1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and workplan)

Project title: From saline soils to a sustainable environment: Exploring the potential of halophytic microorganisms in agriculture and soil recovery

1.1. Project goals

The scientific aim of the project is to identify halophytic microorganism species with the highest potential to stimulate plant growth under salt stress conditions. The practical goal is to develop innovative biopreparations based on selected microorganisms that can be applied in agriculture on degraded and saline areas.

The research will include both laboratory analyses of salt tolerance and field experiments in degraded areas. It is planned to monitor the impact of bioinoculant application on plant growth parameters, soil fertility, and microbiological biodiversity. Molecular techniques, such as genomic sequencing, will be used to identify and characterize halophytic microorganisms. Physicochemical soil analyses (pH, salinity, nutrient content) will allow for assessing the effectiveness of remediation. Biochemical tests will evaluate the ability of microorganisms to produce phytohormones and enzymes that neutralize salt stress.

Within the doctoral project, it is expected to obtain data confirming the ability of selected strains to improve plant growth and soil quality under saline conditions. The project outcome will be the development of a bioinoculant application protocol and guidelines for practical use in agriculture. The project will contribute to a better understanding of the adaptive mechanisms of halophytic microorganisms, which is essential in the context of climate change and the increasing problem of soil degradation. The obtained results may serve as a basis for the implementation of innovative agricultural strategies in regions affected by salt stress. The doctoral project will be carried out within the EU Bidiversa+ SaltyBeats project.

1.2. Outline

Soil salinity problem:
Salinity is one of the major challenges in modern agriculture, as vast areas worldwide are affected by this issue, significantly impacting agricultural productivity, food security, and ecosystem health. According to the Food and Agriculture Organization of the United Nations (FAO, 2021), salinity affects 424 million hectares of topsoil (0–30 cm) and 833 million hectares of subsoil (30–100 cm) in areas covering 73% of mapped lands. Other studies indicate that salinity negatively affects nearly 1 billion hectares of land, including over 20% of all irrigated arable land.

Limitations of traditional methods:
Traditional approaches to managing salinity in agriculture often rely on chemical additives and supportive measures, which may provide short-term results but are generally unsustainable.

Moreover, the use of these substances can, over time, deteriorate soil health, leading to its degradation and contamination. Therefore, it becomes crucial to seek ecological alternatives that enhance plant resilience and support sustainable agricultural practices.

Innovative approach using halophytic microorganisms:
Halophytic microorganisms, naturally adapted to high-salinity environments, represent a promising yet still insufficiently explored biological solution. These extremophilic bacteria often exhibit plant growth-promoting (PGP) traits and bioremediation capabilities, making them potential candidates for improving plant tolerance to salinity and for the rehabilitation of degraded saline soils.

Role of halophytic bacteria:
Saline environments often serve as reservoirs for halotolerant bacteria possessing valuable PGP traits. Under salt stress conditions, certain bacterial groups, especially endophytic ones, tend to become dominant, suggesting their adaptive advantage. Halophytic bacteria play a key role in enhancing plant defense mechanisms under stress conditions. They support salt tolerance through various mechanisms, such as: improved nutrient acquisition, production of phytohormones (e.g., auxins, gibberellins), ACC-deaminase activity, production of siderophores and exopolysaccharides (EPS), phosphate solubilization.

1.3. Work plan

Year 1:

- Conduct a comprehensive literature review and refine the research methodology.
- Monitor soil characteristics, including physical, chemical, and biological parameters.
- Isolate and identify halophytic microorganisms, focusing on soil microorganisms, rhizosphere bacteria, and arbuscular mycorrhizal fungi (AMF).
- Perform in vitro screening for Plant Growth-Promoting Microorganisms (PGPM), assessing traits such as: IAA (Indole-3-acetic acid) production, phosphate solubilization, siderophore activity, ACC deaminase activity, biofilm formation, salt-tolerance characteristics.

Year 2:

- Conduct pot experiments using selected halophytic strains and model plants under salt stress conditions.
- Measure physiological and biochemical responses of plants, with a focus on oxidative stress markers.
- Perform bioremediation assays under laboratory-controlled saline and contaminated conditions, including: Na^+ sequestration, Antioxidant enzyme production, production of exopolysaccharides (EPSs).

- Evaluate the combined effects of PGPM in plant interactions, including: in vitro screening of PGPM traits (e.g., siderophore production, IAA production, biofilm formation), assessment of microbial salt resistance.
- Analyze data and initiate manuscript preparation.

Year 3:

- Perform whole-genome sequencing and metabolic pathway analysis of selected strains, utilizing molecular techniques, bioinformatics, and statistical approaches.
- Conduct molecular identification (16S rRNA) and establish a culture collection of the most beneficial bacterial strains.
- Analyze and interpret the collected data.
- Write scientific publications and draft thesis chapters.

Year 4:

- Complete final data analysis.
- Finalize thesis writing and prepare for defense.
- Present research findings at national and international conferences.

1.4. Literature *(max. 7 listed, as a suggestion for a PhD candidate preliminary study)*

- Egamberdieva, D., Wirth, S., Bellingrath-Kimura, S.D., Mishra, J. and Arora, N.K. 2019. "Salt-Tolerant Plant Growth Promoting Rhizobacteria for Enhancing Crop Productivity of Saline Soils". *Front. Microbiol.* 10:2791. https://doi.org/10.3389/fmicb.2019.02791
- Furtado, B.U., S. Szymańska, and K. Hrynkiewicz. 2019. "A Window into Fungal Endophytism in *Salicornia europaea*: Deciphering Fungal Characteristics as Plant Growth Promoting Agents." *Plant and Soil* 445: 577–594. https://doi.org/10.1007/s11104-019-04315-3.
- Hidri, R., O.M.-B. Mahmoud, W. Zorrig, H. Mahmoudi, A. Smaoui, C. Abdelly, R. Azcon, and A. Debez. 2022. "Plant Growth-Promoting Rhizobacteria Alleviate High Salinity Impact on the Halophyte *Suaeda fruticosa* by Modulating Antioxidant Defense and Soil Biological Activity." *Frontiers in Plant Science* 13: 821475. https://doi.org/10.3389/fpls.2022.821475.
- Kuźniar, A., A. Kruczyńska, K. Włodarczyk, J. Vangronsveld, and A. Wolińska. 2025. "Endophytes as Permanent or Temporal Inhabitants of Different Ecological Niches in Sustainable Agriculture." *Applied Sciences* 15(3): 1253.
- Maitra, P., K. Hrynkiewicz, A. Szuba, A.M. Jagodziński, J. Al-Rashid, D. Mandal, and J. Mucha. 2024. "Metabolic Niches in the Rhizosphere Microbiome: Dependence on Soil Horizons, Root Traits and Climate Variables in Forest Ecosystems." *Frontiers in Plant Science* 15: 1344205. https://doi.org/10.3389/fpls.2024.1344205.
- Negacz, K., Ž. Malek, Arjen de Vos, and P. Vellinga. 2022. "Saline Soils Worldwide: Identifying the Most Promising Areas for Saline Agriculture." *Journal of Arid Environments* 203: 104775. https://doi.org/10.1016/j.jaridenv.2022.104775.
- Zhang, G., J. Bai, Y. Zhai, Jia Jia, Qingqing Zhao, Wei Wang, and Xingyun Hu. 2024. "Microbial Diversity and Functions in Saline Soils: A Review from a Biogeochemical Perspective." *Journal of Advanced Research* 59: 129–140. https://doi.org/10.1016/j.jare.2023.06.015.

1.5. Required initial knowledge and skills of the PhD candidate

I. Basic microbiological techniques (isolation, culturing, and identification of bacteria)
II. Foundational knowledge of plant-microbe interactions
III. Familiarity with molecular biology tools (PCR, electrophoresis, etc.)

IV. Skills in data analysis and scientific writing

V. Proficiency in English and willingness to work in interdisciplinary and international environments

1.6. Expected development of the PhD candidate's knowledge and skills

- Advanced expertise in environmental microbiology and microbial ecology
- Practical skills in microbiome technologies, pot experimentation, and bioinformatics
- Proficiency in scientific communication through conferences and publications
- Development of critical thinking, experimental design, and problem-solving abilities
- Enhanced project management and collaboration skills in multidisciplinary research