Abstract

Plant growth-promoting rhizobacteria (PGPR) represent an environmentally friendly alternative to reducing the use of chemical fertilizers and fungicides. PGPR enhance plant growth by secreting phytohormones, dissolving phosphates, fixing nitrogen, increasing nutrient uptake, and acting as biocontrol agents against pathogens. The effectiveness of PGPR inoculation is linked to their ability to colonize roots, as well as their survival in the presence of native bacterial and fungal communities in the rhizosphere.

The aim of this doctoral thesis was to characterize and identify bacteria isolated from the rhizosphere of canola roots cultivated on two farms located in Górsk in the Kuyavian-Pomeranian Voivodeship and in Ostróda in the Warmian-Masurian Voivodeship, assess the impact of rhizobacteria on improving the growth and development of canola, search for genes contributing to a plant growth promotion and a biocontrol of plant pathogens, and determine the impact of PGPR rhizosphere inoculation on the number of microorganisms associated with the nitrogen cycle and the biodiversity of native bacterial and fungal communities.

In the preliminary studies, an analysis was conducted to determine the abundance of cultivable rhizobacteria from different growth stages of canola (vegetative, flowering, and maturity), cultivated in Górsk and in Ostróda. The studies revealed a complex dynamics of microbial communities, showing differences in the abundance of cultivable rhizobacteria at different growth stages of canola. In the case of the rhizosphere of canola taken from Górsk, the population of cultivable rhizobacteria was highest in the vegetative stage, decreased in the flowering stage, and then increased in the plant maturity stage. Meanwhile, in the rhizosphere of canola taken from Ostróda, the abundance of cultivable rhizosphere bacteria was highest in the flowering stage, after which it decreased successively in the maturity and vegetative stages of the plant.

In this doctoral thesis, 300 bacterial stains were isolated: 150 isolates from the rhizosphere of canola roots cultivated in Górsk and in Ostróda, including 50 isolates from each plant growth stage: vegetative, flowering, and maturity. Subsequently, a characterization of their plant growth-promoting (PGP) properties was conducted, including the production of indole-3-acetic acid (IAA), phosphates, ACC deaminase, siderophores, chitinases, hydrogen cyanide (HCN), and ammonia. The study aimed to check whether there is a relationship between the abundance of rhizobacteria isolated from different plant growth stages and their

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PGP properties. In the canola rhizosphere cultivated in Górsk, the largest population of cultivable rhizobacteria was observed in the vegetative stage of canola, and four out of the seven tested PGP traits, such as the production of ACC deaminase, siderophores, HCN, and chitinases were produced by the highest percentage of isolates also in the vegetative stage of the plant. In the case of the canola rhizosphere cultivated in Ostróda, the highest abundance of rhizosphere microorganisms was recorded in samples taken in the flowering stage of canola, and also from this growth stage of the plant, the most strains capable of producing IAA, ACC deaminase, phosphates, siderophores, and ammonia were isolated.

In the next stage of the research, rhizobacteria that showed high activities of at least four of the seven tested PGP traits were identified and selected for a pot experiment to investigate their ability to promote canola growth under sterile conditions. Pot experiments under sterile conditions allowed to choose strains that best promoted plant growth. These included: *Bacillus paralicheniformis* 2R5, isolated from the rhizosphere of canola in Górsk, and *Peribacillus frigoritolerans* 2RO30 and *Pseudomonas sivasensis* 2RO45 from the rhizosphere of canola in Ostróda. Based on sterile condition experiments, *B. paralicheniformis* 2R5, *P. frigoritolerans* 2RO30, and *P. sivasensis* 2RO45 were selected for pot experiments in non-sterile soil to check if the isolates would also promote canola growth in the presence of native soil microorganisms. Additionally, in non-sterile pot experiments, a consortium consisting of two isolates, *P. frigoritolerans* 2RO30 and *P. sivasensis* 2RO45, was used to check if the microbial consortium would show better promoting effect than individual strains. Pot experiments in non-sterile soil showed that only the individual strains *B. paralicheniformis* 2R5 and *P. sivasensis* 2RO45 promoted canola growth.

Canola growth-promoting rhizobacteria: *B. paralicheniformis* 2R5 and *P. sivasensis* 2RO45 were subjected to genome sequencing analysis to find genes responsible for promoting plant growth and biological control of phytopathogens. Genome analysis of *B. paralicheniformis* 2R5 revealed the presence of genes responsible for the biosynthesis of IAA, phosphate solubilization, siderophore sequestration, and chitinase production. Meanwhile, in the genome of *P. sivasensis* 2RO45, genes responsible for the IAA biosynthesis, phosphate solubilization, ACC deaminase production, and siderophore sequestration were found. In these strains' genomes, many biosynthetic gene clusters coding for biologically active secondary metabolites with antifungal activity were also identified.

Moreover, in the genome of *B. paralicheniformis* 2R5, genes related to the nitrogen cycle, such as *narG* and *nosZ*, were found. Therefore, it was examined whether the inoculation

of *B. paralicheniformis* 2R5 could influence the number of copies of these genes in the canola rhizosphere, and the number of copies of other equally important nitrogen cycle-related genes, such as *nifH*, *nirS*, and *amoA*, were determined. Results showed that the presence of *narG* and *nosZ* genes in the genome of *B. paralicheniformis* 2R5 was not correlated with significant changes in the number of gene copies related to the nitrogen cycle in the rhizosphere. Inoculation with *B. paralicheniformis* 2R5 led to an increase in the number of copies of both *narG* and *nosZ* genes present in the 2R5 genome, as well as an increase in the number of copies of other genes, such as *nifH* and *nirS*.

The next step of the research was to determine the impact of inoculation with *B. paralicheniformis* 2R5 and *P. sivasensis* 2RO45 on the composition and diversity of bacterial and fungal communities in the canola rhizosphere. Results showed that inoculation with *P. sivasensis* 2RO45 did not significantly affect alpha-diversity indices. However, linear discriminant analysis showed that inoculation with *P. sivasensis* 2RO45 changed the taxonomic composition of the canola rhizosphere microbial communities, significantly increasing the number of reads of beneficial microorganisms, such as bacteria from the *Comamonadaceae* and *Vicinamibacteraceae* families, *Streptomyces* genus, and fungi from the *Nectriaceae*, *Didymellaceae* families, *Exophiala* genus, and *Cyphellophora vermispora* and *Mortierella minutissima* species. Additionally, it was observed that inoculation with *B. paralicheniformis* 2R5 initially reduced OTU richness of bacterial communities, while after 44 days of inoculation, the alpha-diversity index increased. Linear discriminant analysis showed that inoculation with *B. paralicheniformis* 2R5 modified the taxonomic composition of the bacterial and fungal communities in the canola rhizosphere, increasing the number of reads of beneficial microorganisms, such as *Nitrospira*, *Ramlibacter*, *Sphingomonas*, *Massilia*, *Terrimonas*, and *Solicoccozyma*, *Schizothecium*, *Cyphellophora*, *Fusicolla*, *Humicola*.

In this doctoral thesis, the impact of inoculation with *P. sivasensis* 2RO45 on the metabolic activity and functional diversity of canola rhizospheric microorganisms was also analyzed. Results showed that inoculation with *P. sivasensis* 2RO45 contributed to an increase in the overall metabolic activity of rhizospheric microorganisms. Additionally, four carbon sources, including phenols, polymers, carboxylic acids, and amino acids, were better metabolized by the microbial community of canola rhizosphere inoculated with *P. sivasensis* 2RO45 than in control samples. Based on the obtained carbon substrate degradation profiles, functional diversity indices were calculated. Results showed that inoculation with *P. sivasensis* 2RO45 caused an increase in the functional diversity of the canola rhizosphere microbiome

measured by the Shannon-Wiener biodiversity index (H') and the Shannon-Wiener evenness index (E).

In summary, the results presented in this doctoral thesis describe for the first time *Bacillus paralicheniformis* and *Pseudomonas sivasensis* as rhizobacteria promoting canola growth. *B. paralicheniformis* 2R5 and *P. sivasensis 2RO45*, by promoting canola growth, may be a promising alternative to chemical fungicides or mineral fertilizers. Their potential ability to change the microbiome by increasing the number of beneficial microorganism groups seems to be important for improving the efficiency of canola cultivation. Further research towards developing a formulation based on these bacteria may contribute to better canola cultivation quality.