Torun, 03.10.2025

Title of the doctoral dissertation (EN):

Potential of *Salicornia europaea* in decreasing colonization of plants by Human Pathogenic Microorganisms

Doctoral dissertation abstract (EN):

This PhD thesis investigates the complex interactions between the halophyte *Salicornia europaea* L. and three major human pathogenic microorganisms (HPMOs): *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* in field and *in vitro* experiments. *S. europaea* was used as model crop based on its nutritional and antimicrobial qualities as a sustainable crop for saline agriculture with possible raw consumption. Given the rising incidence of foodborne illnesses linked to raw vegetables contaminated with HPMOs, there is a critical need for innovative and sustainable antimicrobial strategies in agriculture and food safety. Therefore, the links between the chemical composition of plant shoots (e.g., lignin and lipid content) and soil properties (e.g., salinity and pH) with the colonization of HPMOs were analysed. The persistence of HPMOs in *S. europaea* was investigated using plant transcriptomics. Additionally, plant endophytic and rhizosphere bacteria were investigated on their production of volatile organic compounds (mVOCs) with potential to inhibit the HPMOs.

It was hypothesized that: (i) chemical traits of plants and soils control HPMOs' abundance and can therefore serve as indicators of food safety risk; (ii) the persistence of HPMOs in *S. europaea* can be limited by the plant's native microbiome and internal salinity; (iii) inhibition of HPMOs can be based on mVOCs production of endophytic and rhizosphere bacteria.

To address these hypotheses, three different experimental approaches were employed:

1. field experiment: the chemical composition of *S. europaea* shoots and soils from two French field sites was analysed using GC-MS jointly with the abundance of HPMOs on them to identify potential biomarkers of the HPMOs' contamination;

2. *in vitro* experiment: interactions between *S. europaea* and HPMOs were assessed at varying salinity levels (0, 50, 100 and 200 mM NaCl), focusing on plant growth, HPMOs' abundance using selective media and qPCR of shoot and root samples, and plant transcriptomics of plant samples in responses to HPMOs colonization;

3. functional assay to identify antagonists: endophytic and rhizosphere bacteria isolated from *S. europaea* were tested for their inhibitory effects on HPMOs using mVOC-mediated interactions and identification of volatiles using a bipartite *in vitro* assay coupled with head space - solid phase microextraction - gas chromatography - mass spectrometry (HS-SPME-GC-MS).

Negative correlations were found between the lignin content in shoots and the abundance of *S. enterica*, as well as between the lignin content in bulk soil organic matter and the abundance of *E. coli*, suggesting a potential protective role of lignin. Conversely, shoot lipid content was positively correlated with the abundance of *E. coli*. The soil pH and the soil salinity were negatively correlated with the abundance of HPMOs levels in bulk soil. This indicates the advantage of growth of *S. europaea* on saline soils for food safety. In the *in vitro* experiment a marked decline in the abundance of HPMOs in plant tissues was revealed, despite an initially high bacterial abundance in sterile sand, suggesting a natural reduction from soil to plant. *E. coli* was not found in plant tissues, whereas *S. enterica* and *L. monocytogenes* persisted and induced significant transcriptional changes, notably under specific salinity (0 and 100 mM NaCl). Transcriptomic analysis revealed no substantial changes in the gene expression in response to application of *E. coli*, but notable shifts in response to the other HPMOs (*S. enterica* and *L. monocytogenes*), involving genes linked to stress responses, biosynthesis of secondary metabolite and cellular responses to extracellular stimuli, e.g., biosynthesis of phosphoethanolamine N-

methyltransferase and pentacyclic triterpenoid. Distinct inhibition patterns emerged among bacterial taxa. Bacilli, Actinomycetes, and Gammaproteobacteria predominantly suppressed the growth of *L. monocytogenes* and *E. coli*. Key strains identified for mVOC profiling included *Bacillus pumilus* CSR28, *Xanthomonadales* sp. CSE34, *Streptomyces champavatii* CSR4, and *Bacillus pseudomycoides* CSE4, with dimethyl disulfide (DMDS) highlighted as a principal and potential antimicrobial mVOC. Other detected mVOCs, such as cyclohexane and methylcyclopentane, varied across bacterial and pathogen co-cultures, including in the HPMOs' monocultures.

Concluding, this PhD thesis highlights high diversity of interactions between *S. europaea*-and HPMOs, revealing that only some of these microorganisms are capable of persisting within the plant tissues. Soil pH and salinity emerge as a key controls and indicator of the risk of HPMOs' contamination in *S. europaea*, and can be used to enhance the safety for human consumption. Moreover, HPMOs trigger notable changes in the plant's gene expression, suggesting that the plant actively responds to certain HPMOs. Furthermore, it demonstrates that specific endophytic and rhizosphere bacteria associated with *S. europaea* generate mVOCs which are capable to suppress major foodborne pathogens, positioning these bacteria as viable candidates for the biocontrol of HPMOs for an increased food safety in agriculture.

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Signature of the PhD student