et al. 1956) showing 16S rRNA gene sequence similarities with them of 99.3% (10 nt differences), 99.2% (12 nt differences) and 99.2% (12 nt differences), respectively (Fig. S3). The corresponding sequences between the isolate and the type strains of the remaining *Streptomyces* species ranged from 98.0 (28 nt differences) to 98.9% (15 nt differences; Table S4). The isolate and the *S. kronopolitis* strain formed a well-supported branch in the neighbour-joining tree that was loosely associated with the other *Streptomyces* strains (Fig. S4).

Multilocus sequence analyses of single copies of conserved housekeeping genes provide greater resolution between closely related streptomycetes than corresponding 16S rRNA gene sequence studies as they are based on comparisons of many more nucleotide sequences (Labeda et al. 2012, 2017). In the present study, the isolate was assigned to a subcluster that was supported by a 64% bootstrap value (Fig. 1). This taxon encompassed the type strains of 10 *Streptomyces* species, eight of which featured in the subclade defined in the 16S rRNA gene tree. Isolate SF28^T was most closely related to the type strains of *S. chattanoogensis, Streptomyces inhibens* (Jin et al. 2019), *S. kronopolitis* and *S. lydicus* sharing nucleotide sequence similarities with them of 96.2%, 96.0%, 95.9% and 95.8% (Table S5). The MLSA evolutionary distances between the isolate and the type strains of the most closely related *Streptomyces* species ranged from 0.039 to 0.124 (Table S6), values well above the species threshold of 0.007 used to distinguish between closely related strains, including streptomycetes (Rong and Huang 2014). Based on these data isolate SF28^T was not closely related to the *S. chattanoogensis, S. inhibens* and *S. kronopolitis* strains as it showed evolutionary distance values with them of 0.039, 0.040 and 0.042, respectively. A corresponding distance score of 0.43 was recorded between the isolate and the type strains of *Streptomyces celluloflavus* (Nishimura and Kimura 1953; Madhaiyan et al. 2020), and *S. lyidicus*.

Greater confidence can be placed in the topology of phylogenomic trees than in corresponding 16S rRNA and MLSA trees as they are based on millions not thousands of unit characters (Nouioui et al. 2018). It is evident from the phylogenomic tree (Fig. 2) that the sequence generated for isolate SF28^T and corresponding data available on the type strains of its phylogenetic relatives showed that the isolate formed

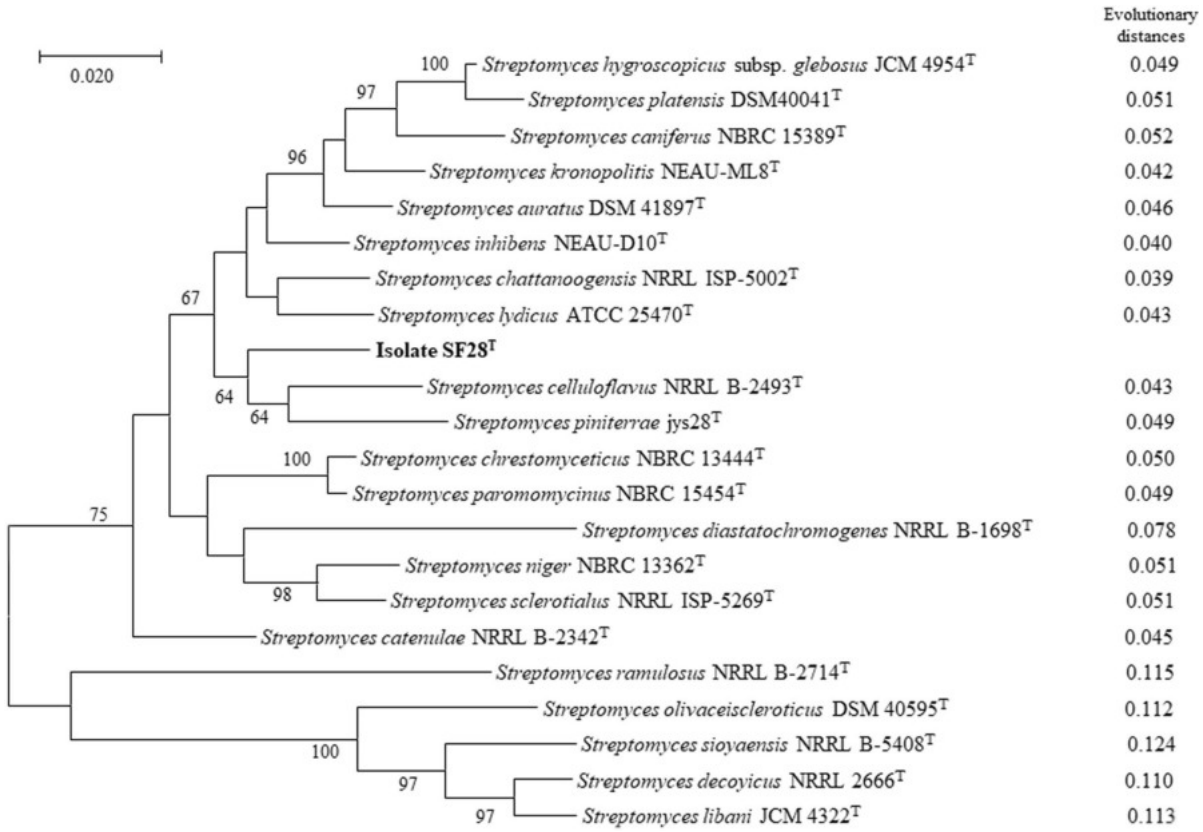

Fig. 1 Maximum-likelihood tree based on MLSA analysis of concatenated sequences from the 16S rRNA gene and five housekeeping genes (*atpD*, *gyrB*, *recA, rpoB* and *trpB)* showing relationships between isolate SF28^T and the type strains of related *Streptomyces* species. The numbers at the nodes are bootstrap support values over 60%. Bar, 0.020 substitutions per nucleotide position

a singleton in a well-supported subclade which included the type strains of 14 *Streptomyces* species, including those representing *S. chattanoogensis, S. kronopolitis* and *S. lyidicus*.

Comparison of genomes

The draft genome sequence of isolate SF28^T has been deposited in GenBank (accession number JAJCXB000000000). It is composed of 133 contigs, has 6594 protein coding genes, 74 RNA genes and L50 and N50 scores of 21 and 117,612, respectively. The total genome size was found to be 7.85 Mbp and the digital (d) G + C content 71.5%. The major classes of functional gene clusters in the genome of the isolate were associated with amino acids and derivatives (441), carbohydrates (297) and protein (237) metabolism, as shown in Figure S5. In general, the genome of the isolate was of a similar size to those of its evolutionary neighbours, as exemplified by *S. chattanoogensis* NRRL ISP-5002 ^{T} (9.1 Mbp; Burns and Holtman 1959), *S. celluloflavus* NRRL B-2493 ^{T} (8.65 Mbp; Madhaiyan et al. 2020), *Streptomyces decoyicus* NRRL 2666 ^{T} (8.6 Mbp; Kumar and Goodfellow 2010), *S. inhibens* NEAU-D10^T (9.5 Mbp; Jin et al. 2019), *S. kronopolitis* NEAU-ML8^T (7.8 Mbp; Liu et al. 2016), *S. lydicus* ATCC 25470 ^{T} (7.9 Mbp; De Boer et al. 1956) and *S. piniterrae* jys28^T (8.5 Mbp; Zhuang et al. 2020).

The dDDH relatedness values between the isolate and its evolutionary relatives fell within the range 21.7 to 28.7% (Table S7), which is well below the 70% threshold for the assignment of bacterial strains to the same species (Wayne et al. 1987). It can also be seen from this Table that the corresponding ANI similarities ranged from 80.5 to 85.1%, similarities

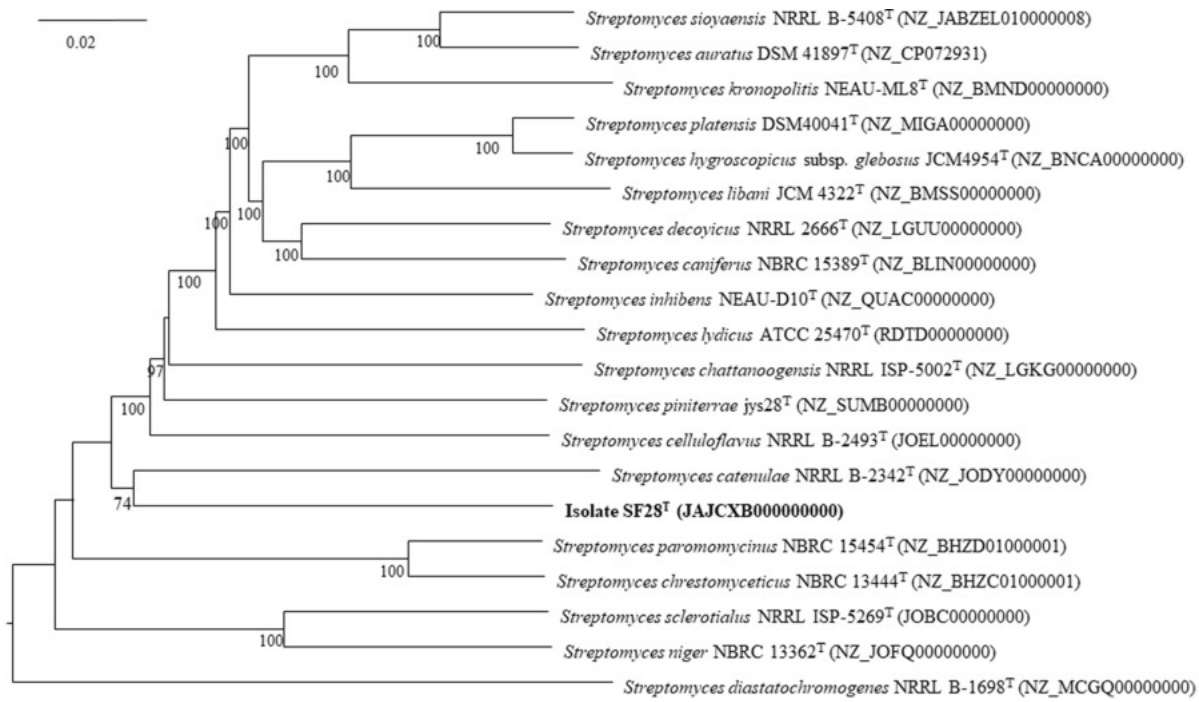

Fig. 2 Phylogenomic tree showing relationships between isolate SF28^T and the type strains of the most closely related *Streptomyces* species constructed using the TYGS server. The numbers above the branches are GBDP pseudo-bootstrap support values greater than 60% from 100 replications with an average branch support of 95.4% The tree was rooted at the midpoint (Farris 1972)

below the recommended threshold (95–96%) for species delineation (Richter and Rosselló-Móra 2009; Lee et al. 2016). These results indicated that isolate SF28^T represents a new *Streptomyces* species which is only loosely associated with its evolutionary relatives. It seems likely that the position of the isolate and its closest phylogenetic/phylogenenomic relatives will only be settled by the addition of new species to this unstable part of *Streptomyces* gene trees. Other *Streptomyces* species found to form distinct lineages in *Streptomyces* gene trees include *Streptomyces adelaidensis* (Kaewkla et al. 2021), *Streptomyces leeuwenhoekii* (Busarakam et al. 2014) and *Streptomyces tardus* (Králová et al. 2021).

Phenotypic properties

Closely related species can usually be distinguished using a broad-range of phenotypic properties (Komaki and Tamura, 2019; Kusuma et al. 2021). Cultural and morphological properties have been shown to be particularly predictive in this respect, as exemplified in extensive phylogenetic analyses of streptomyces species (Labeda et al. 2012, 2017). In the present study, isolate SF28^T was examined for cultural and key morphological features, and for its ability to metabolize a broad range of carbon and nitrogen sources, enzymes and growth characteristics. It is particularly encouraging that identical results were recorded for the duplicated and triplicated cultures. Comparison of some of these properties with corresponding data acquired for *S. decoyicus* NRRL 2666 ^{T}, its closest phylogenomic neighbour based on dDDH similarity, showed that the strains can be distinguished readily using cultural and morphological properties, as well as by associated phenotypic properties that were also recorded using media and methods described by Williams et al. (1983). It is significant that these strains showed different properties when grown on oatmeal agar and exhibit markedly different spore chain morphologies (Table 1). In addition, only the isolate grew at 10 °C and at pH 4.0, and 5.0; it also showed more activity than *S. decoyicus* NRRL 2666 ^{T} when grown on the sole carbon sources.

Table 1 Phenotypic features which separate isolate SF28^T from the type strain of *S. decoyicus*

	Isolate SF28^T	*S. decoyicus** NRRL 2666 ^{T}
Pigments formed on oatmeal agar		
Aerial spore mass	Bluish grey	Grey, becoming black and moist
Substrate mycelium	Brownish grey	Deep yellow
Diffusible pigment	Greyish yellow	None
Morphology		
Spore chains	Rectiflexibles	Spiral
Degradation of		
Xylan, xanthine	+	−
Growth on sole carbon sources (1% w/v)		
Adonitol, amygdalin, *meso*-erythritol, inulin, α-lactose, D-raffinose, L-rhamnose, D-salicin	+	−
D-Xylose	−	+
Tolerance tests		
Growth tests:		
pH 4 and 5	+	−
10 °C	+	−
Growth on presence of:		
NaCl 13% w/v	−	+
DNA G + C content (%)	71.5	70.9
Genome size (Mbp)	7.85	8.63

*Properties of *S. decoyicus* strain are from Kumar and Goodfellow (2010). DNA G + C content and genome size are from GenBank. Both strains formed spores with smooth surfaces, degraded adenine, gelatin, casein, hypoxanthine, L-tyrosine and uric acid, used D-arabitol, D-cellobiose, dextrin, D-fructose, D-galactose, glycogen, D-glucose, glycerol, *myo*-inositol, D-maltose, D-melezitose, D-ribose, sucrose and D-trehalose as sole carbon sources, and L-alanine, L- glutamic acid, L-histidine, L-*iso*leucine, DL-methionine, L-phenylalanine, L-threonine and L-valine as sole nitrogen sources, and grew at pH 9 and 10 and 30 °C. Neither of the strains hydrolysed allantoin, degraded starch or used L-arabinose as a sole carbon source

The isolate was distinguished from the type strains of related *Streptomyces* species listed in Table S7 as they showed different cultural features on oatmeal agar and formed spiral chains of spores, albeit ones with smooth surfaces (Kämpfer, 2012; Liu et al. 2016; Komaki and Tamura 2019; Madhaiyan et al. 2020). It was also be separated from the *S. chattanoogensis, S. hygroscopicus* subsp. *glebosus*, *S. inhibens* and *S. piniterrae* strains as these strains formed spiny, ridged or wrinkled spores in spiral chains (Kämpfer 2012; Jin et al. 2019; Zhuang et al. 2020).

Antimicrobial activity

Good congruence was found between antimicrobial screens that were either duplicated or triplicated. Isolate SF28^T formed zones of inhibition against the *B. subtilis* (9.7 ± 1.2 mm), *E. coli* (3.0 ± 1.0 mm), *K. pneumoniae* (2.0 ± 0.0 mm), *M. luteus* (3.0 ± 0.1 mm), *P. aeruginosa* (3.0 ± 0.1 mm) and *S. aureus* (6.2 ± 0.6 mm) strains in the agar plug assays (Fig. S6). Similar results have been recorded by members of novel *Streptomyces* species isolated from natural habitats, including extreme hyper-arid Atacama Desert soils (Sharma et al. 2014; Goodfellow et al. 2017; Abdelkader et al. 2018; Le Roes-Hill et al. 2018). Similarly, the isolate inhibited the growth of diverse fungal pathogens, including representatives of several *Fusarium* and *Phythophthora* species, as shown in Table 2 and Figure S7. These results provided further evidence that novel *Streptomyces* species from different habitats (Sharma et al. 2014; le Roes Hill et al. 2018), including extreme hyper- and Atacama Desert soils (Goodfellow et al. 2017; Abdelkoder et al. 2018) produce new natural products, especially antifungal antibiotics that inhibit the growth of phytopathogens (Zhao et al. 2017; Singh and Dubey 2018; Qi et al. 2019; Peng et al. 2021)

Table 2 Antimicrobial activity of isolate SF28^T against fungal and fungal-like organisms evaluated using the co-cultured method

Fungi and fungi-like organisms	I (%)
Plant pathogens	
Alternaria alternata IOR 1783	85.6 ± 2.1
Botritis cinerea IOR 1873	82.4 ± 1.4
Chalara fraxinea	61.8 ± 0
Fusarium culmorum	91.9 ± 2.1
Fusarium culmorum D	91.0 ± 0.6
Fusarium graminearum A	91.3 ± 2.3
Fusarium graminearum D	92.5 ± 2.0
Fusarium oxysporum IOR 342	80.3 ± 0
Fusarium poae A	58.1 ± 2.1
Fusarium solani IOR 825	88.9 ± 1.4
Fusarium tricinctum A	50.2 ± 2.1
Phoma lingam IOR 2284	89.7 ± 0
Phytophthora cactorum IOR 1925	90.5 ± 0
Phytophthora cryptogea IOR 2080	60.2 ± 0.7
Phytophthora megasperma IOR 404	52.1 ± 2.8
Phytophthora plurivora	92.2 ± 0
Rhizoctonia solani	94.4 ± 0.6
Sclerotina sclerotiorum IOR 2242	89.3 ± 0.7
Human pathogens	
Trichophyton erinacei DSM 25,374	92.0 ± 0
T. thuringense DSM 25,373	78.7 ± 2.3

I; % inhibition of fungal growth that was calculated using the formula: I (%) = (C-T/C) × 100, where C is the diameter of pathogen growth in the control sample and T the diameter of the pathogen growth in each of the co-culture samples

and which thereby show promise as biocontrol agents (Cao et al. 2020). It is also interesting that the isolate showed pronounced activity against representatives of the two *Trichophyton* species (Table 2, Fig. S7).

Genome mining

AntiSMASH predicts BGCs and potential products based on the percentage of genes from the closest known bioclusters showing significant BLAST hits against corresponding clusters in the genomes of strains under consideration (Blin et al. 2021). The genome of isolate SF28^T was found to contain 29 BGCs, notably ones predicted to encode for druggable molecules such as non-ribosomal peptide synthetases (Table 3) which may be involved in the results of the antimicrobial screening studies considered above. Eleven of the bioclusters showed at least 50% gene identity with known compounds, as exemplified by those associated with the synthesis of anantin C (75% gene identity), a peptide antagonist of the atrial natriuretic factor (Tietz et al. 2017), desferrioxamine E (100% gene identity), a siderophore which forms stable hexadentate complexes with ferric ions (Barona-Gómez et al. 2004), ectoine (100% gene identity), which protects against osmotic stress and desiccation (Prabhu et al. 2004), ethylenediaminesuccinic acid hydroxyarginine (EDHA) (100% gene identity), a second line siderophore (Spohn et al. 2018), and lugdunomycin (74% gene identity), a novel aromatic polyketide with antibacterial activity (Wu et al. 2019). Similarly, several bioclusters were predicted to encode for a range of products, as exemplified by those involved in the synthesis of alkylresorcinol (100% gene identity) which has multiple biological functions (Funabashi et al. 2008; Nakano et al. 2012), a heat-stable antifungal factor (75% gene identity) that inhibits the growth of diverse fungi (Yu et al. 2007) and the tallysomycins (TLMs) (60% gene identity), which are antitumor antibiotics (Tao et al. 2007). Other bioclusters found in the genomes of the isolate were associated with the synthesis of 3,7-dihydroxytropolone (33% gene identity) which shows antimicrobial, anticancer and antiviral activity (Chen et al. 2018), lankacidin C (26% gene identity) that is known to have antibacterial and antitumor properties (Ayoub et al. 2019) and stenothricin (13% gene identity), a cyclic depsipeptide that exhibits antibacterial activity (Liu et al. 2014). Other BGCs encoded for antibacterial compounds such as dutomycin and lactonamycin showed 4 and 3% gene identity, respectively, with known compounds (Matsumoto et al. 1999; Sun et al. 2016). Moreover, the genome of isolate SF 28^T included 7 bioclusters that were predicted to encode for unknown compounds.

The SEED analyses (Aziz et al. 2012) showed that the genome of isolate SF28^T contained 61 putative stress related genes, notably those linked to cold and heat shock responses, and DNA repair and oxidative stress (Table S8, Fig. S5). This complement of genes included *cspC* and *cspE* which express for cold shock proteins (Etchegaray and Inouye 1999) and chaperone genes such as *clpB, clpC, clpX* and *hrcA* that are involved in responses to heat shock (Li et al. 2011). In turn, genes such as *betA, betB* and *proU* are involved in the uptake of betaine and choline, metabolites,

Table 3 The distribution of BGCs in the genome of isolate SF28^T

Gene type	Product	Span (nt)	Gene similarity (%)	Most similar biosynthetic gene cluster is from	Similarity (%)
Ectoine	Ectoine	123,024–133,437	100	*Streptomyces kasugaensis* BCRC 12,349	100
Lassopeptide	Anantin C	1–14,4	75	*Geodermatophilus siccatus* DSM 45,419	25
				Streptomyces cyaneogriseus subsp. *noncyanogenus*	25
				Streptomyces leeuwenhoekii	25
				Streptomyces kasugaensis BCRC 12,349	25
NAPAA	Eethylenediaminesuccinic acid hydroxyarginine (EDHA)	1–38,693	100	*Kitasatospora* sp. CB02891	37
NRPS/ Type 1 PKS (T1PKS)	Tallysomycin A	9,621–84,434	60	*Actinosynnema mirum* DSM 43,827	39
NRPS/ Type 1 PKS (T1PKS)	Heat-stable antifungal factor	130,781–204,146	75	*Streptomyces* sp. CS090A	30
				Streptomyces fulvissimus DSM 40,593	30
Siderophore	Desferrioxamine E	71,733–81,169	100	*Streptomyces* sp. MOE7	83
				Streptomyces lydicus GS93	83
				Streptomyces lydicus 103	83
				Streptomyces lydicus WYEC 108	83
				Streptomyces sp. RPA4-5	83
				Streptomyces platensis ATCC 23,948	83
				Streptomyces sp. NEAU-S7GS2	83
				Streptomyces lydicus strain A02	83
				Streptomyces sp. GS7	83
				Streptomyces sp. 2323.1	83
Type 2 PKS (T2PKS)	Lugdunomycin	37,499–80,898	74	*Streptomyces* sp. CB02414	36
Type 2 PKS (T2PKS)	Spore pigment	84,575–157,09	83	*Streptomyces* sp. PAMC 26,508	23
				Streptomyces microflavus CG 893	23
				Streptomyces pratensis ATCC 33,331	23
				Streptomyces sp. Root1304	23
Type 3 PKS (T3PKS)	Naringenin	213,991–241,849	100	*Streptomyces griseorubens* JSD-1	26
Type 3 PKS (T3PKS)/ Terpene	Alkylresorcinol	59,464–105,812	100	*Nonomuraea* sp. WYY166	22
Terpene/Lanthipeptide class I/NRPS	Hopene	221,191–291,727	69	*Streptomyces platensis* ATCC 23,948	100

which have a role in responses to osmotic stress (Boncompagni et al. 1999; Nau-Wagner et al. 2012), as are enzymes expressed by genes *katE* and *soxR* (Normand et al. 2012; Golińska et al. 2020), and the products of genes *trx* and *trxR* (Kim et al. 2008). The genome of the isolate also included genes such as *RecF, RecO and RecR*, *UvrD* which are associated with DNA repair and stabilizing (Hickson 2003; Kang and Blaser 2006). The detection of a *CoxG* gene, which encodes for a subunit of carbon monoxide dehydrogenase suggests that the isolate may be able to adapt to a chemolitotrophic lifestyle by using carbon monoxide as a carbon and energy source (Lorite et al. 2000), as is the case with the type strains of *Streptomyces thermocarboxydovorans* and *Streptomyces thermocarboxydus* (Kim et al. 1998). The genome of the isolate is also rich in genes that express for DNA polymerase Sigma factors, as shown in Table S8. Some of these genes, such as *sigB,* which is involved in osmotic stress, are upregulated under acidic conditions (Kim et al. 2008). These authors also showed that this applies to heat shock genes, including those found in the genome of the isolate, notably ones supressing proteins belonging to the DnaK family and chaperones, such as GroEL2. It is also interesting that the genome of the isolate contained gene *atpA,* which is involved in a transmembrane protein transport system and is known to enhance survival of bacteria under acidic conditions (Guan and Liu 2020). These results provide further evidence that pH is a major factor governing the survival and distribution of streptomycetes in acidic soils (Williams et al. 1971; Goodfellow and Williams 1983).

Conclusions

Isolate SF28^T showed antimicrobial activity, notably against fungal pathogens, has a large genome rich in BGCs predicted to encode for a broad range of specialised metabolites, especially putatively new antibiotics, and stress related genes, notably ones associated with adaptation to acidic conditions. It is also evident from the sequence data that isolate SF28^T forms a distinct lineage within the evolutionary radiation occupied by *Streptomyces* species. It is only loosely associated with its closest phylogenetic/phylogenomic neighbours, a point underlined by corresponding low ANI and dDDH similarities. It can also be distinguished from these organisms using key cultural and micromorphological properties. Consequently, the isolate is considered to represents a novel *Streptomyces* species for which the name *Streptomyces pinistramenti* sp. nov. is proposed.

Description of *Streptomyces pinistramenti* sp. nov.

Streptomyces pinistramenti (pi.ni.stra.men'ti. L. fem. n. *pinus*, pine tree; L. neut. n. *stramentum*, litter; N.L. gen. n. *pinistramenti*, of pine litter).

Aerobic, Gram stain-positive actinomycete which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long chains of smooth surfaced spores. Grows from 4 to 30 °C, optimally around 25 °C, from pH 4–12, abundantly from pH 5–11, and in the presence of 0–11% NaCl w/v, optimally without NaCl. Grows well on yeast extract-malt extract agar forming a brownish grey substrate mycelium, a greenish grey aerial spore mass and a greyish yellow diffusible pigment. Hydrolyses aesculin and arbutin, but not allantoin or urea. Nitrate is reduced. Adenine, casein, gelatin, hypoxanthine, Tweens 40 and 60, L-tyrosine, uric acid, xanthine and xylan are degraded, but not chitin, elastin, guanine, starch, Tweens 20 or 80. Positive for cystine, leucine and valine arylamidases, α- chymotrypsin, acid- and alkaline- phosphatases, α-galactosidase, β-glucosidase, α-mannosidase, naphthol-AS-BI-phosphohydrolase and trypsin, but negative for esterase (C4), esterase lipase (C8), α-fucosidase, β-galactosidase, N-*acetyl*-β-glucosaminidase α-glucosidase, β-glucuronidase and lipase (C14) (API-ZYM tests). Adonitol, amygdalin, D-arabitol, D-cellobiose, dextrin, *meso*-erythritol, D-fructose, D-galactose, D-glucosamine, D-glucose, glycerol, glycogen, *meso*-inositol, inulin, D-lactose, D-maltose, D-mannitol, D-melezitose, D-melibiose, α- and β-*methyl*-D-glucosides, D-raffinose, L-rhamnose, D-ribose, D-salicin, D-sucrose, D-trehalose and xylitol are metabolized as sole carbon sources, but not L-arabinose, L-arabitol, D-glucuronic acid or D-xylose (all at 1% w/v). Metabolizes acetate, citrate, fumarate, hippurate, propionate, pyruvate and succinate, but not adipate, benzoate, butyrate or oxalate (sodium salts) or *para*-hydroxybenzoic acid (all at 0.1% w/v). L-alanine, L-arginine, L-asparagine, L-cysteine, ethanolamine, L-glutamic acid, L-histidine, L-hydroxyproline,

L-*iso*leucine, L-methionine, L-phenylalanine, L-serine, L-threonine and L-valine are used as sole nitrogen sources, but not acetamide or L-aspartic acid (all at 0.1% w/v).

The cell wall peptidoglycan contains *LL*-diaminopimelic acid, the whole organism sugars are glucose and ribose, the major fatty acids are *anteiso*-$C_{15:0}$, $C_{16:0}$, *iso*-$C_{16:0}$ and *anteiso*-$C_{17:0}$, the predominant menaquinones MK-9 (H_6) and MK-9 (H_8), and the major polar lipids diphosphatidylglycerol, glycophospholipids, phosphatidylethanolamine (diagnostic component), phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylinositol, and 3 unknown lipids.

The type strain SF28^T (= DSM 113360 ^{T} = PCM 3163 ^{T}) was isolated from partially decomposed needles under *Pinus sylvestris* trees growing on the southern slope of an inland sand dune in the Toruń Basin, Poland.

Novel streptomycetes isolated from extreme habitats are a rich source of new specialised metabolites, including antibiotics (Rateb et al. 2018; Sivalingam et al. 2019; Sivakala et al. 2021). It is, therefore, surprising that streptomycetes known to be common in coniferous forest soils (Golińska et al. 2022) have received little attention, especially since they have been shown to be antagonistic to fungal pathogens (Golińska and Dahm 2013; Cao et al. 2020) and can form mutualistic associations with the pine beetle, *Dendroctonus frontalis* (Strzelczyk and Szpotański 1989; Scott et al. 2008). It is also interesting that *S. piniterrae* jys28^T, an isolate from the rhizosphere soil of *Pinus yunnanensis*, contains a putative gene cluster that encodes for the synthesis of heliquinomycins which belong to the rubromycin family of compounds (Zhuang et al. 2020). Consequently, the discovery that *S. pinistramenti* SF28^T shows pronounced activity against diverse fungal plant pathogens provides further evidence that novel *Streptomyces* species isolated from coniferous forest soils merit greater attention as a source of new bioactive metabolites. It is also interesting that *S. pinistramenti* SF28^T and *S. piniterrae* jys28^T are associated with strains that synthesise novel antibiotics, as exemplified by *S. decoyicus* NRRL ISP-5087 ^{T} which produces psicofuranine, a purine nucleoside antibiotic that shows antibacterial and antitumor activity (Eble et al. 1959; Vavra et al 1959).

Acknowledgements We are grateful to Professor Aharon Oren (Hebrew University of Jerusalem) for suggesting the species name.

Author Contributions PG and MG conceived the study, PG and MŚ designed it and MŚ carried out all the experiments and was responsible for the data analyses. MŚ wrote the first draft of the manuscript which was revised by PG and MG. The final version of the manuscript was approved by all of the authors.

Funding This work was supported by Grants No. 1207-B and No. 90-SIDUB.6102.40.2021.G4NCUS1 from Nicolaus Copernicus University.

Data Availability The GenBank accession numbers for the 16S rRNA and the whole-genome sequences of strain SF28^T are OK576049 and JAJCXB000000000, respectively.

Declarations

Conflict of interest The authors declare that they do not have any conflicts of interest.

Ethical approval This article does not contain any studies with human participants and/or animals performed by any authors. Formal consent is not required in this study.

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Supplementary material

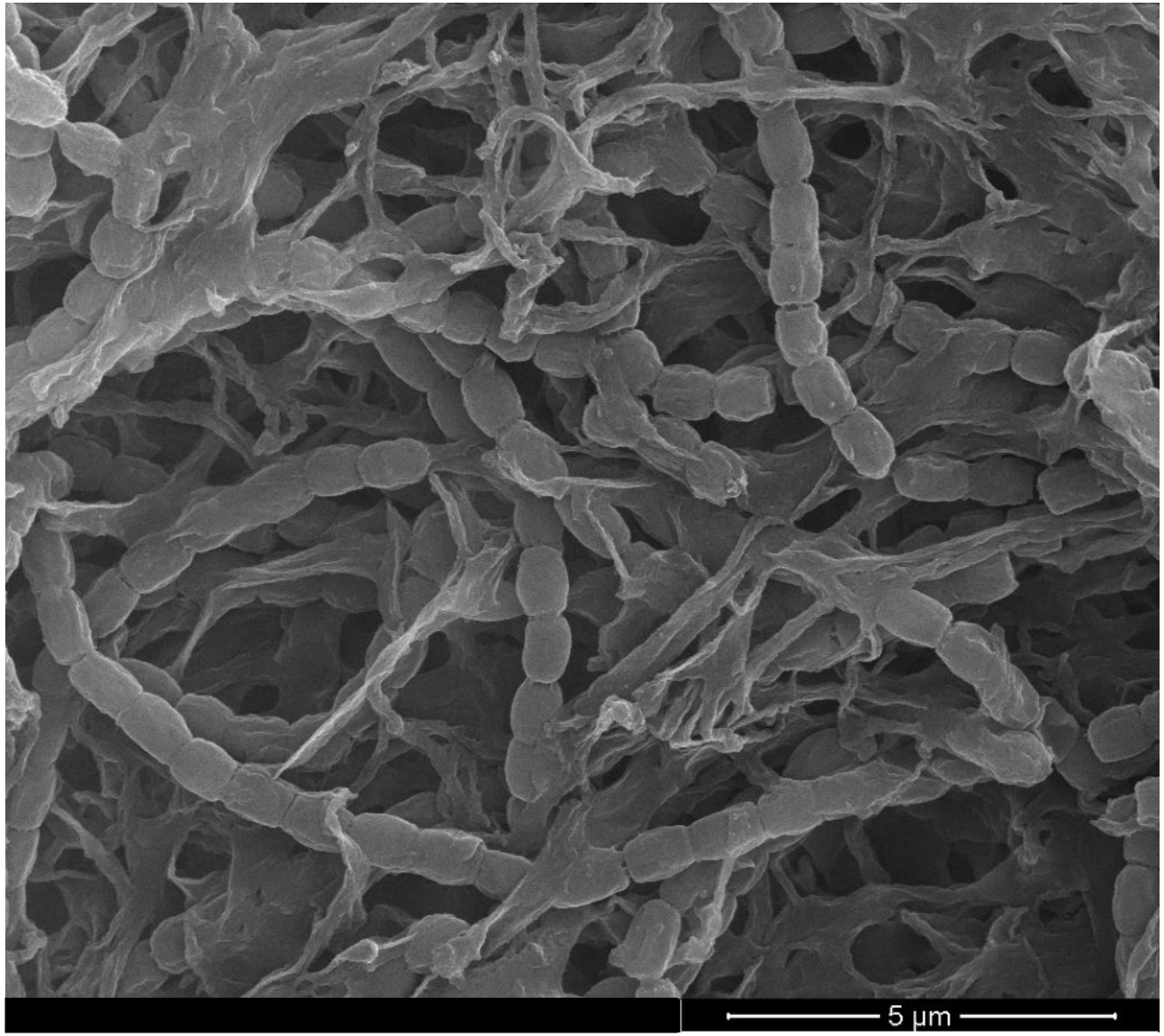

Fig. S1. Scanning electron micrograph of isolate SF28^T showing straight chains of cylindrical spores with smooth surfaces following growth on ISP 2 agar for 4 weeks at 28 °C. Bar, 5 µm

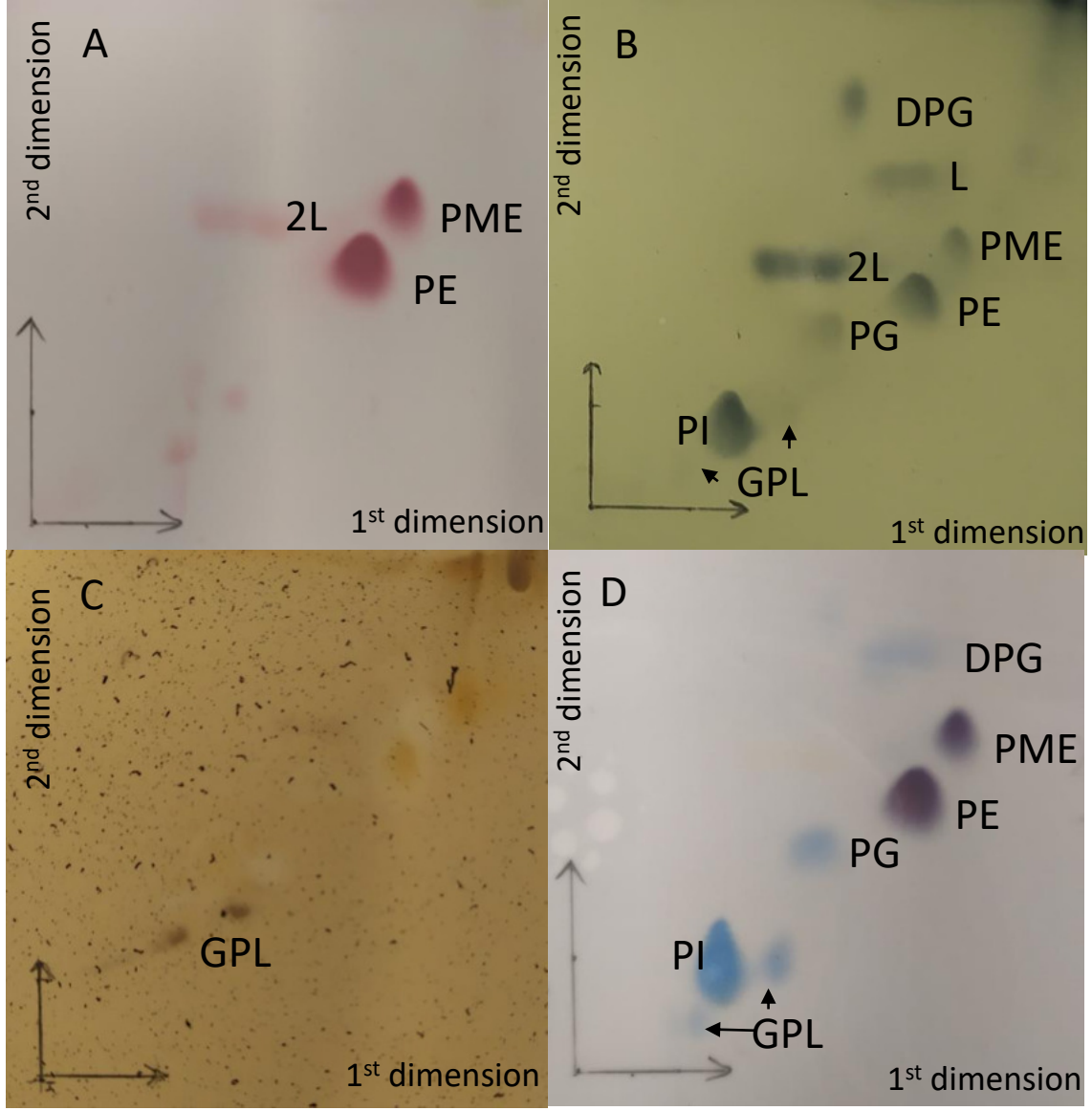

Fig. S2. Polar lipid profile of isolate SF28^T obtained using two dimensional thin-layer chromatography. TLC plates were stained with ninhydrin (A), molybdatophosphoric acid (B), α-naphthosulfuric acid (C) and molybdenum blue (Sigma) (D) for the detection of total polar lipids. Chloroform:methanol:water (32.5:12.5:2.0 v/v) was used in the first direction and chloroform:acetic acid:methanol: water (40:7.5:6:2 v/v) in the second direction. DPG diphosphatidylglycerol, GPL glycophospholipids, PE phosphatidylethanolamine, PG phosphatidylglycerol, PI phosphatidylinositol, PIM phosphatidylinositol mannoside, PME phosphatidylmethylethanolamine, L unknown lipid.

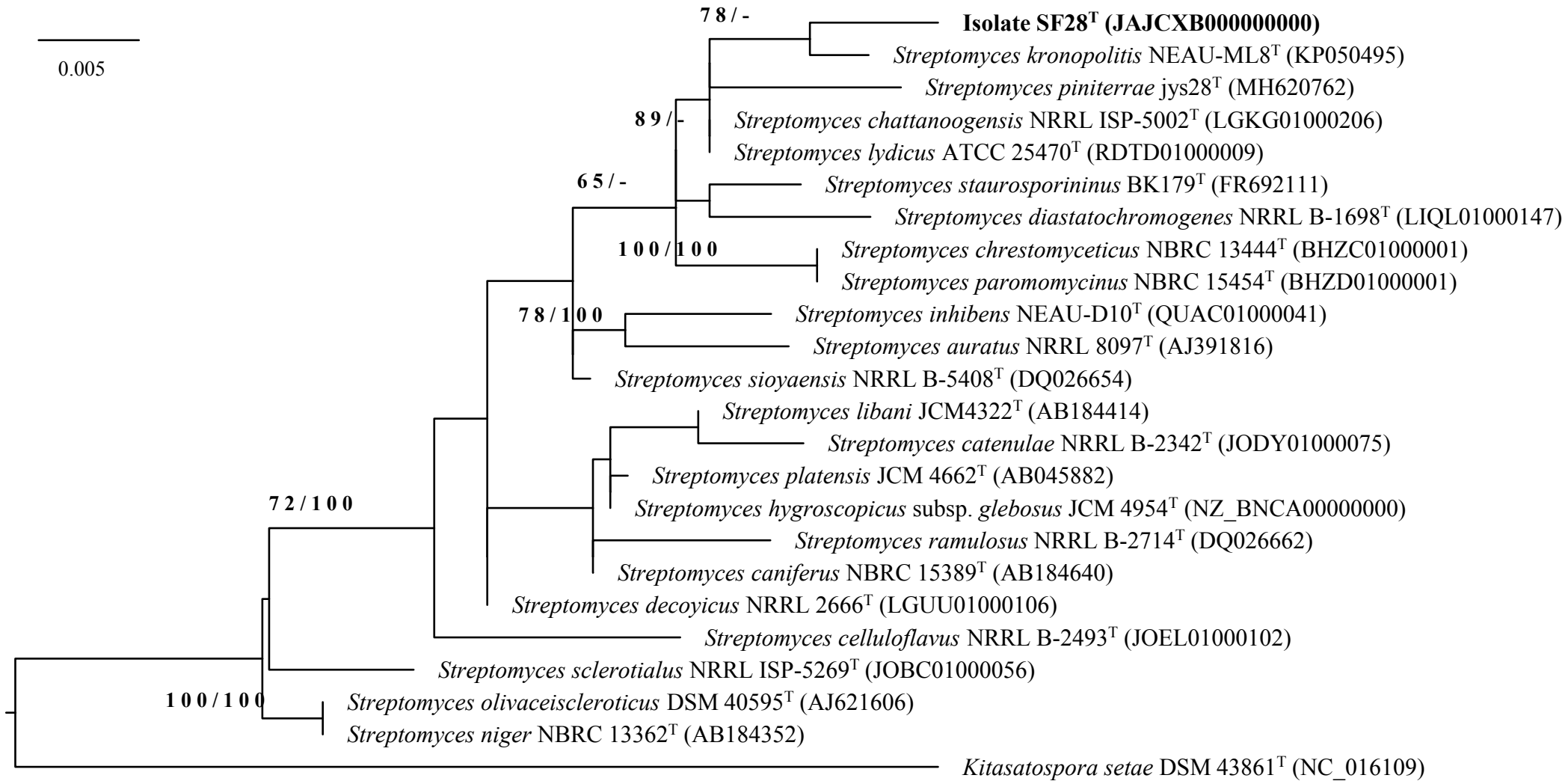

Fig. S3. Maximum-likelihood and maximum-parsimony tree based on nearly complete 16S rRNA gene sequences showing relationships between isolate SF28^T and the type strains of closely related *Streptomyces* species. The numbers above the branches are bootstrap support values greater than 60% for ML (left) and MP (right). Bar 0.005 substitutions per nucleotide position. The root position of the tree was determined using *Kitasatospora setae* DSM 43861^T.

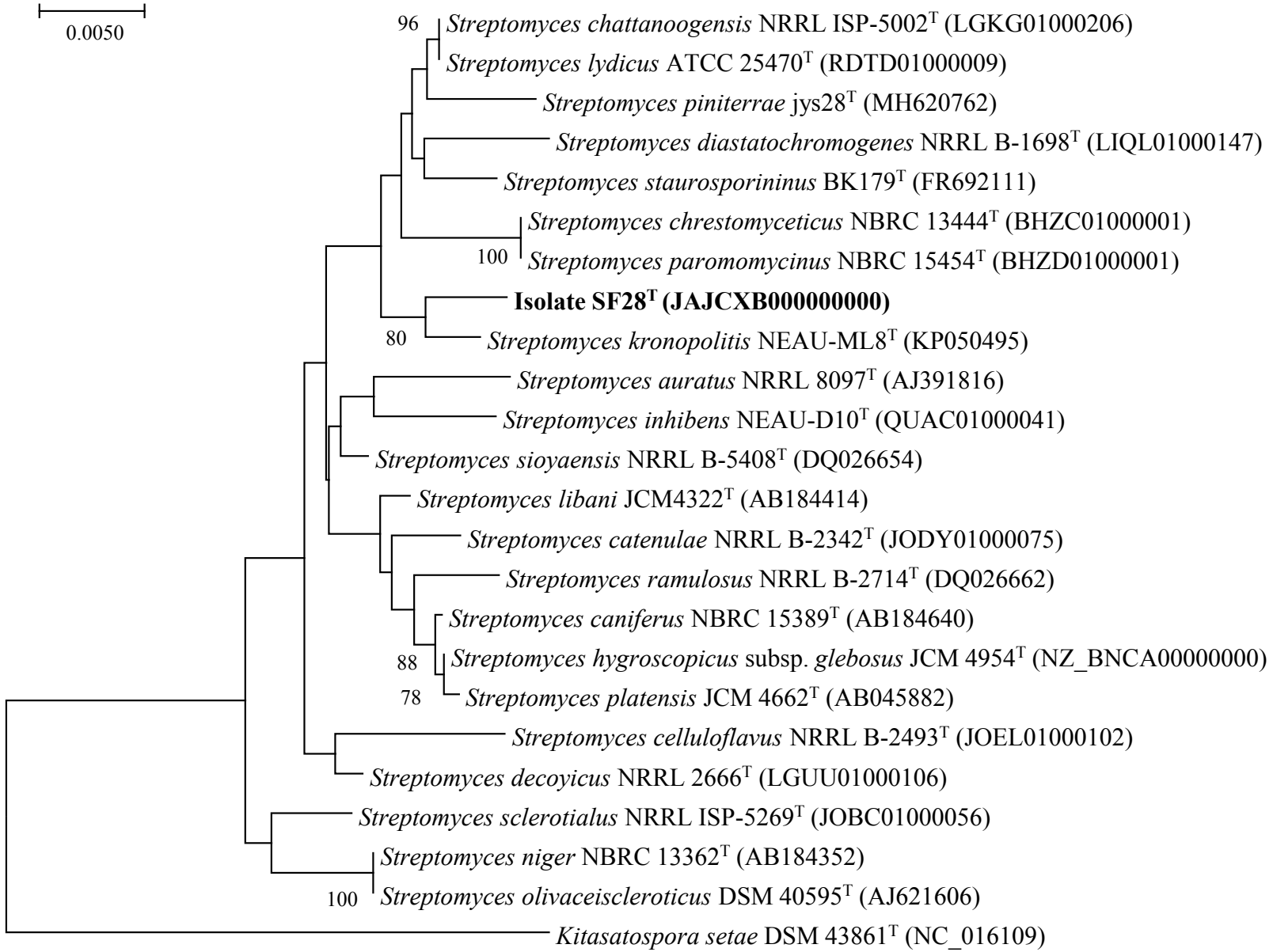

Fig. S4. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between isolate SF28^T and the type strains of closely related *Streptomyces* species. The numbers above the branches are bootstrap support values greater than 60%. Bar 0.005 substitutions per nucleotide position. The root position of the tree was determined using *Kitasatospora setae* DSM 43861^T.

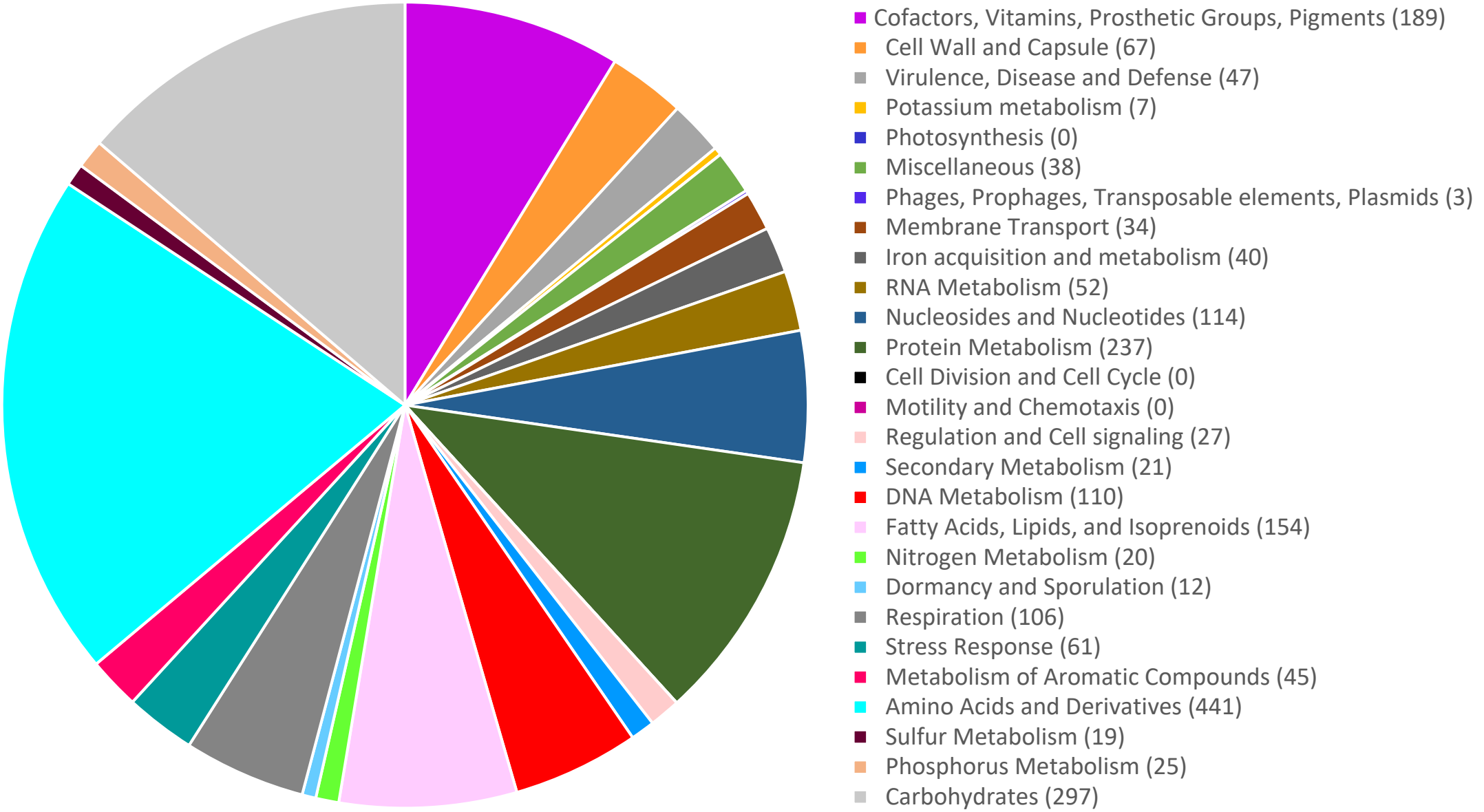

Fig. S5. Distribution of functional gene classes found in the genome of isolate SF28^{T} generated using the RAST-SEED webserver at https://rast.nmpdr.org/.

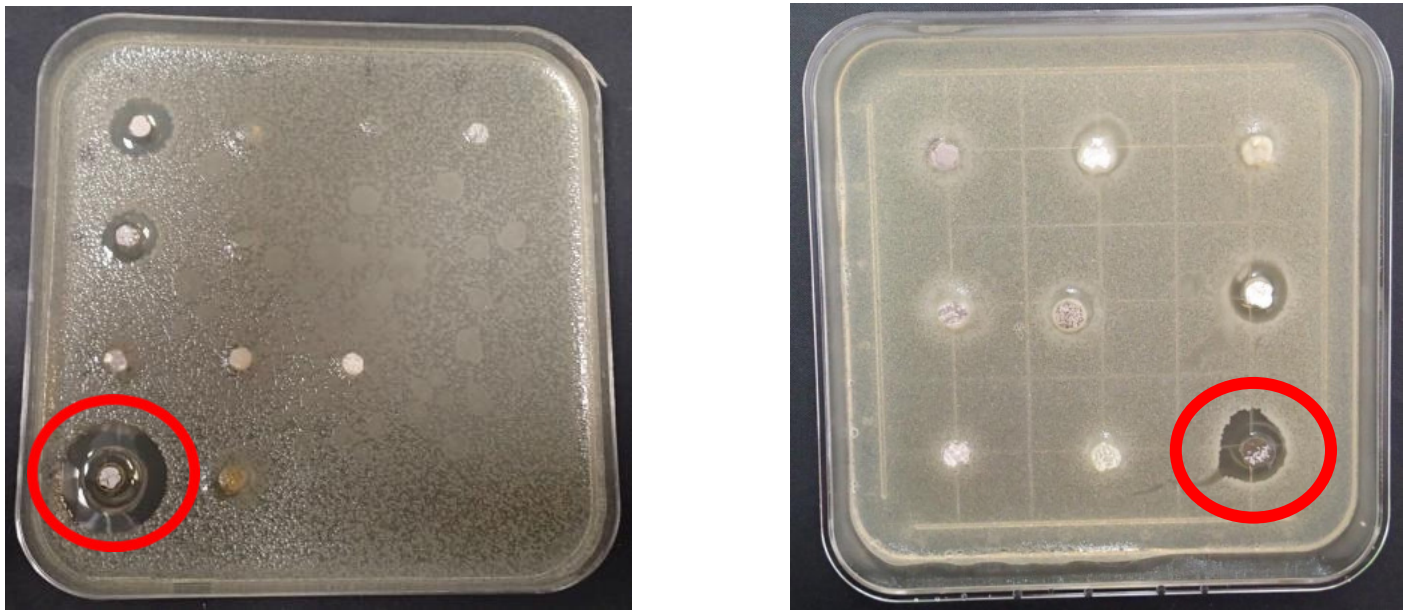

Fig. S6. Antibacterial activity of isolate SF28^{T} (in a red circle) against A, *B. subtilis* and B, *S. aureus*.

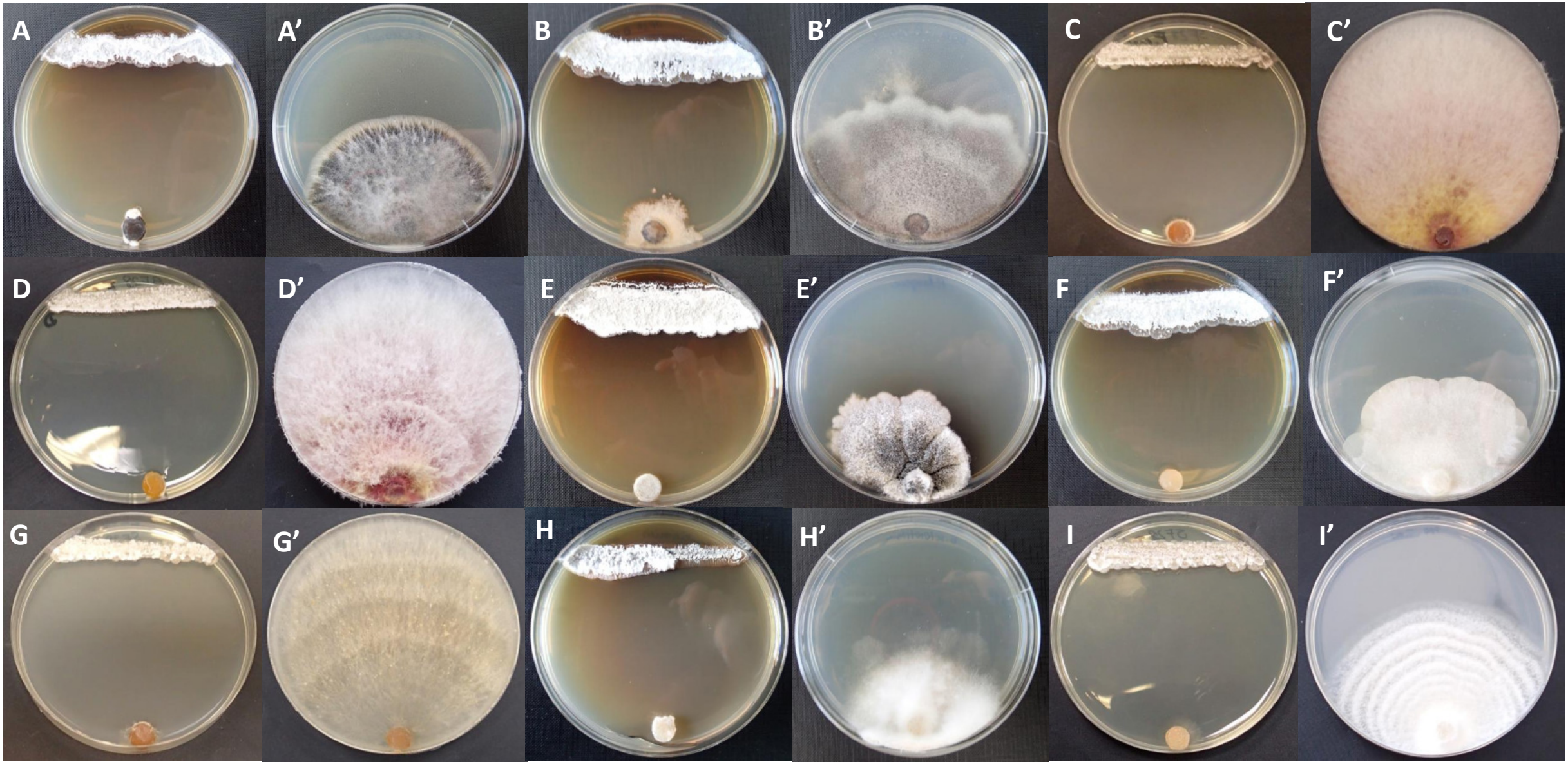

Fig. S7. Antimicrobial activity of isolate SF28^{T} against A, *A. alternata*; B, *B. cinerea*; C, *F. culmorum; D, F. graminearum* D; E, *P. lingam*; F, *P. cactorum*; G, *R. solani*; H, *S. sclerotiorum*; I, *T. erinacei* in co-cultures. A’-I’ are corresponding negative controls.

Table S1. GenBank accession numbers of 16S rRNA, *atpD, gyrB, recA, rpoB* and *trpB* gene sequences of isolate SF28^{T} and its closest phylogenetic relatives.

Strain	16Sr RNA	*atpD*	*gyrB*	*recA*	*rpoB*	*trpB*
Isolate SF28^{T}	OK576049	JAJCXB000000000	JAJCXB000000000	JAJCXB000000000	JAJCXB000000000	JAJCXB000000000
Streptomyces auratus DSM 41897^{T}	AJ391816	NZ_CP072931	NZ_CP072931	NZ_CP072931	NZ_CP072931	NZ_CP072931
Streptomyces caniferus NBRC 15389^{T}	AB184640	NZ_BLIN00000000	NZ_BLIN00000000	NZ_BLIN00000000	NZ_BLIN00000000	NZ_BLIN00000000
Streptomyces catenulae NRRL B-2342^{T}	JODY01000075	NZ_JODY00000000	NZ_JODY00000000	NZ_JODY00000000	NZ_JODY00000000	NZ_JODY00000000
Streptomyces celluloflavus NRRL B-2493T	JOEL01000102	JOEL00000000	JOEL00000000	JOEL00000000	JOEL00000000	JOEL00000000
Streptomyces chattanoogensis NRRL ISP-5002^{T}	LGKG01000206	NZ_LGKG00000000	NZ_LGKG00000000	NZ_LGKG00000000	NZ_LGKG00000000	NZ_LGKG00000000
Streptomyces chrestomyceticus NBRC 13444^{T}	BHZC01000001	BHZC01000001	BHZC01000001	BHZC01000001	BHZC01000001	BHZC01000001
Streptomyces decoyicus NRRL 2666^{T}	LGUU01000106	NZ_LGUU00000000	NZ_LGUU00000000	NZ_LGUU00000000	NZ_LGUU00000000	NZ_LGUU00000000
Streptomyces diastatochromogenes NRRL B-1698^{T}	LIQL01000147	NZ_MCGQ00000000	NZ_MCGQ00000000	NZ_MCGQ00000000	NZ_MCGQ00000000	NZ_MCGQ00000000
Streptomyces hygroscopicus subsp. *glebosus* JCM 4954^{T}	NZ_BNCA00000000	NZ_BNCA00000000	NZ_BNCA00000000	NZ_BNCA00000000	NZ_BNCA00000000	NZ_BNCA00000000
Streptomyces inhibens NEAU-D10^{T}	QUAC01000041	NZ_QUAC00000000	NZ_QUAC00000000	NZ_QUAC00000000	NZ_QUAC00000000	NZ_QUAC00000000
Streptomyces kronopolitis NEAU-ML8^{T}	KP050495	NZ_BMND00000000	NZ_BMND00000000	NZ_BMND00000000	NZ_BMND00000000	NZ_BMND00000000
Streptomyces libani JCM 4322^{T}	FJ406169	FJ406169	FJ406225	FJ406281	FJ406337	FJ406392
Streptomyces lydicus ATCC 25470^{T}	RDTD01000009	RDTD00000000	RDTD00000000	RDTD00000000	RDTD00000000	RDTD00000000
Streptomyces niger NBRC 13362^{T}	AB184352	NZ_JOFQ00000000	NZ_JOFQ00000000	NZ_JOFQ00000000	NZ_JOFQ00000000	NZ_JOFQ00000000
Streptomyces olivaceiscleroticus DSM 40595^{T}	AJ621606	JF424198	JF424104	JF424058	JF424011	JF423964
Streptomyces paromomycinus NBRC 15454^{T}	BHZD01000001	NZ_BHZD01000001	NZ_BHZD01000001	NZ_BHZD01000001	NZ_BHZD01000001	NZ_BHZD01000001
Streptomyces piniterrae jys28^{T}	MH620762	NZ_SUMB00000000	NZ_SUMB00000000	NZ_SUMB00000000	NZ_SUMB00000000	NZ_SUMB00000000
Streptomyces platensis DSM 40041^{T}	AB045882	NZ_MIGA00000000	NZ_MIGA00000000	NZ_MIGA00000000	NZ_MIGA00000000	NZ_MIGA00000000
Streptomyces ramulosus NRRL B-2714^{T}	DQ026662	MF581785	KC954564	MF581787	MF581789	MF581783
Streptomyces sclerotialus NRRL ISP-5269^{T}	JOBC01000056	JOBC00000000	JOBC00000000	JOBC00000000	JOBC00000000	JOBC00000000

Streptomyces sioyaensis NRRL B-5408T	DQ026654|	NZ_JABZEL010000008	FJ406188	NZ_JABZEL010000008	MG881217	MG881219

Table S2. Fatty acid profiles (>10%) of isolate SF28^T and those available for the type strains of related species.

	$C_{14:0}$	***iso*-$C_{14:0}$**	**$C_{15:0}$**	***anteiso*-$C_{15:0}$**	***iso*-$C_{15:0}$**	**$C_{16:0}$**	**$C_{16:1}\omega7c$**	***iso*-$C_{16:0}$**	***anteiso*-$C_{17:0}$**	**$C_{18:0}$**	**Database**	**References**
Isolate SF28^T				26.2		20.0		11.0	11.5		ACTIN13.80	
***S. celluloflavus* NRRL B-2493^T**				+		+		+	+		no data	Madhaiyan et al. 2020
***S. chattanoogensis* NRRL ISP-5002^T**						30.0	16.2			41.4	NIST 14	Zhuang et al. 2020
***S. kronopolitis* NEAU-ML8^T**			10.4	18.6		15.0		17.7			no data	Liu et al. 2016
***S. lydicus* ATCC 25470^T**	14.3		18.2			28.4		12.7	11.1		NIST 14	Zhuang et al. 2020
***S. paromomycinus* NBRC 15454^T**				23.0	9.0	10.0		13.0	19.0		TSBA	Komaki and Tamura 2019
***S. piniterrae* jys28^T**			15.8	11.7			11.9	25.3			NIST 14	Zhuang et al. 2020
***S. staurosporininus* BK179^T**				16.8		17.0		19.5			no data	Kim et al. 2012
***S. inhibens* NEAU-D10^T**		12.5		16.5		17.8		21.0			no data	Jin et al. 2019

+ the quantity was not given

Table S3. Growth of isolate SF28^{T} on standard growth media.

Medium	SF28 ^{T}
Bennett agar (BA):	
Growth	+++
Aerial mycelial colour	Pale blue
Substrate mycelial colour	Dark yellowish brown
Diffusible pigment	Moderate yellow
Glycerol-asparagine agar (ISP5):	
Growth	+
Substrate mycelial colour	Greyish yellow
Diffusible pigment	Greyish yellow
HSA5 agar:	
Growth	+
Aerial mycelial colour	Pale blue (scant)
Substrate mycelial colour	Deep brown
Inorganic – salt - starch agar (ISP4):	
Growth	+
Aerial mycelial colour	Light bluish grey
Substrate mycelial colour	Greyish yellow
Diffusible pigment	Greyish yellow
Modified Bennett's agar (MBA):	
Growth	+++
Aerial mycelial colour	Pale blue
Substrate mycelial colour	Dark yellowish brown
Diffusible pigment	Dark yellow
Nutrient agar (NA)	
Growth	++
Substrate mycelial colour	Yellowish white
Nutrient agar (NA) 100-fold diluted:	
Growth	+
Aerial mycelial colour	Greyish green
Substrate mycelial colour	Yellowish grey
Oatmeal agar (ISP 3):	
Growth	++
Aerial mycelial colour	Bluish grey
Substrate mycelial colour	Brownish grey
Diffusible pigment	Greyish yellow
Peptone – yeast extract iron agar (ISP 6):	
Growth	+++
Substrate mycelial colour	Greyish yellow
Tap water agar (TWA):	
Growth	+
Aerial mycelial colour	Pale blue
Substrate mycelial colour	Yellowish white
Tryptone-yeast extract agar (ISP 1):	
Growth	++
Substrate mycelial colour	Yellowish white
Tyrosine agar (ISP 7):	
Growth	+

Aerial mycelial colour	White (scant)
Substrate mycelial colour	Greyish brown
Diffusible pigment	Dark orange yellow
Yeast extract - malt extract agar (ISP 2):	
Growth	+++
Aerial mycelial colour	Greenish grey
Substrate mycelial colour	Brownish grey
Diffusible pigment	Greyish yellow

Key: +++, abundant growth; ++, good growth; +, weak growth; A, absent. Aerial hyphae were not formed on ISP media 1, 5 or 6 or on nutrient agar.

Table S4. 16S rRNA gene sequence similarity analysis for strains phylogenetically near to isolate SF28^{T} and related isolates generated using PHYDIT.

No.	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	Isolate SF28^{T}	---	25/1410	19/1411	26/1412	28/1412	12/1412	20/1412	15/1412	21/1412	19/1410	26/1411	10/1412	24/1412	12/1412	21/1412	21/1412	20/1412	20/1412	20/1412	19/1412	15/1412	21/1412	17/1405	66/1408
2	*S. auratus*	98.23	---	23/1440	21/1441	28/1441	20/1441	22/1441	17/1441	25/1441	23/1417	17/1441	28/1441	18/1433	20/1441	23/1441	23/1441	22/1441	24/1441	29/1441	25/1441	27/1441	13/1441	20/1426	69/1438
3	*S. caniferus*	98.65	98.40	---	9/1448	21/1448	17/1448	23/1448	6/1448	26/1448	1/1419	22/1446	16/1448	6/1435	17/1448	22/1448	22/1448	23/1448	2/1448	28/1448	10/1448	24/1448	12/1448	22/1432	63/1443
4	*S. catenulae*	98.16	98.54	99.38	---	27/1451	17/1451	17/1451	15/1451	24/1451	10/1420	22/1449	25/1451	6/1436	17/1451	29/1450	29/1451	17/1451	11/1451	28/1451	13/1451	27/1451	12/1451	22/1435	66/1446
5	*S. celluloflavus*	98.02	98.06	98.55	98.14	---	28/1451	34/1451	16/1451	33/1451	21/1420	25/1449	26/1451	25/1436	28/1451	25/1450	25/1451	34/1451	22/1451	25/1451	24/1451	29/1451	19/1451	25/1435	66/1446
6	*S. chattanoogensis*	99.15	98.61	98.83	98.83	98.07	---	10/1451	13/1451	11/1451	17/1420	19/1449	9/1451	12/1436	0/1451	29/1450	29/1451	10/1451	18/1451	11/1451	27/1451	23/1451	9/1451	9/1435	68/1446
7	*S. chrestomyceticus*	98.58	98.47	98.41	98.83	97.66	99.31	---	19/1451	17/1451	23/1420	25/1449	19/1451	18/1436	10/1451	25/1450	25/1451	0/1451	24/1451	21/1451	27/1451	13/1451	15/1451	15/1435	66/1446
8	*S. decoyicus*	98.94	98.82	99.59	98.97	98.90	99.10	98.69	---	22/1451	6/1420	16/1449	12/1451	11/1436	13/1451	16/1450	16/1451	19/1451	7/1451	24/1451	16/1451	20/1451	6/1451	18/1435	64/1446
9	*S. diastatochromogenes*	98.51	98.27	98.20	98.35	97.73	99.24	98.83	98.48	---	26/1420	26/1449	20/1451	21/1436	11/1451	36/1450	36/1451	17/1451	27/1451	20/1451	32/1451	30/1451	18/1451	14/1435	67/1446
10	*S. hygroscopicus* subsp. *Glebosus*	98.65	98.38	99.93	99.30	98.52	98.80	98.38	99.58	98.17	---	22/1418	15/1420	5/1420	17/1420	22/1420	22/1420	23/1420	1/1420	28/1420	11/1420	24/1420	12/1420	22/1405	64/1415
11	*S. inhibens*	98.16	98.82	98.48	98.48	98.27	98.69	98.27	98.90	98.21	98.45	---	28/1449	17/1434	19/1449	26/1448	26/1449	25/1449	23/1449	26/1449	26/1449	30/1449	12/1449	17/1434	61/1446
12	*S. kronopolitis*	99.29	98.06	98.90	98.28	98.21	99.38	98.69	99.17	98.62	98.94	98.07	---	20/1436	9/1451	26/1450	26/1451	19/1451	17/1451	18/1451	26/1451	20/1451	16/1451	18/1435	68/1446
13	*S.libani*	98.30	98.74	99.58	99.58	98.26	99.16	98.75	99.23	98.54	99.65	98.81	98.61	---	12/1436	27/1436	27/1436	18/1436	6/1436	23/1436	16/1436	29/1436	7/1436	17/1420	66/1431
14	*S.lydicus*	99.15	98.61	98.83	98.83	98.07	100.00	99.31	99.10	99.24	98.80	98.69	99.38	99.16	---	29/1450	29/1451	10/1451	18/1451	11/1451	27/1451	23/1451	9/1451	9/1435	68/1446
15	*S. niger*	98.51	98.40	98.48	98.00	98.28	98.00	98.28	98.90	97.52	98.45	98.20	98.21	98.12	98.00	---	0/1450	25/1450	23/1450	33/1450	18/1450	12/1450	20/1450	26/1434	60/1445
16	*S. olivaceiscleroticus*	98.51	98.40	98.48	98.00	98.28	98.00	98.28	98.90	97.52	98.45	98.21	98.21	98.12	98.00	100.00	---	25/1451	23/1451	33/1451	18/1451	12/1451	20/1451	26/1435	60/1446
17	*S. paromomycinus*	98.58	98.47	98.41	98.83	97.66	99.31	100.00	98.69	98.83	98.38	98.27	98.69	98.75	99.31	98.28	98.28	---	24/1451	21/1451	27/1451	13/1451	15/1451	15/1435	66/1446
18	*S. platensis*	98.58	98.33	99.86	99.24	98.48	98.76	98.35	99.52	98.14	99.93	98.41	98.83	99.58	98.76	98.41	98.41	98.35	---	29/1451	12/1451	25/1451	13/1451	23/1435	65/1446
19	*S. piniterrae*	98.58	97.99	98.07	98.07	98.28	99.24	98.55	98.35	98.62	98.03	98.21	98.76	98.40	99.24	97.72	97.73	98.55	98.00	---	31/1451	27/1451	18/1451	14/1435	74/1517
20	*S. ramulosus*	98.65	98.27	99.31	99.10	98.35	98.14	98.14	98.90	97.79	99.23	98.21	98.21	98.89	98.14	98.76	98.76	98.14	99.17	97.86	---	16/1451	22/1451	22/1435	63/1446
21	*S. sclerotialus*	98.94	98.13	98.34	98.14	98.00	98.41	99.10	98.62	97.93	98.31	97.93	98.62	97.98	98.41	99.17	99.17	99.10	98.28	98.14	98.90	---	24/1451	20/1435	62/1446
22	*S. sioyaensis*	98.51	99.10	99.17	99.17	98.69	99.38	98.97	99.59	98.76	99.15	99.17	98.90	99.51	99.38	98.62	98.62	98.97	99.10	98.76	98.48	98.35	---	14/1435	65/1446
23	*S. staurosporininus*	98.79	98.60	98.46	98.47	98.26	99.37	98.95	98.75	99.02	98.43	98.81	98.75	98.80	99.37	98.19	98.19	98.95	98.40	99.02	98.47	98.61	99.02	---	68/1431
24	*K. setae*	95.31	95.20	95.63	95.44	95.44	95.30	95.44	95.57	95.37	95.48	95.78	95.30	95.39	95.30	95.85	95.85	95.44	95.50	95.12	95.64	95.71	95.50	95.25	---

1540 nucleotides analysed

Lower-left triangle contains [NT] Similarity.

Uppper-right triangle contains [NT] Different/Total nucleotides.

Table S5. MLSA similarity analysis for strains phylogenetically near to isolate SF28^T and related isolates generated using PHYDIT.

No.	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	Isolate SF28^T	---	179/3931	201/3932	175/3933	169/3933	151/3933	193/3933	396/3863	298/3933	190/3932	158/3933	162/3933	408/3863	166/3933	198/3933	436/3864	191/3933	187/3933	196/3933	445/3914	200/3933	448/3870
2	*S. auratus*	95.45	---	139/3931	204/3932	191/3932	130/3932	206/3932	349/3862	286/3932	123/3931	122/3932	99/3932	346/3862	151/3932	202/3932	436/3863	207/3932	202/3932	133/3932	481/3913	210/3932	348/3869
3	*S. caniferus*	94.89	96.46	---	204/3935	216/3935	181/3935	207/3935	344/3865	295/3935	110/3934	152/3933	139/3935	354/3865	171/3935	232/3935	457/3866	212/3935	201/3935	141/3935	481/3916	226/3935	400/3872
4	*S. catenulae*	95.55	94.81	94.82	---	216/3936	184/3936	191/3936	411/3866	299/3936	196/3935	188/3934	195/3936	414/3866	176/3936	210/3936	439/3867	188/3936	213/3936	221/3936	378/3917	201/3936	439/3873
5	*S. celluloflavus*	95.70	95.14	94.51	94.51	---	178/3936	201/3936	410/3866	303/3936	200/3935	162/3934	190/3936	419/3866	185/3936	227/3936	445/3867	198/3936	180/3936	201/3936	481/3917	238/3936	460/3873
6	*S. chattanoogensis*	96.16	96.69	95.40	95.33	95.48	---	186/3936	383/3866	261/3936	155/3935	120/3934	131/3936	379/3866	116/3936	212/3936	431/3867	187/3936	161/3936	152/3936	469/3917	199/3936	402/3873
7	*S. chrestomyceticus*	95.09	94.76	94.74	95.15	94.89	95.27	---	423/3866	279/3936	215/3935	192/3934	197/3936	436/3866	165/3936	196/3936	427/3867	29/3936	217/3936	213/3936	490/3917	180/3936	449/3873
8	*S. decoyicus*	89.75	90.96	91.10	89.37	89.39	90.09	89.06	---	482/3873	336/3865	374/3864	358/3866	74/3873	388/3866	436/3866	221/3872	422/3866	422/3866	356/3866	456/3854	434/3866	154/3873
9	*S. diastatochromogenes*	92.42	92.73	92.50	92.40	92.30	93.37	92.91	87.55	---	287/3935	273/3934	268/3936	493/3873	267/3936	285/3936	499/3873	278/3936	300/3936	291/3936	560/3917	282/3936	490/3894
10	*S. hygroscopicus* subsp. *glebosus*	95.17	96.87	97.20	95.02	94.92	96.06	94.54	91.31	92.71	---	154/3933	124/3935	320/3865	143/3935	223/3935	448/3866	218/3935	197/3935	45/3935	481/3916	221/3935	385/3872
11	*S. inhibens*	95.98	96.90	96.14	95.22	95.88	96.95	95.12	90.32	93.06	96.08	---	136/3934	375/3864	121/3934	194/3934	418/3865	195/3934	183/3934	162/3934	461/3915	195/3934	411/3871
12	*S. kronopolitis*	95.88	97.48	96.47	95.05	95.17	96.67	94.99	90.74	93.19	96.85	96.54	---	371/3866	130/3936	212/3936	429/3867	194/3936	193/3936	131/3936	470/3917	197/3936	374/3873
13	*S.libani*	89.44	91.04	90.84	89.29	89.16	90.20	88.72	98.09	87.27	91.72	90.30	90.40	---	385/3866	453/3866	241/3872	438/3866	425/3866	341/3866	460/3854	453/3866	143/3873
14	*S.lydicus*	95.78	96.16	95.65	95.53	95.30	97.05	95.81	89.96	93.22	96.37	96.92	96.70	90.04	---	190/3936	411/3867	165/3936	188/3936	141/3936	469/3917	183/3936	408/3873
15	*S. niger*	94.97	94.86	94.10	94.66	94.23	94.61	95.02	88.72	92.76	94.33	95.07	94.61	88.28	95.17	---	307/3867	193/3936	243/3936	237/3936	484/3917	96/3936	444/3873
16	*S. olivaceiscleroticus*	88.72	88.71	88.18	88.65	88.49	88.85	88.96	94.29	87.12	88.41	89.18	88.91	93.78	89.37	92.06	---	421/3867	452/3867	462/3867	476/3855	340/3867	229/3873
17	*S. paromomycinus*	95.14	94.74	94.61	95.22	94.97	95.25	99.26	89.08	92.94	94.46	95.04	95.07	88.67	95.81	95.10	89.11	---	211/3936	218/3936	485/3917	176/3936	444/3873
18	*S. platensis*	95.25	94.86	94.89	94.59	95.43	95.91	94.49	89.08	92.38	94.99	95.35	95.10	89.01	95.22	93.83	88.31	94.64	---	227/3936	482/3929	242/3936	455/3873
19	*S. piniterrae*	95.02	96.62	96.42	94.39	94.89	96.14	94.59	90.79	92.61	98.86	95.88	96.67	91.18	96.42	93.98	88.05	94.46	94.23	---	507/3917	228/3936	399/3873
20	*S. ramulosus*	88.63	87.71	87.72	90.35	87.72	88.03	87.49	88.17	85.70	87.72	88.22	88.00	88.06	88.03	87.64	87.65	87.62	87.73	87.06	---	477/3917	495/3861
21	*S. sclerotialus*	94.91	94.66	94.26	94.89	93.95	94.94	95.43	88.77	92.84	94.38	95.04	94.99	88.28	95.35	97.56	91.21	95.53	93.85	94.21	87.82	---	445/3873
22	*S. sioyaensis*	88.42	91.01	89.67	88.67	88.12	89.62	88.41	96.02	87.42	90.06	89.38	90.34	96.31	89.47	88.54	94.09	88.54	88.25	89.70	87.18	88.51	---

4007 nucleotides analysed
Lower-left triangle contains [NT] Similarity.
Uppper-right triangle contains [NT] Different/Total nucleotides.

Table S6. MLSA distances for strains phylogenetically near to isolate SF28^{T} and related isolates.

No.	Strain	MLSA (Kimura 2-paramenter) distance																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	Isolate SF28^{T}	-																					
2	*S. auratus*	0.046																					
3	*S. caniferus*	0.052	0.036																				
4	*S. catenulae*	0.045	0.053	0.054																			
5	*S. celluloflavus*	0.043	0.050	0.057	0.056																		
6	*S. chattanoogensis*	0.039	0.034	0.048	0.048	0.046																	
7	*S. chrestomyceticus*	0.050	0.053	0.054	0.049	0.052	0.048																
8	*S. decoyicus*	0.110	0.096	0.094	0.114	0.113	0.106	0.118															
9	*S. diastatochromogenes*	0.078	0.075	0.078	0.078	0.079	0.068	0.074	0.135														
10	*S. hygroscopicus* subsp. *glebosu*	0.049	0.032	0.029	0.051	0.053	0.041	0.056	0.092	0.076													
11	*S. inhibens*	0.040	0.031	0.040	0.048	0.042	0.031	0.050	0.103	0.071	0.040												
12	*S. kronopolitis*	0.042	0.025	0.037	0.051	0.050	0.035	0.051	0.098	0.070	0.033	0.035											
13	*S.libani*	0.113	0.095	0.097	0.115	0.116	0.105	0.122	0.019	0.138	0.087	0.104	0.102										
14	*S.lydicus*	0.043	0.039	0.046	0.046	0.048	0.030	0.042	0.107	0.070	0.038	0.031	0.034	0.107									
15	*S. niger*	0.051	0.052	0.060	0.053	0.058	0.054	0.051	0.121	0.074	0.057	0.050	0.054	0.127	0.048								
16	*S. olivaceiscleroticus*	0.122	0.122	0.127	0.122	0.124	0.120	0.118	0.058	0.139	0.125	0.116	0.119	0.064	0.113	0.084							
17	*S. paromomycinus*	0.049	0.054	0.055	0.048	0.051	0.049	0.008	0.117	0.074	0.057	0.051	0.050	0.122	0.042	0.050	0.116						
18	*S. piniterrae*	0.049	0.054	0.054	0.056	0.047	0.043	0.057	0.118	0.079	0.053	0.048	0.052	0.119	0.050	0.063	0.126	0.055					
19	*S. platensis*	0.051	0.035	0.037	0.058	0.054	0.040	0.055	0.097	0.076	0.011	0.043	0.034	0.093	0.037	0.061	0.129	0.056	0.061				
20	*S. ramulosus*	0.115	0.125	0.124	0.093	0.124	0.121	0.127	0.128	0.147	0.124	0.119	0.121	0.130	0.121	0.126	0.135	0.125	0.122	0.132			
21	*S. sclerotialus*	0.051	0.054	0.058	0.050	0.061	0.051	0.046	0.121	0.074	0.056	0.050	0.050	0.127	0.046	0.025	0.093	0.045	0.062	0.058	0.123		
22	*S. sioyaensis*	0.124	0.094	0.110	0.122	0.127	0.110	0.125	0.041	0.135	0.105	0.113	0.102	0.038	0.113	0.123	0.060	0.123	0.127	0.109	0.140	0.123	-

Table S7. Average nucleotide identity (ANI) and digital DNA:DNA hybridization (dDDH) values found between isolate SF28^{T} and their closest evolutionary neighbours

Subject strain	**dDDH [%]**	**ANI [%]**
Streptomyces decoyicus NRRL ISP-5087^{T}	28.7	85.1
Streptomyces celluloflavus NRRL B-2493^{T}	28.2	85.5
Streptomyces inhibens NEAU-D10 ^{T}	28.0	85.3
Streptomyces piniterrae jys28^{T}	27.8	85.3
Streptomyces caniferus NBRC 15389^{T}	27.8	85.3
Streptomyces libani JCM 4322^{T}	27.5	85.1
Streptomyces platensis DSM 40041^{T}	27.4	85.0
Streptomyces hygroscopicus subsp. *glebosus* JCM 4954^{T}	27.4	85.1
Streptomyces catenulae NRRL B-2342^{T}	27.4	85.0
Streptomyces sioyaensis NRRL B-5408^{T}	27.4	85.0
Streptomyces lydicus ATCC 25470^{T}	27.3	84.9
Streptomyces kronopolitis CGMCC 4.7323^{T}	27.3	85.0
Streptomyces *auratus* DSM 41897^{T}	27.3	84.9
Streptomyces chattanoogensis NRRL ISP-5002^{T}	26.8	84.6
Streptomyces *paromomycinus* NBRC 15454^{T}	25.7	83.6
Streptomyces chrestomyceticus NBRC 13444^{T}	25.6	83.6
Streptomyces sclerotialus NRRL-ISP 5269^{T}	24.6	82.9
Streptomyces niger NBRC 13362^{T}	24.5	82.8
Streptomyces diastatochromogenes NRRL B-1698^{T}	21.7	80.5

Table S8. Stress response genes found in the genome of isolate SF28^T using the RAST-SEED webserver (https://rast.nmpdr.org/)

Stress response genes	Chromosome region	Encoded protein
Osmotic stress response		
betA	fig|6666666.874822.peg.2043	Choline dehydrogenase
betB	fig|6666666.874822.peg.158	Betaine aldehyde dehydrogenase
proU	fig|6666666.874822.peg.3917 fig|6666666.874822.peg.3918 fig|6666666.874822.peg.6537 fig|6666666.874822.peg.6539 fig|6666666.874822.peg.6540	Glycine betaine-binding protein
Oxidative stress response		
katE	fig|6666666.874822.peg.1076 fig|6666666.874822.peg.1803 fig|6666666.874822.peg.6671 fig|6666666.874822.peg.7113	Catalase synthesis
soxR	fig|6666666.874822.peg.3772 fig|6666666.874822.peg.4547	Redox-sensitive transcriptional activator
trx	fig|6666666.874822.peg.2619 fig|6666666.874822.peg.2877 fig|6666666.874822.peg.4485 fig|6666666.874822.peg.4549	Putative thioredoxin
trxR	fig|6666666.874822.peg.35 fig|6666666.874822.peg.2546 fig|6666666.874822.peg.2878 fig|6666666.874822.peg.4548 fig|6666666.874822.peg.6861	Thioredoxin reductase
Heat stress response		
clpB	fig|6666666.874822.peg.1578 fig|6666666.874822.peg.2101	Protein chaperones, ATP-dependent subunit
clpC	fig|6666666.874822.peg.2068 fig|6666666.874822.peg.2990 fig|6666666.874822.peg.7292 fig|6666666.874822.peg.2425 fig|6666666.874822.peg.4318 fig|6666666.874822.peg.4319	Protein chaperones, ATP-dependent subunit
clpX	fig|6666666.874822.peg.4320	Protein chaperones, ATP-dependent subunit
DnaK	fig|6666666.874822.peg.1032 fig|6666666.874822.peg.1585	Protein chaperones, macromolecule protection and repair

DnaJ	fig|6666666.874822.peg.1583 fig|6666666.874822.peg.2481 fig|6666666.874822.peg.4385	Protein chaperones,
grpE	fig|6666666.874822.peg.1033 fig|6666666.874822.peg.1584	Protein chaperones, heat shock protein
Hsp10	fig|6666666.874822.peg.6171	Chaperone family GroES
Hsp60	fig|6666666.874822.peg.1196 fig|6666666.874822.peg.6170	Chaperone family GroEL
hrcA	fig|6666666.874822.peg.4384	Heat-inducible transcription repressor
Cold stress response		
csp	fig|6666666.874822.peg.558 fig|6666666.874822.peg.669 fig|6666666.874822.peg.1011 fig|6666666.874822.peg.1197 fig|6666666.874822.peg.1224 fig|6666666.874822.peg.4645 fig|6666666.874822.peg.4829	Cold shock protein family
cspC	fig|6666666.874822.peg.3619	Cold shock protein family
cspE	fig|6666666.874822.peg.1126	Cold shock protein family
DNA stabilizing and repair		
RecF	fig|6666666.874822.peg.2862	DNA Recombination and repair protein
RecO	fig|6666666.874822.peg.4425	DNA Recombination and repair protein
RecR	fig|6666666.874822.peg.581	DNA Recombination and repair protein
Putative DNA binding genes	fig|6666666.874822.peg.595 fig|6666666.874822.peg.932 fig|6666666.874822.peg.1143 fig|6666666.874822.peg.1153 fig|6666666.874822.peg.1190 fig|6666666.874822.peg.1272 fig|6666666.874822.peg.1970 fig|6666666.874822.peg.2296 fig|6666666.874822.peg.2302 fig|6666666.874822.peg.2303 fig|6666666.874822.peg.2387 fig|6666666.874822.peg.2853 fig|6666666.874822.peg.4125 fig|6666666.874822.peg.4440	DNA protction proteins
DNA binding genes	fig|6666666.874822.peg.652 fig|6666666.874822.peg.1071 fig|6666666.874822.peg.1593	DNA protection proteins
uvrD	fig|6666666.874822.peg.1824 fig|6666666.874822.peg.5494	ATP-dependent helicase
Sigma factors		

rpoD	fig|6666666.874822.peg.3248 fig|6666666.874822.peg.4033 fig|6666666.874822.peg.4474	RNA polymerase Sigma factor, Transcription initiation
rpoE	fig|6666666.874822.peg.130 fig|6666666.874822.peg.2540	RNA polymerase Sigma factor, Transcription initiation
Sig	fig|6666666.874822.peg.2	ECF subfamily
Sig	fig|6666666.874822.peg.1994	ECF subfamily
SigB	fig|6666666.874822.peg.1766 fig|6666666.874822.peg.2128 fig|6666666.874822.peg.2709 fig|6666666.874822.peg.4959 fig|6666666.874822.peg.4960 fig|6666666.874822.peg.6505 fig|6666666.874822.peg.7087	RNA polymerase Sigma factor, Transcription initiation
SigE	fig|6666666.874822.peg.6667	Alternative RNA polymerase
ECF-type Sig	fig|6666666.874822.peg.26 fig|6666666.874822.peg.530 fig|6666666.874822.peg.577 fig|6666666.874822.peg.985 fig|6666666.874822.peg.1792	ECF subfamily, *RNA polymerase*
atpA	fig|6666666.874822.peg.3292	Potassium-transporting F0F1-ATPase proton pumps
coxG	fig|6666666.874822.peg.1978	Carbon monoxide dehydrogenase subunit

V Dyskusja i podsumowanie uzyskanych wyników

1. Dereplikacja i wstępna identyfikacja wybranych izolatów na podstawie sekwencji nukleotydowej genu 16S rRNA

Promieniowce wyizolowane z trzech środowisk: silnie wysuszonych gleb pustyni Atakama w Chile, alkalicznej i zasolonej gleby jeziora Lonar w Indiach oraz kwaśnej gleby z północnego i południowego stoku wydmy śródlądowej w lesie sosnowym w okolicach Torunia poddano dereplikacji, grupując szczepy na podstawie zabarwienia grzybni substratowej i powietrznej oraz koloru barwnika dyfundującego do podłoża i wytwarzania barwnika melaninowego (Williams i in., 1983), aby uzyskać wgląd w ich różnorodność taksonomiczną na poziomie rodzaju wśród szczepów wyizolowanych z każdego z wymienionych stanowisk. Procedury dereplikacji mają za zadanie wyselekcjonowanie z rozbudowanych bibliotek (kolekcji) szczepów tych reprezentatywnych wykorzystywanych do dalszych analiz eliminując ich przypadkowy wybór (Goodfellow i in., 2018). W badaniach nad promieniowcami środowisk ekstremalnych (pustynia Atakama) wykazano, że liczba grup barwnych, zawierających promieniowce odzwierciedla ich różnorodność, umożliwiając w ten sposób wybór reprezentatywnych izolatów do testów przeciwdrobnoustrojowych, chemicznych badań przesiewowych i wielofazowych badań taksonomicznych (Pathom-aree i in., 2006; Goodfellow i Fiedler, 2010; Busarakam i in., 2014; Idris, 2016; Goodfellow i in., 2018). W związku z tym spośród izolatów z pustyni Atakama wybrano 28 szczepów reprezentatywnych, ze stoku północnego i południowego wydmy śródlądowej w lesie sosnowym wybrano odpowiednio 36 i 35 izolatów reprezentatywnych, zaś z gleby krateru Lonar, ze względu na niewielką liczbę (16) pozyskanych izolatów, zdecydowano o włączeniu wszystkich tych szczepów do dalszych analiz. W sumie do dalszych analiz wybrano 115 izolatów (publikacja I).

Analizy sekwencjonowania genów 16S rRNA przeprowadzono na wybranych przedstawicielach grup kolorystycznych w celu określenia ich przynależności do znanych i prawidłowo opublikowanych gatunków lub potencjalnie nowych gatunków, a uzyskane dane filogenetyczne wykorzystano do ustalenia zgodności taksonomicznej izolatów przypisanych do poszczególnych grup kolorystycznych. Wśród analizowanych promieniowców dominował rodzaj *Streptomyces*, niezależnie od źródła izolacji. Pozostałe szczepy z pustyni Atakama należały do rodzajów *Modestobacter*, *Pseudonocardia, Kribbella* i *Micromonospora*; z krateru Lonar do rodzaju *Nocardiopsis*

natomiast z gleb wydmy śródlądowej do rodzajów *Streptacidiphilus*, *Actinacidiphila*, *Catenulispora*, *Kitasatospora i Pilimelia* (stok N) oraz *Pilimelia*, *Actinacidiphila, Nocardia*, *Actinospica* i *Streptacidiphilus* (stok S). Wykazano wysoką korelację pomiędzy przynależnością taksonomiczną izolatów a ich przynależnością do grup kolorystycznych. Na podstawie uzyskanych wyników należy przypuszczać, że do określonej grupy kolorystycznej należą wyłącznie szczepy reprezentujące ten sam rodzaj, a często wykazujące najbliższe podobieństwo do określonego gatunku. 28 ze 115 analizowanych izolatów (24%) wykazywało podobieństwo sekwencji genu 16S rRNA z najbliżej spokrewnionymi gatunkami na poziomie ≤ 99% wskazując, że mogą one być rozważane jako potencjalnie nowe gatunki rodzajów *Actinacidiphila*, *Actinospica, Kribbella, Nocardia, Nocardiopsis, Pilimelia, Pseudonocardia, Streptacidiphilus* i *Streptomyces* (publikacja I).

Uzyskane wyniki potwierdzają, że selektywna izolacja, dereplikacja i wstępna charakterystyka izolatów są skuteczną metodą selekcji potencjalnie nowych oraz rzadkich taksonów promieniowców pochodzących z mało poznanych środowisk (Liu i in., 2021).

2. Potencjał przeciwdrobnoustrojowy promieniowców

Promieniowce, szczególnie z rodzaju *Streptomyces*, będące głównym producentem związków o charakterze antybiotycznym, znane są ze swojej aktywności przeciwbakteryjnej i przeciwgrzybowej (Barka i in., 2016). Uważa się, że związki te mogą pomóc w rozwiązaniu problemu narastającej antybiotykooporności (Hui i in., 2021). Jednak poszukiwanie nowych metabolitów, zwłaszcza od promieniowców ze środowisk dobrze poznanych, jest dużym wyzwaniem, ponieważ często prowadzi do reizolacji znanych już taksonów i związków. Z tego powodu duże nadzieje wiąże się z mikroorganizmami, w tym szczególnie z promieniowcami, środowisk ekstremalnych i mało poznanych, które posiadają potencjał do syntetyzowania nowych, nieodkrytych jeszcze związków o aktywności biologicznej, w tym przeciwdrobnoustrojowej (Hui i in., 2021; Sivakala i in., 2021). W ostatnich latach silną aktywność przeciwdrobnoustrojową wykazano u promieniowców wyizolowanych z takich środowisk jak pustynia Atakama (Abdelkader i in., 2018), osady fiordu Trondheim (Králová i in., 2021) oraz kwaśna gleba leśna (Sharma i Thakur 2020).

W niniejszej pracy, szczepy reprezentatywne ze wszystkich analizowanych środowisk przebadano pod względem aktywności wobec bakterii Gram-dodatnich

i Gram-ujemnych oraz *Candida albicans*. 79 ze 115 zbadanych izolatów wykazywało aktywność wobec przynajmniej jednego ze szczepów testowych. Aktywność ta zależała również od podłoża, na którym hodowano promieniowce. Najmniejszą aktywność przeciwdrobnoustrojową wykazywały izolaty z gleby jeziora Lonar w Indiach, a największą izolaty z kwaśnej gleby leśnej w Polsce. Żaden ze szczepów wyizolowanych ze środowisk alkalicznych (pustynia Atakama, jezioro Lonar) nie posiadał zdolności do hamowania wzrostu *K. pneumoniae* i *P. mirabilis*; izolaty z jeziora Lonar nie wykazywały również aktywności wobec *E. coli* i *S. aureus*. Szczepy wyizolowane z kwaśnej gleby lasu sosnowego posiadały zdolność do hamowania wzrostu wszystkich zbadanych bakterii i *C. albicans* (publikacje I, V i VII). Największy odsetek szczepów o aktywności przeciwbakteryjnej zanotowano wśród promieniowców wyizolowanych ze stoku południowego wydmy śródlądowej lasu sosnowego, natomiast tych o aktywności przeciwdrożdżakowej wśród izolatów ze stoku północnego (publikacja I).

W przeprowadzonych badaniach zaobserwowano większą aktywność promieniowców wobec bakterii Gram-dodatnich i *C. albicans* niż bakterii Gram-ujemnych. Wyniki te są zbieżne z otrzymanymi przez innych badaczy (Deshmukh i Vidhale, 2015). Największą strefę zahamowania wzrostu bakterii Gram-dodatnich, mianowicie *Bacillus subtilis* PCM 2021 (12-14 mm) i *Staphylococcus aureus* PCM 2054 (12-14,3 mm) wykazywały izolaty z kwaśnej gleby leśnej, a *Micrococcus luteus* ATCC 10240 (14-17,3 mm) izolaty z jeziora Lonar i północnego stoku wydmy śródlądowej lasu sosnowego. Najsilniejszą aktywność wobec *C. albicans* (14-15 mm) zaobserwowano u promieniowców z kwaśnej gleby leśnej oraz z pustyni Atakama, zaś wobec *Escherichia coli* PCM 2057, *Klebsiella pneumoniae* ATCC 700603, *Proteus mirabilis* i *Salmonella infantis* (strefa zahamowania wzrostu odpowiednio 5-7, 7-8, 6-8,3 i 12-15,3 mm) u szczepów z gleby leśnej (publikacja I).

Izolat NH11^{T}, zidentyfikowany jako nowy gatunek z rodzaju *Catenulispora* (*C. pinisilvae*), zbadano również wobec Gram-dodatnich szczepów dzikich (*Bacillus subtilis*, *Micrococcus luteus* i *Staphylococcus aureus*) wyizolowanych od pacjentów niewykazujących objawów chorobowych, wykazując jego aktywność przeciwdrobnoustrojową wobec tych bakterii (strefy zahamowania wzrostu od 6-10 mm) (publikacja V).

Promieniowce kwaśnych gleb leśnych charakteryzowała silna aktywność wobec grzybów i lęgniowców fitopatogennych. 62 z 71 analizowanych izolatów wykazywało co najmniej 50% stopień zahamowania wzrostu przynajmniej jednego ze zbadanych

patogenów (publikacja I). Badane promieniowce wykazywały zdolność do hamowania wzrostu grzybów z rodzajów *Alternaria, Botritis, Chalara, Colletotrichum, Fusarium, Phoma, Rhizoctonia, Sclerotina* oraz lęgniowców z rodzaju *Phytophthora*. Aktywność promieniowców wobec takich fitopatogenów potwierdzają liczne doniesienia literaturowe (Vurukonda i in., 2018; Tamreihao i in. 2018; Elshafie i Camele, 2022; Díaz-Díaz i in., 2022), wskazując na ich duży potencjał biokontrolujący. Największą aktywnością odznaczał się szczep SF28^{T}, *Streptomyces pinistramenti*, wyizolowany z południowego stoku wydmy śródlądowej lasu sosnowego, który hamował wzrost 17 z 20 zbadanych patogenów roślin, w tym 13 patogenów w stopniu wyższym niż 70% (publikacje I i VII). Interesujący jest fakt, że *S. pinistramenti* silnie hamował również dermatofity *Trichophyton erinacei* DSM 25374 i *T. thuringense* DSM 25373 (publikacja VII).

Innymi szczególnie aktywnymi szczepami, wykazującymi >70% zahamowanie wzrostu fitopatogenów były izolaty NA10a i NH17 (hamujące wzrost 11 ze zbadanych patogenów), SA7 i SF8 (hamujące wzrost 10 ze zbadanych patogenów), NH7 i SL3 (hamujące wzrost 9 ze zbadanych patogenów), SH15 i NH28 (hamujące wzrost 8 ze zbadanych patogenów). Izolaty NH17 i NH28 stanowią dwa potencjalnie nowe gatunki z rodzaju *Streptomyces*, natomiast wszystkie pozostałe szczepy zostały zidentyfikowane z wysokim podobieństwem do *Streptomyces celluloflavus* (publikacja I), potwierdzając doniesienia literaturowe o znacznej aktywności przeciwgrzybowej promieniowców należących do tego rodzaju (Barka i in., 2016; Ebrahimi-Zarandi i in., 2022). Jednak coraz częściej odnotowuje się silną aktywność przeciwdrobnoustrojową promieniowców należących do innych rodzajów (Martínez-Hidalgo i in., 2015; Ebrahimi-Zarandi i in., 2022). Na co wskazują również niniejsze badania, w których zanotowano silną aktywność izolatów należących do rodzajów *Catenulispora*, *Kitasatospora*, *Streptacidiphilus* czy *Nocardia* wobec zbadanych fitopatogenów.

Aktywność promieniowców wobec fitopatogenów polega głównie na zdolności promieniowców do wytwarzania metabolitów przeciwdrobnoustrojowych i enzymów degradujących ściany komórkowe mikroorganizmów, ale także na hiperpasożytnictwie czy indukcji odporności roślin (Vurukonda i in., 2018; Tamreihao i in., 2018; Boukhatem i in., 2022).

3. Aktywność hydrolityczna promieniowców i ich potencjał do promowania wzrostu roślin

Promieniowce są źródłem cennych dla procesów przemysłowych enzymów hydrolitycznych (Mukhtar i in., 2017; Jin i in., 2019). Mikroorganizmy te wydzielają enzymy hydrolityczne, w szczególności celulazy i chitynazy, przekształcające nierozpuszczalne polimery w składniki odżywcze, które działają jak naturalne nawozy i promują wzrost roślin (Jog i in., 2016; Nouioui i in., 2019). Enzymy hydrolityczne promieniowców odgrywają również ważną rolę w eliminacji patogenów poprzez degradację ścian i/lub błon komórkowych mikroorganizmów (Barka i in., 2016; Tamreihao i in., 2018; Boukhatem i in., 2022; Elshafie i Camele, 2022).

Izolaty pozyskane ze wszystkich środowisk wytwarzały celulazy, chitynazy, lipazy i proteinazy, ale aktywność pektynaz odnotowano głównie wśród szczepów izolowanych z gleb pustyni Atakama. Wśród zbadanych promieniowców 76,5% wytwarzało lipazy, 64,3% ureazy i 61,7% proteazy. Celulazy, chitynazy i pektynazy były rzadziej wytwarzane, odpowiednio przez 36,5, 22,6 i 13,9% izolatów (publikacja I). Wszystkie szczepy promieniowców z jeziora Lonar wytwarzały lipazy i proteazy, a 62,5% z nich również celulazy. Aktywność chitynolityczna występowała najczęściej wśród szczepów wyizolowanych z gleb leśnych oraz gleb pustyni Atakama (21,4-27,8% izolatów). Jednak indeks aktywności enzymatycznej dla chitynaz, niezależnie od środowiska izolacji promieniowców, był niski (Wact 0,4-3.1). Ureazy najczęściej wytwarzane były przez szczepy wyizolowane z gleb leśnych (83,3-91,4%) (publikacja I).

Poza aktywnością hydrolityczną, zwiększającą żyzność gleby, mikroorganizmy mogą promować wzrost i rozwój roślin poprzez stymulację reakcji obronnych rośliny, zwiększanie dostępności związków mineralnych oraz produkcję hormonów roślinnych (Boukhatem i in., 2022).

Wśród 115 badanych promieniowców zdolność do produkcji sideroforów zaobserwowano u 50,4% szczepów, a amoniaku u 39,1% izolatów. Zdolności te najrzadziej notowano wśród szczepów z jeziora Lonar. Wytwarzanie auksyn odnotowano u 30,4% zbadanych promieniowców a zdolność do solubilizacji fosforanów u 8,7% izolatów. Takich właściwości nie zaobserwowano u izolatów z jeziora Lonar. Cyjanowodór syntetyzowany był tylko przez szczep NL8^{T}, opisany jako nowy gatunek z rodzaju *Catenulispora*, *C. pinistramenti*.

Podsumowując, największy odsetek szczepów wykazujących zdolność do promowania wzrostu roślin zaobserwowano wśród promieniowców wyizolowanych z

gleb leśnych zarówno stoku północnego (syntetyzowanie auksyn, cyjanowodoru i solubilizacja fosforanów) jak i południowego (produkcja amoniaku i sideroforów) wydmy śródlądowej (publikacja I).

Potencjał promieniowców do wykorzystywania jako czynniki promujące wzrost roślin był już wielokrotnie opisywany, dzięki ich zdolności do syntetyzowania amoniaku, cyjanowodoru, enzymów hydrolitycznych, hormonów roślinnych, sideroforów, solubilizacji fosforanów czy wiązania azotu (Barka i in., 2016; Vurukonda i in., 2018; Boukhatem i in., 2022; Elshafie i Camele, 2022). Dodatkowo wiele szczepów promieniowców wykazuje jednocześnie zdolność do biokontrolowania rozwoju fitopatogenów, jednak na rynku dostępnych jest niewiele środków promujących wzrost roślin opartych o te mikroorganizmy (Boukhatem i in., 2022). W związku z tym, warto poświęcić im więcej uwagi, ponieważ mogą stanowić skuteczną i bezpieczniejszą dla zdrowia ludzi i zwierząt oraz dla środowiska alternatywę dla chemicznych środków ochrony roślin.

4. Nowe gatunki promieniowców ze środowisk ekstremalnych i mało poznanych

Na podstawie wyników identyfikacji w oparciu o sekwencję nukleotydową genu 16S rRNA wybrano 17 izolatów promieniowców (6 z gleby pustyni Atakama - publikacja II i III, 6 z gleby jeziora Lonar - publikacja IV, 4 z gleby stoku północnego wydmy śródlądowej lasu sosnowego - publikacje V i VI oraz 1 z gleby stoku południowego wydmy śródlądowej w lesie sosnowym - publikacja VII) stanowiących potencjalnie nowe gatunki reprezentujące rzadkie rodzaje promieniowców i/lub wykazujące różną aktywność biologiczną (enzymatyczną, przeciwdrobnoustrojową, zdolność do wytwarzania sideroforów, cyjanowodoru, auksyn, solubilizację fosforanów; publikacja).

4.1. Rodzaj *Modestobacter*

Szczepy 1G4^T, 1G6^T, 1G14, 1G50, 1G51 i 1G52 wyizolowane z gleby pustyni Atakama w Chile na podstawie analizy sekwencji nukleotydowej genu 16S rRNA zidentyfikowano do rodzaju *Modestobacter* (publikacje II i III).

Dalsze analizy filogenetyczne tych izolatów wykazały, że tworzą one dwa siostrzane klady w drzewach 16S rRNA, obejmujących przedstawicieli rodziny *Geodermatophilaceae,* wygenerowanych metodą najbliższego sąsiada oraz największej wiarygodności i największej parsymonii. Izolaty stanowią dwa blisko spokrewnione

ze sobą taksony. Pierwszy z kladów obejmował izolaty 1G6^T, 1G14, 1G50, których najbliższym filogenetycznym sąsiadem był *Modestobacter italicus* DSM 44449^T (99,8-99,9% podobieństwa sekwencji nukleotydowej genu 16S rRNA). Drugi klad obejmujący izolaty 1G4^T, 1G51 i 1G52 był najbliżej spokrewniony ze szczepami 1G6^T, 1G14, 1G50 (99,7-99,9% podobieństwa sekwencji). Wszystkie wartości podobieństwa były znacznie powyżej progu 98,7%, rekomendowanego przez Kim i wsp. (2014) do wyodrębnienia nowego gatunku, wskazując, że analizowane szczepy nie spełniają tej przesłanki. Jednak jak pokazują wcześniejsze badania nad gatunkiem *M. italicus* (Montero-Calasanz i in., 2019) ta wartość progowa nie zawsze ma zastosowanie do bardzo blisko spokrewnionych ze sobą taksonów. Ponadto, inni autorzy sugerują stosowanie wyższych wartości progowych takich jak 98,7–99,0% (Stackebrandt i Ebers, 2006) oraz 98,2–99,0% (Meier-Kolthoff i in., 2013a). Analizy porównawcze w oparciu o sekwencje całych genomów potwierdziły, że izolat 1G6^T był najbliżej spokrewniony z gatunkiem *M. italicus* DSM 44449^T, jednak wartości *in silico* hybrydyzacji DNA:DNA i średniego podobieństwa nukleotydów (ANI) były znacznie poniżej progów wynoszących odpowiednio 70% (Wayne i in., 1987) i 95-96% (Goris i in., 2007; Chun i Rainey, 2014), wskazując na przynależność izolatów do nowego gatunku, któremu nadano nazwę *Modestobacter excelsi,* ze szczepem wzorcowym 1G6^T. Podobnie, analizy dDDH i ANI izolatu 1G4^T wykazały najbliższe podobieństwo z gatunkiem *M. excelsi* 1G6^T, jednak na tyle niskie by wyodrębnić nowy gatunek, któremu nadano nazwę *Modestobacter altitudinis*, ze szczepem wzorcowym 1G4^T. Poza tym, porównawcze analizy fenotypowe izolatów pomiędzy sobą oraz izolatów z ich najbliższymi filogenomowymi sąsiadami z rodzaju *Modestobacter* wykazały, że analizowane szczepy nie są klonami oraz że na tej podstawie można je odróżnić od najbliżej spokrewnionych gatunków (publikacje II i III).

Modestobacter to rzadki rodzaj należący do rodziny *Geodermatophilaceae*, razem z rodzajami *Blastococcus*, *Geodermatophilus* oraz *Klenkia* i obejmuje jedenaście poprawnie opublikowanych gatunków (https://lpsn.dsmz.de/, ang. List of Prokaryotic names with Standing in Nomenclature, LPSN; data dostępu 30.11.2022; Parte i in., 2020). Gatunki te w większości wyizolowano z ekstremalnych, oligotroficznych środowisk takich jak gleba pustyni Atakama w Chile - *M. caceresii* (Busarakam i in., 2016), *M. excelsi* (publikacja II) i *M. altitudinis* (publikacja III), gleba Wyżyny Kolorado - *M. versicolor* (Reddy i in., 2007), osady denne oceanu Atlantyckiego - *M. marinus* (Xiao i in., 2011), gleba Antarktyki - *M. multiseptatus* (Mevs i in., 2000), zdegradowane piaskowce zabytkowej budowli w Hiszpanii - *M. lapidis* i *M. muralis* (Trujillo i in., 2015)

oraz z powierzchni marmuru we włoskim kamieniołomie - *M. italicus* (Montero-Calasanz i in., 2019). Pozostałe dwa szczepy (*M. roseus* i *M. lacusdianchii*) wyizolowano odpowiednio z *Salicornia europea* (Qin i in., 2013) i mikroalg (Zhang i in., 2016).

Bakterie z rodzaju *Modestobacter* mają kształt pałeczek lub ziarniaków z tendencją do agregacji i nie wytwarzają spor. Do komórkowych markerów chemotaksonomicznych tego rodzaju należą kwas *mezo*-diaminopimelinowy oraz glukoza i ryboza. Występowanie innych cukrów takich jak arabinoza, galaktoza, mannoza i ramnoza jest cechą zmienną (Mevs i in., 2000; Montero-Calasanz i in., 2019). Dominującymi kwasami tłuszczowymi są $C_{18:1}$, *izo*-$C_{16:0}$ i *anteizo*-$C_{17:0.}$ Profil lipidów polarnych składa się z fosfatydyloetanoloaminy, fosfatytyloinozytolu oraz difosfatydyloglicerolu (Mevs i in., 2000). Dominującym chinonem izoprenoidowym jest MK-9 (H_4), jednak w mniejszej ilości mogą występować również inne chinony z ośmioma, dziewięcioma lub dziesięcioma jednostkami izoprenoidowymi (MK-8 (H_4), MK-9, MK-9 (H_2), MK-9 (H_6) oraz MK-10 (H_4)) (Montero-Calasanz i in., 2019). Zawartość par G+C w DNA przedstawicieli tego rodzaju wynosi około 70% (Reddy i in., 2007).

Zbadane w publikacjach II i III właściwości chemotaksonomiczne, hodowlane i morfologiczne izolatów były charakterystyczne dla przedstawicieli *Modestobacter* i wskazują na ich przynależność do tego rodzaju (Mevs i in., 2000; Montero-Calasanz i in., 2019).

4.2. Rodzaj *Streptomyces*

Szczepy OF1^T, OF3 i OF8 oraz IF11, IF17 i IF19 wyizolowane z gleby jeziora Lonar w Indiach, a także szczep SF28^T wyizolowany z kwaśnej gleby lasu sosnowego w okolicach Torunia, na podstawie analizy sekwencji nukleotydowej genu 16S rRNA zidentyfikowano do rodzaju *Streptomyces* i rozpatrywano jako trzy potencjalnie nowe gatunki (publikacje IV i VII).

Izolaty OF1^T, OF3 i OF8 w drzewach filogenetycznych opartych na sekwencjach genu 16S rRNA wygenerowanych metodami najbliższego sąsiada, największej wiarygodności i największej parsymonii utworzyły dobrze wspieraną gałąź razem ze swoim najbliższym filogenetycznym sąsiadem *S. durbertensis* DSM 104538^T. Izolaty wykazały 99,3% podobieństwa sekwencji genu 16S rRNA z tym gatunkiem. Szczepy IF11, IF17 i IF19 były najbliżej spokrewnione ze *Streptomyces alkaliphilus* DSM 42118^T wykazując 99,6-99,7% podobieństwa sekwencji nukleotydowej genu 16S rRNA z tym

gatunkiem oraz ze *Streptomyces calidiresistens* DSM 42108^T (99,4-99,5% podobieństwa sekwencji genu). Izolaty IF11, IF17 i IF19 tworzyły osobny klad ze *S. alkaliphilus* i *S. calidiresistens* w drzewach filogenetycznych wygenerowanych na podstawie sekwencji nukleotydowej genu 16S rRNA z wykorzystaniem algorytmów najbliższego sąsiada oraz największej wiarygodności i największej parsymonii (publikacja IV).

Szczep SF28^T był blisko spokrewniony z trzema gatunkami rodzaju *Streptomyces*, mianowicie *S. kronopolitis* CGMCC 4.7323^T, *S. chattanoogensis* NRRL ISP-5002^T i *S. lydicus* ATCC 25470^T, z którymi wykazywał odpowiednio 99,3, 99,2 i 99,2% podobieństwa sekwencji nukleotydowej genu 16S rRNA. W drzewach filogenetycznych wygenerowanych metodą najbliższego sąsiada oraz największej wiarygodności i największej parsymonii opartych na analizie sekwencji tego genu, izolat SF28^T tworzył wspólną gałąź razem ze *S. kronopolitis* CGMCC 4.7323^T (publikacja VII).

Podobnie jak w przypadku nowo opisanych gatunków *Modestobacter*, wszystkie izolaty rodzaju *Streptomyces* pomimo podobieństwa sekwencji nukleotydowej genu 16S rRNA powyżej wartości 98,7% rekomendowanej przez Kim i wsp. (2014) poddano dalszym analizom w celu ustalenia ich pozycji taksonomicznej.

Porównawcza analiza sekwencji genomów izolatu OF1^T i *S. durbertensis* DSM 104538^T z wykorzystaniem hybrydyzacji DNA:DNA *in silico* oraz ANI wykazała podobieństwo, odpowiednio 45,2 i 92,0%, zatem zacznie poniżej wartości progowych (odpowiednio 70 i 95-96%) rekomendowanych przez Wayne'a i wsp. (1987), Gorisa i wsp. (2007) oraz Chuna i Raineya (2014), wskazując przynależność izolatu OF1^T do nowego gatunku z rodzaju *Streptomyces*, któremu nadano nazwę *Streptomyces alkaliterrae* (publikacja IV).

Wartość hybrydyzacji DNA:DNA przeprowadzonej w warunkach laboratoryjnych pomiędzy szczepem IF17 i jego najbliższym filogenetycznym sąsiadem, *S. alkaliphilus* DSM 42118^T, wynosiła 63,6%, a więc poniżej wartości 70% rekomendowanej przez Wayne'a i współpracowników (1987), wskazując, że izolat IF17 reprezentuje nowy gatunek w obrębie rodzaju *Streptomyces*. Jednak wyniki DDH bliskie wartości progowej otrzymane metodą laboratoryjną mogą być wątpliwe, ponieważ ta metoda obarczona jest błędem eksperymentalnym, który jest minimalizowany w bardziej wiarygodnej metodzie DDH *in silico,* opartej na analizach bioinformatycznych (Auch i in., 2010). Hybrydyzacja DNA:DNA *in silico* przeprowadzona pomiędzy szczepem IF17 i *S. alkaliphilus* wykazała podobieństwo sekwencji genomowego DNA na poziomie 79,6%, wskazując jednoznacznie, że szczepy należą do jednego gatunku. Przynależność

tych szczepów do jednego gatunku potwierdzono również na podstawie wysokiej wartości ANI (98,0%), a więc znacznie powyżej progu 95-96% zalecanego przez Gorisa i wsp. (2007) oraz Chuna i Raineya (2014) (publikacja III).

Pomimo, że analiza filogenetyczna w oparciu o sekwencje nukleotydową genu 16S rRNA wykazała najbliższe pokrewieństwo izolatu SF28^T ze *Streptomyces kronopolitis* CGMCC 4.7323^T, *Streptomyces chattanoogensis* NRRL ISP-5002^T i *Streptomyces lydicus* ATCC 25470^T to wielolokusowa analiza sekwencji nukleotydowej genów metabolizmu podstawowego (*atpD*, *gyrB*, *recA* i *rpoB*) i genu 16S rRNA (ang. Multilocus Sequence Analysis, MLSA) wykazała, że najbliższymi filogenetycznymi sąsiadami izolatu SF28^T, oprócz *S. kronopolitis, S. chattanoogensis* i *S. lydicus*, jest również *Streptomyces inhibens* NEAU-D10^T. Jednak podobieństwo sekwencji nukleotydowych analizowanych genów (95,8-96,2%) oraz odległości ewolucyjne pomiędzy izolatem a najbliżej spokrewnionymi gatunkami (0,032-0,042) były znacznie poniżej wartości 98,7% rekomendowanej przez Kima i współpracowników (2014) oraz powyżej wartości 0,007 rekomendowanej przez Ronga i Huanga (2012, 2014), wskazując, że badany izolat nie był blisko spokrewniony z żadnym z wymienionych gatunków. Natomiast DDH *in silico*, oparta o analizę milionów nukleotydów, wykazała, że izolat jest najbliżej spokrewniony ze *Streptomyces deycoicus* NRRL 2666^T, wykazując 28,7% podobieństwa sekwencji DNA z tym gatunkiem. Średnie podobieństwo nukleotydów (ANI) pomiędzy analizowanymi szczepami wynosiło 85,1%. Oba analizowane parametry miały wartość znacznie poniżej wartości progowej rekomendowanej przy opisywaniu nowych gatunków bakterii (Wayne i in., 1987; Goris i in., 2007; Chun i Rainey, 2014), wskazując że izolat SF28^T reprezentuje nowy gatunek w obrębie rodzaju *Streptomyces*, który nazwano *Streptomyces pinistramenti* (publikacja VII).

Analiza porównawcza sekwencji całych genomów jest znacznie bardziej wiarygodnym narzędziem przy wyodrębnianiu nowych gatunków, ponieważ opiera się na milionach a nie tysiącach analizowanych nukleotydów, jak ma to miejsce w przypadku analizy MLSA lub sekwencji 16S rRNA (Nouioui i in., 2018).

Wyniki analiz chemicznych markerów komórkowych i właściwości morfologicznych potwierdziły przynależność izolatów OF1^T, OF3, OF8, IF11, IF17 i IF19 oraz SF28^T do rodzaju *Streptomyces.* Natomiast analiza sekwencji powtarzalnych (BOX-PCR) i właściwości fizjologicznych pozwoliły na wykazanie różnic pomiędzy izolatami reprezentującymi ten sam gatunek (publikacja IV), a w przypadku właściwości

fizjologicznych również pomiędzy izolatami a ich najbliżej spokrewnionymi gatunkami (publikacje IV i VII).

Rodzaj *Streptomyces* jest najliczniejszym taksonem wśród promieniowców (Gao i Gupta, 2012), obejmującym obecnie 699 poprawnie opisanych gatunków (LPSN, https://lpsn.dsmz.de/, data dostępu 30.11.2022; Parte i in., 2020) i wraz z rodzajami *Actinacidiphila, Allostreptomyces, Embleya, Kitasatospora, Mangrovactinospora, Peterkaempfera, Phaeacidiphilus, Streptacidiphilus, Streptantibioticus, Wenjunlia* i *Yinghuangia* należy do rodziny *Streptomycetaceae* (Madhaiyan i in., 2022).

Bakterie z rodzaju *Streptomyces* powszechnie występują w glebie i w większości są gatunkami pożytecznymi uczestniczącymi w obiegu pierwiastków w przyrodzie i produkującymi antybiotyki (Kämpfer, 2012). Nieliczne gatunki *Streptomyces* są patogenami roślin np. *Streptomyces scabiei* (Lambert i Loria, 1989b) i *Streptomyces acidiscabies* (Lambert i Loria, 1989a) oraz ludzi i zwierząt jak np. *Streptomyces somaliensis* (Kämpfer, 2012).

Przedstawiciele tego rodzaju mają silnie rozgałęzioną grzybnię substratową i powietrzną. W obrębie grzybni powietrznej znajdują się strzępki sporonośne zakończone łańcuszkami spor składającymi się z 3 lub więcej zarodników. Czasami tego typu krótkie łańcuszki występują również w grzybni substratowej. Kwas diamnopimelinowy występuje głównie w formie *LL*, ale możliwe jest występowanie mniejszej ilości formy *mezo* (Kämpfer, 2012). Dominującymi chinonami izoprenoidowymi błon komórkowych są sześcio- i ośmiouwodornione chinony z dziewięcioma jednostkami izoprenoidowymi (MK-9 (H6), (H8)), jednak w mniejszej ilości mogą być obecne także czterouwodornione menachinony z dziewięcioma jednostkami izoprenoidowymi oraz ośmio- i dziesięcio-izoprenoidowe chinony (MK-8 i MK-10) (Kämpfer, 2012; Nouioui i in., 2018). Profil lipidów polarnych składa się z difosfatydyloglicerolu, fosfatydyloglicerolu, fosfatydyloetanoloaminy (fosfolipid diagnostyczny), fosfatydyloinozytolu oraz mannozydu fosfatydyloinozytolu (Wellington i in., 1992; Kämpfer, 2012; Nouioui i in., 2018). Kwasy tłuszczowe w większości są rozgałęzione, wśród których dominują $C_{14:0}$, *izo*-$C_{14:0}$, $C_{15:0}$, *anteizo*- $C_{15:0}$, $C_{16:0}$, *izo*-$C_{16:0}$, *anteizo*- $C_{17:0}$ (Kämpfer, 2012; Nouioui i in., 2018). Zawartość par G+C w DNA wynosi od 66 do 78% (Wellington i in., 1992; Nouioui i in., 2018).

4.3. Rodzaj *Catenulispora*

Szczepy NF3, NF23, NH11^T i NL8^T wyizolowane z kwaśnej gleby lasu sosnowego z okolic Torunia na podstawie analizy sekwencji nukleotydowej genu 16S rRNA zidentyfikowano do rodzaju *Catenulispora* (publikacje V i VI).

Analiza filogenetyczna na podstawie sekwencji nukleotydowej genu 16S rRNA wykazała, że szczepy są ze sobą blisko spokrewnione chociaż tworzą dwie pary izolatów, odpowiednio NF3 i NH11^T oraz NF23 i NL8^T, reprezentujących potencjalnie dwa nowe gatunki. Izolaty każdej pary tworzyły osobną, dobrze wspieraną gałąź w drzewie filogenetycznym obejmującym przedstawicieli rzędu *Catenulisporales* wygenerowanym zarówno metodą najbliższego sąsiada jak i największej wiarygodności i największej parsymonii (publikacje V i VI). Izolaty NF3 i NH11^T wykazywały 99,0 i 98,8% podobieństwa sekwencji nukleotydowej genu 16S rRNA z najbliższymi filogenetycznymi sąsiadami, odpowiednio *Catenulispora acidiphila* DSM 44928^T i *Catenulispora rubra* DSM 44948^T. Hybrydyzacja DNA:DNA *in silico* i analiza średniego podobieństwa nukleotydów (ANI) pomiędzy szczepami NF3 i NH11^T a najbliżej spokrewnionymi gatunkami *C. acidiphila* i *C. rubra* wykazała podobieństwo odpowiednio 86,3 i 29,6% oraz 86,8-86,9% i 30,6-30,8%, zatem wartości znacznie poniżej wartości progowych zaproponowanych przez Wayne'a i wsp. (1987), Gorisa i wsp. (2007) oraz Chuna i Raineya (2014) stosowanych do wyodrębnienia nowych gatunków bakterii. Na podstawie uzyskanych wyników badań zaproponowano utworzenie nowego gatunku rodzaju *Catenulispora*, któremu nadano nazwę *Catenulispora pinisilvae* z izolatem NH11^T jako szczepem wzorcowym tego gatunku (publikacja V).

Analizy filogenetyczne izolatów NF23 i NL8^T w oparciu o sekwencje nukleotydową genu 16S rRNA wykazały ich najbliższe pokrewieństwo z opisanym powyżej gatunkiem *C. pinisilvae* (izolatem NH11^T; 99,9% podobieństwa sekwencji genu). Poza tym, izolaty były również blisko spokrewnione z *C. acidiphila* i *C. rubra* (99,1% podobieństwa sekwencji genu 16S rRNA). Pomimo dużego podobieństwa sekwencji nukleotydowej genu 16S rRNA izolatów NF23 i NL8^T ze szczepem NH11^T, analizy porównawcze sekwencji genomów wykazały, że wartości dDDH oraz Ortho ANI wynosiły odpowiednio 54,6-54,7% i 93,9% (publikacja VI) i były znacznie poniżej wartości progowych przyjętych dla tych parametrów (Wayne i in., 1987; Goris i in., 2007; Chun i Rainey, 2014), potwierdzając przynależność analizowanych szczepów do nowego

gatunku w obrębie rodzaju *Catenulispora*, któremu nadano nazwę *C. pinistramenti,* z izolatem NL8^T jako szczepem wzorcowym (publikacja VI).

Ponadto, właściwości chemotaksonomiczne i morfologiczne izolatów były charakterystyczne dla rodzaju *Catenulispora* (Busti i in., 2006; Tamura i in., 2008), a właściwości fizjologiczne i molekularny odcisk palca (ang. molecular fingerprint; BOX-PCR) pozwoliły na wykazanie różnic pomiędzy izolatami, a w przypadku właściwości fizjologicznych również pomiędzy izolatami a ich najbliżej spokrewnionymi gatunkami.

Rzadko występujący rodzaj *Catenulispora* obejmuje obecnie osiem poprawnie opisanych gatunków (LPSN, https://lpsn.dsmz.de/, data dostępu 30.11.2022; Parte i in., 2020), mianowicie *C. acidiphila* (Busti i in., 2006), *C. fulv*a (Lee i Whang, 2016), *C. rubra* (Tamura i in., 2007), *C. pinisilvae* (publikacja V) i *C. pinistramenti* (publikacja VI) pochodzące z kwaśnej gleby lasów szpilkowych, *C. graminis* (Lee i in., 2012) wyizolowany z lasu bambusowego, a także *C. subtropica* i *C. yoronensis* pochodzące odpowiednio z gleby pola ryżowego i gleby leśnej (Tamura i in., 2008).

Większość przedstawicieli rodzaju *Catenulispora* należy do mikroorganizmów kwasolubnych (Busti i in., 2006). Promieniowce z tego rodzaju zawierają formę *LL*-kwasu diaminopimelinowego w peptydoglikanie ściany komórkowej, arabinozę, mannozę i galaktozę jako cukry dominujące w hydrolizatach komórek, *izo*-$C_{16:0}$ i *anteizo*-$C_{17:0}$ jako dominujące kwasy w profilu kwasów tłuszczowych oraz difosfatydyloglicerol, fosfatydyloinnozytol i mannozyd fosfatydyloinozytolu w profilu lipidów polarnych. Chinony izoprenoidowe to cztero- i sześcio-uwodornione chinony z dziewięcioma jednostkami izoprenoidowymi (MK-9 (H4), (H6)) i w mniejszej ilości ośmio-uwodornione (MK-9 (H8)). Zawartość par G+C w DNA waha się od 69 do 72% (Tamura i in., 2008).

5. Analiza genomów nowych gatunków promieniowców

5.1 Geny stresu związane z przystosowaniem promieniowców do życia w ekstremalnym lub nietypowym środowisku

Analiza genomu *M. excelsi* 1G6^T (publikacja II) i *M. altitudinis* 1G4^T (publikacja III) wykazała obecność odpowiednio 59 i 64 genów stresu. Wśród nich zidentyfikowano geny związane ze stresem oksydacyjnym, szokiem cieplnym i zimna, opornością na promieniowanie UV i niedoborem węgla organicznego. Jednocześnie obecność genów odpowiedzialnych za wychwytywanie tlenku węgla wskazuje, że szczepy te mogą

być chemolitoautotrofami. U zbadanych szczepów nie znaleziono genów stresu suszy, ale stwierdzono obecność genów kodujących wychwytywanie trehalozy, która zwiększa oporność na ciepło i suszę u bakterii (Reina-Bueno i in., 2010). Podobne wyniki uzyskała Busarakam i współpracownicy (2016) analizując genom *Modestobacter caceresii,* promieniowca wyizolowanego z gleb pustyni Atakama.

Szczep OF1^T (publikacja IV) posiadał 44 geny stresu zorganizowane w subsystemy, w tym głównie geny stresu oksydacyjnego i kodujące czynnik transkrypcyjny Sigma B (SigB) włączany podczas stresu wywoływanego czynnikami fizycznymi i brakiem związków odżywczych. Geny stresu występujące u promieniowców ze słonych alkalicznych jezior nie zostały jeszcze dobrze zbadane. Ich analizie należy poświęcić w przyszłości więcej uwagi.

Wstępna analiza genów stresu u promieniowców z rodzaju *Catenulispora* wykazała obecność 54-58 genów stresu u *C. pinisilvae* (publikacja V), 53-56 u *C. pinistramenti* (publikacja VI), natomiast u blisko związanych z nimi gatunków, *C. acidiphila* i *C. rubra,* odpowiednio 69 i 56.

Gatunek *Streptomyces pinistramenti* (publikacja VII), reprezentowany przez szczep SF28^T wyizolowany z kwaśnej gleby leśnej, posiadał 61 genów związanych ze stresem. W genomie tego izolatu potwierdzono obecność genów kodujących białka związane z odpowiedzią komórki na szok cieplny i zimna, stres oksydacyjny oraz genów odpowiedzialnych za naprawę i stabilizację DNA. Obecność genu odpowiedzialnego za wychwytywanie tlenku węgla wskazuje, że szczep ten może prowadzić chemolitoautotroficzny tryb życia. Szczególnie interesująca była obecność genu *atpA,* kodującego białko międzybłonowe uczestniczące w transporcie i zwiększające adaptację do życia w kwaśnych środowiskach (Guan i Liu, 2020) oraz genu (*sigB*) związanego ze stresem osmotycznym, kodującego czynnik sigma B*,* który jest silnie aktywowany w kwaśnych warunkach środowiska (Kim i in., 2008).

Obecność genów odpowiedzi na stres środowiskowy może ułatwiać mikroorganizmom zasiedlanie trudnych środowisk (Busarakam i in., 2016) i zapewnić im przewagę nad innymi mikroorganizmami w konkurencji o niszę i składniki odżywcze (Shivlata i Satyanarayana, 2015). Jednak dokładne poznanie molekularnych mechanizmów przystosowania bakterii do panujących warunków środowiska wymaga dalszych analiz.

5.2. Biosyntetyczne klastry genowe (BGCs) kodujące metabolity wtórne

Wielkość genomu może być bezpośrednio związana ze zdolnością mikroorganizmów do kodowania dużej ilości związków biologicznie czynnych. Szczególnie interesujące pod tym względem są promieniowce, które często mają genomy bliskie 8,0 Mbp lub większe (Baltz, 2017).

Wielkość genomu izolatów 1G6^T (publikacja II) i 1G4^T (publikacja III), reprezentujących nowe gatunki rodzaju *Modestobacter*, pozyskanych z gleby pustyni Atakama była podobna i wynosiła odpowiednio 5,3 i 5,2 Mbp. Niewiele większe genomy (5,9-6,0 Mbp) miały potencjalnie nowe gatunki *Streptomyces* wyizolowane z jeziora Lonar (publikacja IV). Zatem te izolaty nie potwierdzają powyższej reguły. Jednak u promieniowców wyizolowanych z kwaśnej gleby leśnej należących do rodzaju *Catenulispora* stwierdzono obecność genomów znacznie większych (od 10,8 do 11,5 Mbp) (publikacje V i VI), zaś u szczepu SF28^T należącego do rodzaju *Streptomyces* genom o wielkości 7,9 Mbp (publikacja VII).

Analiza niewielkich jak na promieniowce genomów, u szczepów z rodzaju *Modestobacter* wykazała nielicznie występujące klastry genów odpowiedzialnych za syntezę biologicznie czynnych metabolitów wtórnych (ang. Biosynthetic Gene Clusters, BGCs), wśród których dominowały klastry genów kodujące betalaktony, terpeny i syntazy poliketydowe typu 2 i 3 (publikacje II i III). Uzyskane wyniki analiz BGCs są zgodne z tymi, które opublikowano dla *M. caceresii* (Busarakam i in., 2016).

Izolaty OF1^T, OF3 i OF8 oraz IF17 należące do rodzaju *Streptomyces* posiadały w swoim genomie od 24 do 31 klastrów genów kodujących metabolity wtórne, głównie siderofory, terpeny, nierybosomalne peptydy, syntazy poliketydowe i lantipeptydy, co jest zgodne z doniesieniami literaturowymi, podającymi, że przedstawiciele tego rodzaju posiadają powyżej 20 takich klastrów w swoim genomie (Barka i in., 2016). Intersujące jest występowanie u tych izolatów dużej ilości klastrów hybrydowych składających się z co najmniej 2 klastrów. Metabolity kodowane przez takie klastry wykazują odmienne właściwości, mają unikalną strukturę i mechanizm działania w porównaniu do związków kodowanych przez pojedyncze klastry genów (Fischbach i Walsh, 2006). U izolatów OF1^T, OF3 i OF8 wykryto obecność klastrów genów o dużym podobieństwie sekwencji (75-100%) do tych, które kodują znane związki przeciwbakteryjne, takie jak streptofenazyna (Bauman i in., 2019) i tiolaktomycyna (Slayden i in., 1996), a także przeciwnowotworowe i obniżające poziom cholesterolu we krwi, jak naringenina (Álvarez-Álvarez i in., 2015). Na szczególną uwagę zasługują

niescharakteryzowane klastry genów oraz takie, które wykazują małe podobieństwo sekwencji do poznanych klastrów genów, ponieważ mogą kodować nieznane metabolity wtórne (publikacja IV).

Analiza biosyntetycznych klastrów genów w genomach izolatów rodzaju *Catenulispora* wykazała obecność 50, 40, 40 i 34 takich klastrów u szczepów NF3, NH11^T (publikacja V), NF23 i NL8^T (publikacja VI). Genomy dwóch blisko spokrewnionych gatunków z rodzaju *Catenulispora*, mianowicie *C. acidiphila* i *C. rubra* posiadały 26 i 52 klastry (publikacje V i VI). Ogólnie klastry te były odpowiedzialne za syntezę nierybosomalnych peptydów, syntaz poliketydowych, bakteriocyn, lantipeptydów, sideroforów i terpenów. Ponadto, podobnie jak u izolatów z jeziora Lonar, w genomach zbadanych izolatów stwierdzono występowanie hybrydowych klastrów genowych (publikacje V i VI). Izolaty NF3 i NH11^T w swoich genomach posiadają klastry genowe, których sekwencje są identyczne z sekwencjami kodującymi rhizomide A–C, wykazującymi działanie przeciwdrobnoustrojowe i przeciwnowotworowe (Wang i in., 2018) oraz alkilrezorcynol o wielu funkcjach biologicznych (Nakano i in., 2012; publikacja V).

Izolat SF28^T należący do rodzaju *Streptomyces* zawierał w genomie 29 biosyntetycznych klastrów genowych, z których większość kodowała syntetazy nierybosomalnych peptydów, syntazy poliketydowe, a ponadto również klastry hybrydowe. W genomie *S. pinistramenti* SF28^T zanotowano klastry genów, których sekwencje były w 100% podobne do sekwencji rhizomide A–C (Wang i in., 2018) i alkilrezorcynolu (Nakano i in., 2012) oraz klaster genowy, którego sekwencja była w 75% podobna do opornego na ciepło czynnika przeciwgrzybowego (Yu i in., 2007) (publikacja VII).

Analizując klastry genów występujące u izolatów leśnych, podobnie jak w przypadku izolatów z gleby jeziora Lonar, najbardziej interesującymi wydają się klastry genów kodujące nieznane dotychczas związki lub te o małym podobieństwie do znanych metabolitów, zwłaszcza, że genomy tych izolatów posiadają blisko połowę takich klastrów (publikacje V – VII).

VI Wnioski

1. Identyfikacja izolatów z gleby pustyni Atakama w Chile, jeziora Lonar w Indiach i lasu sosnowego w okolicy Torunia w oparciu o sekwencję genu 16S rRNA wykazała, że są one zróżnicowane taksonomicznie, należą zarówno do powszechnie jaki i rzadko występujących rodzajów i mogą stanowić potencjalnie nowe gatunki promieniowców.
2. Wielofazowa analiza wybranych izolatów wyodrębniła 6 nowych gatunków promieniowców z rodzajów *Catenulispora, Modestobacter* i *Streptomyces*, mimo, że podobieństwo sekwencji genu 16S rRNA do najbliżej spokrewnionego, sąsiada u większości z nich, było wyższe niż rekomendowana wartość progowa, wskazując, że takich gatunków wśród zbadanych szczepów może być znacznie więcej.
3. Genomy izolatów z rodzaju *Modestobacter* wyizolowanych z pustyni Atakama charakteryzował niewielki rozmiar i nielicznie występujące biosyntetyczne klastry genów (BGCs) w przeciwieństwie do genomów nowych gatunków z rodzajów *Streptomyces* i *Catenulispora*.
4. W genomach nowych gatunków *Modestobacter* wyizolowanych z silnie nasłonecznionych i silnie wysuszonych gleb pustyni Atakama zanotowano największą liczbę genów stresu związanych z adaptacją od ekstremalnych warunków środowiska w porównaniu z genomami nowych gatunków *Streptomyces* i *Catenulispora*.
5. Najczęściej występujące BGCs w genomach nowych gatunków kodowały nierybosomalne syntetazy polipeptydowe (NRPS), syntazy poliketydowe (PKS), siderofory, terpeny oraz należały do klas hybrydowych.
6. Wśród zbadanych promieniowców szczepy wyizolowane z kwaśnej gleby leśnej wykazywały największą aktywność przeciwdrobnoustrojową i promującą wzrost roślin.
7. Zbadane promieniowce wykazywały zdolność do wytwarzania celulaz, chitynaz, lipaz i proteinaz, niezależnie od źródła izolacji; wytwarzanie pektynaz odnotowano wśród izolatów z gleb pustyni Atakama.
8. Przeprowadzone badania przesiewowe na szczepach reprezentatywnych pozwoliły na wygenerowanie biblioteki szczepów o różnej aktywności biologicznej i potencjale biotechnologicznym.

Przeprowadzone doświadczenia pozwoliły na weryfikację postawionych hipotez badawczych i potwierdzenie, że gleby pustyni Atakama, jeziora Lonar i lasu sosnowego są nadal źródłem nowych gatunków promieniowców, zarówno w obrębie powszechnie, jak i rzadko występujących rodzajów (hipoteza 1).

Wśród zbadanych izolatów ze wszystkich środowisk większość z nich wykazywała zróżnicowaną aktywność biologiczną (przeciwdrobnoustrojową, enzymatyczną i/lub promującą wzrost roślin), co potwierdza, że słabo poznane i ekstremalne biomy są bogatym rezerwuarem promieniowców o dużej aktywności wskazującej na ich potencjał do zastosowania w medycynie, biotechnologii czy rolnictwie (hipoteza 2).

Analizy genomów większości nowych gatunków promieniowców potwierdziły obecność licznych klastrów genowych kodujących niezidentyfikowane metabolity lub wykazujące małe podobieństwo do znanych metabolitów mogących stanowić potencjalnie nowe związki biologicznie aktywne. Genomy te charakteryzował duży rozmiar (~ 8 Mbp lub większy) w przeciwieństwie do genomów gatunków rodzaju *Modestobacter* (~ 5 Mbp), u których wykryto nieliczne klastry genów odpowiedzialnych za biosyntezę wyspecjalizowanych metabolitów wtórnych (hipoteza 3).

Analiza genów stresu w genomach nowych gatunków promieniowców wykazała obecność genów związanych ze stresem oksydacyjnym, naprawą DNA, stresem promieniowania UV (u promieniowców z pustyni Atakama). Uzyskane wyniki są zbieżne z wynikami uzyskanymi u innych izolatów z tych samych środowisk potwierdzając hipotezę, że geny te mogą być zaangażowane w adaptację izolatów do trudnych warunków środowiska. Jednocześnie obecność nielicznych biosyntetycznych klastrów genowych oraz licznych genów stresu u izolatów z pustyni Atakama potwierdza, że sukces kompetycyjny mikroorganizmów w tym ekstremalnym środowisku nie jest związany z antybiozą, lecz z opornością na stres środowiskowy, pozwalającą im na przystosowanie się do panujących warunków (hipoteza 4).

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193. Zhuang X, Wang Z, Peng C, Su C, Gao C, Wang Y, i in. (2020) Chracterization of *Streptomyces piniterrae* sp. nov. and identification of the putative gene cluster encoding the biosynthesis of heliquinomycins. *Microorganisms* 8(4):495.

VIII *Curriculum Vitae*

Dane osobowe:

Imię i nazwisko: Magdalena Świecimska

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Przebieg wykształcenia:

Studia doktoranckie z zakresu nauk biologicznych, Wydział Nauk Biologicznych i Weterynaryjnych Uniwersytet Mikołaja Kopernika w Toruniu, od 2017.

Studia magisterskie: Biotechnologia, Wydział Biologii i Ochrony Środowiska Uniwersytet Mikołaja Kopernika w Toruniu, 2015-2017.

Praca magisterska: „Wytwarzanie nanocząstek tlenku cynku i selenu przez promieniowce środowisk ekstremalnych".

Technik farmaceutyczny: Policealna Szkoła Farmaceutyczna Studium Kształcenia Kadr w Bydgoszczy, 2015-2017.

Studia licencjackie: Biotechnologia, Wydział Biologii i Ochrony Środowiska Uniwersytet Mikołaja Kopernika w Toruniu, 2012-2015.

Praca licencjacka: „Bakteriocyny i ich zastosowanie".

Kursy i szkolenia:

- Kurs dokształcający w zakresie nowoczesnych technik stosowanych w biologii molekularnej i biotechnologii 2013-2015.
- Warsztaty z obsługi programu QGIS 15-17.09.2021.

Doświadczenie zawodowe:

- 12.2019-12.2022 członek zespołu Emerging Field "Nauki o glebie, mikrobiologia, genetyka w rolnictwie i jakość pożywienia".
- 25.10.2019-27.11.2020 zatrudnienie na stanowisku asystent w grupie pracowników badawczo-dydaktycznych w Katedrze Mikrobiologii, Uniwersytet Mikołaja Kopernika w Toruniu.
- Współpraca badawczo-naukowa z Instytutem Ochrony Roślin - Państwowego Instytutu Badawczego w Poznaniu od 2018.

- Współpraca badawczo-naukowa z Instytutem Badawczym Leśnictwa w Sękocinie Starym pod Warszawą od 2018.

Projekty badawcze:

- Grant „Actinomycetes isolated from coniferous soils as a plant growth promoters and biocontrol factor for fungal pathogens of pine seedlings" (90SIDUB.6102.40.2021.G4NCUS1) w ramach IDUB Grants4NCUStudents finansowanego przez Uniwersytet Mikołaja Kopernika (11.05.2021-31.05.2022) - kierownik i wykonawca
- Grant „Poszukiwanie nowych gatunków promieniowców o właściwościach przeciwgrzybowych w glebach kwaśnych i alkalicznych" (B-1207) finansowany przez Uniwersytet Mikołaja Kopernika (6.08.-14.12.2018) - kierownik i wykonawca

Staże naukowe:

Uniwersytet Komeńskiego w Bratysławie w ramach projektu ERASMUS+ (17.06.–16.08.2019).

Publikacje i monografie:

1. **Świecimska M**, Golińska P, Goodfellow M. (2022) Generation of a high quality library of bioactive filamentous actinomycetes from extreme biomes using a culture-based bioprospecting strategy. Frontiers in Microbiology. doi:10.3389/fmicb.2022.1054384.
2. **Świecimska M**, Golińska P, Goodfellow M. (2022) Genome-based classification of *Streptomyces pinistramenti* sp. nov., a novel actinomycete isolated from a pine forest soil in Poland with a focus on its biotechnological and ecological properties. Antonie van Leeuwenhoek 115, 783–800.
3. **Świecimska M**, Golińska, P, Wypij M, Goodfellow M. (2021) *Catenulispora pinistramenti* sp. nov., novel actinobacteria isolated from pine forest soil in Poland. International Journal of Systematic and Evolutionary Microbiology 71, 5063.
4. **Świecimska M**, Golińska P, Wypij M, Goodfellow M. (2021) Genomic-based classification of *Catenulispora pinisilvae* sp. nov., novel actinobacteria isolated from a pine forest soil in Poland and emended description of *Catenulispora rubra.* Systematic and Applied Microbiology 44, 126164.
5. **Świecimska M**, Golińska P, Nouioui I, Wypij M, Rai M, Sangal V, Goodfellow M. (2020) *Streptomyces alkaliterrae* sp. nov., isolated from an alkaline soil, and emended

descriptions of *Streptomyces alkaliphilus*, *Streptomyces calidiresistens* and *Streptomyces durbertensis*. Systematic and Applied Microbiology 43.

6. **Świecimska M**, Tulik M, Šerá B, Golińska P, Tomeková J, Medvecká V, Bujdáková H, Oszako T, Zahoranová A, Šerý M. (2020) Non-thermal plasma can be used in disinfection of Scots pine (*Pinus sylvestris* L.) seeds infected with *Fusarium oxysporum*. Forests 11, 8.
7. Golińska P, **Świecimska M,** Montero-Calasanz MC, Yaramis A, Igual JM, Bull AT, Goodfellow M. (2020) *Modestobacter altitudinis* sp. nov., a novel actinobacterium isolated from Atacama Desert soil. International Journal of Systematic and Evolutionary Microbiology 70.
8. Golińska P, Montero-Calasanz MC, **Świecimska M**, Yaramis A, Igual JM, Bull AT, Goodfellow M. (2020) *Modestobacter excelsi* sp. nov., a novel actinobacterium isolated from a high altitude Atacama Desert soil. Systematic and Applied Microbiology 43, 1.
9. Golińska P, **Świecimska M.** *Pythium*: Diseases and its Management, W: *Pythium*: Diagnosis, Diseases and Management red.: Rai M, Abd-Elsalam K, Ingle A, CRC press/Taylor and Francis, USA, 2020, 29-43.
10. Wypij M, **Świecimska M**, Dahm H, Rai M, Golińska P. (2019) Controllable biosynthesis of silver nanoparticles using actinobacterial strain. Green Process Synth 8, 207-214.
11. Golińska P, **Świecimska M**, Wypij M. Ecotoxicity of Metal Nanoparticles on Microorganisms, W: Nanomaterials: Ecotoxicity, Safety, and Public Perception red.: Rai M, Biswas JK, Springer International Publishing, 2018, 77-94.
12. Wypij M, **Świecimska M**, Czarnecka J, Dahm H, Rai M, Golińska P. (2018) Antimicrobial and cytotoxic activity of silver nanoparticles synthesized from two haloalkaliphilic actinobacterial strains alone and in combination with antibiotics. Journal of Applied Microbiology 124, 1411-1424.
13. Wypij M, Czarnecka J, **Świecimska M**, Dahm H, Rai M, Golińska P. (2018) Synthesis, characterization and evaluation of antimicrobial and cytotoxic activities of biogenic silver nanoparticles synthesized from *Streptomyces xinghaiensis* OF1 strain. World Journal of Microbiology and Biotechnology 34, 23.

Konferencje naukowe:

1. „Plant productivity and food safety: Soil science, Microbiology, Agricultural Genetics and Food quality" Toruń 15-16.09.2022. Poster: Wachnowicz B, **Świecimska M,**

Golińska P, Goodfellow M. *Streptomyces pinistramenti*, sp. nov. as a potential biocontrol agents against phytopathogens.

2. „Plant productivity and food safety: Soil science, Microbiology, Agricultural Genetics and Food quality" Toruń 15-17.09.2021. Poster: **Świecimska M**, Golińska P, Goodfellow M. Novel species of Actinobacteria as a potential biocontrol agents against fungal phytopathogens.
3. 54 Konferencja „Mikroorganizmy Różnych Środowisk" Lublin 20-21.09.2021. Referat: **Świecimska M**, Golińska P, Wypij M, Goodfellow M. Nowe gatunki promieniowców wyizolowane z kwaśnej gleby leśnej i ich aktywność przeciwbakteryjna i przeciwgrzybowa.
4. 54 Konferencja „Mikroorganizmy Różnych Środowisk" Lublin 20-21.09.2021. Poster: **Świecimska M**, Golińska P, Wypij M, Goodfellow M. *Streptomyces* sp. nov. wyizolowany z osadu oceanu Antarktycznego i jego właściwości przeciwdrobnoustrojowe.
5. V Sympozjum Naukowe „Metagenomy różnych środowisk" Warszawa 17-18.06.2021. Referat: **Świecimska M**, Golińska P. Rai M, Goodfellow M. Klasyfikacja szczepów promieniowców wyizolowanych ze słonego, alkalicznego jeziora Lonar w Indiach w oparciu o analizę genomów.
6. XIX Konferencja „Biotechnologia Dziś na Uniwersytecie Technologiczno-Przyrodniczym, Jutro w Regionie Kujawsko Pomorskim" Bydgoszcz 10.06.2021. Poster: **Świecimska M**, Żak J, Regulska O, Golińska P. Analiza genomowa promieniowców wyizolowanych z kwaśnych gleb leśnych.
7. XI Ogólnopolska Konferencja Hydromikrobiologiczna „Mikroorganizmy wodne zagrożenie i nadzieje" Sopot 9-11.06.2021. Referat: **Świecimska M**, Golińska P, Rai M, Goodfellow M. Nowy gatunek z rodzaju *Streptomyces*, o dużym potencjale przeciw drobnoustrojowym, wyizolowany z osadów dennych Oceanu Antarktycznego.
8. "The 66th Annual Conference on Soilborne Plant Pathogens And The 51st California Nematology Workshop" Kalifornia 23-24.03.2021. Referat: **Świecimska M**, Golińska P. Novel species of actinobacteria isolated from acid forest soil with high activity against fungal phytopathogens.
9. "Scientific Anniversary Conference 25 years of Trakia University" Bułgaria 15.05.2020. Referat: **Świecimska M.** Antimicrobial potential of novel *Catenulispora* species isolated from pine forest soil.

10. COST Conference 2019 „PineStrenght final meeting" Skopje, Macedonia 26-28.03.2019 Referat: Davydenko K, Nowakowska JA, Raitelaitytė K, **Świecimska M**, Markovskaja S, Burokienė D, Oszako T. Susceptibility of Lithuanian, Polish and Ukrainian provenances of *P. sylvestris* to *Fusarium circinatum* and possible strategies for biocontrol.
11. III Ogólnopolskie Sympozjum Mikrobiologiczne „Metagenomy różnych środowisk" Lublin 28-29 czerwca 2018. Poster: **Świecimska M**, Wypij M, Carro L, Goodfellow M, Golińska P. *Micromonospor*a species isolated from soils of Atacama desert.
12. NanoBiomateriały teoria i praktyka, Toruń 6-8 czerwca 2018. Poster: **Świecimska M**. Wypij M. Czarnecka J. Golińska P. Antifungal activity of biostable selenium nanoparticles produced by *Streptomyces palmae* strain OF1.
13. International Conference on Natural and Medical Sciences. Young scientists, PhD students and students. Lublin 1-3.12.2017. Poster: **Świecimska M**, Wypij M, Golińska P. Antifungal activity of biostable selenium nanoparticles produced by haloalkalophilic actinobacterial OF1 strain.
14. Ogólnopolska konferencja młodych naukowców "New Challenges for Polish Science" Gdańsk 10.09.2017. Referat: Wypij M, Golińska P, **Świecimska M**, Dahm. Antimicrobial and cytotoxic activity of silver nanoparticles produced by the acidophilic actinomycetes strain SL19.
15. XVI Konferencji „Biotechnologia: dziś na Uniwersytecie Technologiczno-Przyrodniczym, jutro w regionie kujawsko-pomorskim" Bydgoszcz 8.06.2018. Wykład na zaproszenie: **Świecimska M,** Wypij M, Golińska P. Przeciwgrzybowa aktywność biostabilnych nanocząstek selenu wytwarzanych przez haloalkalofilny szczep promieniowca OF1.
16. „Toruńskie Sympozjum Doktorantów Nauk Przyrodniczych" Toruń 24-25.03.2018. Poster: **Świecimska M**, Wypij M, Czarnecka J, Golińska P. Antifungal activity of biostable selenium nanoparticles produced by *Streptomyces palmae* strain OF1.
17. 51 Ogólnopolska Konferencja Mikrobiologiczna "Mikrobiologia środowiskowa szansą bezpiecznego życia i postępu biotechnologicznego" Toruń-Ciechocinek 5-8.09.2017. Referat: Składanowski M, Rudnicka K, Śmigielska E, Golińska P, **Świecimska M**, Dahm H. Cytotoksyczność wobec komórek prokariotycznych i eukariotycznych nanocząstek srebra i złota wytwarzanych przez promieniowce gleb leśnych.
18. 51 Ogólnopolska Konferencja Mikrobiologiczna "Mikrobiologia środowiskowa szansą bezpiecznego życia i postępu biotechnologicznego" Toruń-Ciechocinek 5-8.09.2017.

Referat: Wypij M, Golińska P, **Świecimska M**, Czarnecka J, Dahm H. Aktywność przeciwdrobnoustrojowa i cytotoksyczna nanocząstek srebra wytwarzanych przez acidofilny szczep promieniowca SL19.

Działalność dydaktyczna (prowadzenie zajęć laboratoryjnych):

- Podstawy mikrobiologii na kierunku chemia medyczna (S1)
- Mikrobiologia sądowa na kierunku biologia sądowa (S1)
- Mikrobiologia ogólna na kierunkach biotechnologia i biologia (S1)
- Czynniki patogenności bakterii klinicznych na kierunku biotechnologia (S1)
- Mikrobiom człowieka i zwierząt na kierunku biotechnologia (S2)
- Mikrobiologia środowiskowa na kierunku biotechnologia (S2)
- Wirusologia na kierunku biotechnologia (S2)
- Zastosowanie mikroorganizmów w biotechnologii farmaceutycznej na kierunku chemia medyczna (S1)
- Mikrobiologiczna analiza środowiska na kierunku biologia (S1)

Działalność popularyzatorska i organizacyjna:

- Członek komitetu organizacyjnego drugiej edycji międzynarodowej konferencji „Plant productivity and food safety: Soil science, Microbiology, Agricultural Genetics and Food quality" Toruń 15-16.09.2022
- Członek komitetu organizacyjnego pierwszej edycji międzynarodowej konferencji „Plant productivity and food safety: Soil science, Microbiology, Agricultural Genetics and Food quality" Toruń 15-17.09.2021
- Członek komitetu organizacyjnego VIII Kopernikańskiego Sympozjum Studentów Nauk Przyrodniczych i IV Toruńskiego Sympozjum Doktorantów Nauk Przyrodniczych Uniwersytetu Mikołaja Kopernika w Toruniu 24-25.03.2018
- Prowadzenie zajęć w ramach „Nocy Biologów" Toruń, 12.01.2018, 11.01.2019, 10.01.2020, 8.01.2021, 14.01.2022
- Prowadzenie zajęć w ramach „Fascynującego Dnia Roślin" Toruń, 17.05.2019 i 21.05.2021
- Prowadzenie zajęć w ramach „Dnia Otwartego Wydziału Biologii i Ochrony Środowiska" Toruń 2.10.2017, 28.09.2018 i 29.09.2022,

Nagrody, wyróżnienia i stypendia:

- Nagroda za wystąpienie „Nowe gatunki promieniowców wyizolowane z kwaśnej gleby leśnej i ich aktywność przeciwbakteryjna i przeciwgrzybowa*”* w sesji młodych naukowców na 54. Konferencji Mikrobiologicznej „Mikroorganizmy Różnych Środowisk” 2021 w Lublinie.
- Wyróżnienie za poster „*Streptomyces* sp. nov. wyizolowany z osadu oceanu Antarktycznego i jego właściwości przeciwdrobnoustrojowe” na 54. Konferencji Mikrobiologicznej „Mikroorganizmy Różnych Środowisk” 2021 w Lublinie.
- Zwiększenie stypendium doktoranckiego w ramach programu „Inicjatywa doskonałości-uczelnia badawcza”(rok akademicki 2020/2021, 2021/2022).
- Stypendium rektora dla najlepszych doktorantów (rok akademicki, 2018/2019, 2020/2021, 2021/2022).
- Stypendium naukowe dla doktorantów (rok akademicki 2018/2019, 2019/2020, 2020/2021, 2021/2022, 2022/2023).
- Stypendium projakościowe (rok akademicki 2018/2019, 2019/2020, 2020/2021, 2021/2022).

Znajomość języków obcych:

- Język hiszpański - bardzo dobra (2012 título de bachiller -Matura hiszpańska na poziomie dwujęzycznym)
- Język angielski - dobra