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

Project title:
A salty face of endophytic bacteria and fungi in alleviating the stress of grass salinity

1.1. Project goals

G-1 Screening and selection of potential endophytic bacteria and fungi from the microbial collection.
G-2 Assessment of the effect of various inoculation methods on the physiological and biochemical features of plants, root morphology and endophyte colonization under salinity stress.
G-3 Development of various methods of microbial inoculum delivery to optimize endophyte function in plants.

1.2. Outline

Some economically important grass species such as wheat and/or perennial ryegrass are affected by soil salinity damaging various physiological and metabolic processes thus affecting plant yield. Moreover, most plants are associated with beneficial endophytic bacteria and fungi. Therefore our driving interest to select endophytes that can be used as bioinoculants to improve root penetration in soil for nutrient and water, improve plant salt tolerance and promote plant growth in poor soils.

Studies by Furtado et al (2019 a, b) and Koczorski et al (2021) showed host plants *Salicornia europaea* and *Salix* species harbor specialized endophytic microbiome having plant growth promoting (PGP) properties. This fact makes the endophytic microbiome in these plants interesting to explore. The study on fungal endophytes of *S. europaea* funded by EU Horizon 2020 research under the Marie Skłodowska-Curie grant (project leader Prof. Hrynkiewicz 2016-19) reported strains with high salt tolerance (**>**600 mM NaCl) and PGP traits (7,8). Another project on bacterial endophytes of *Salix* sps (project leader Prof. Hrynkiewicz in collaboration with researchers from Sweden and Germany 2018-2022) reported strains with PGP properties and phosphate-solubilizing activity (11).

Hence, the **main aim of this project** is to assess the role of endophytic bacteria (*Salix* sps.) and fungi (*S. europaea*) and its interaction (as single strains of bacteria or fungi and mixed inoculations) on the growth of non host plants (ryegrass or wheat) under salinity. The novelty of this proposal lies in optimizing the selection of best microbial candidates contributing to selected non host plant traits (i.e. increased biomass and salt tolerance) in which use of bioinoculants offer sustainable productivity benefits.

1.3. Work plan

M-1 Screening and selection of endophytic bacteria and fungi:
Bacteria (*Salix* sps.) and fungi (*S. europaea*) strains available in the culture collection at Department of Microbiology (NCU) will be used. Twenty strains (each for bacteria and fungi) to be screened for PGP properties: e.g. indole acetic acid (14), ACC deaminase activity (15), ammonia production (6), phosphorous

solubilization (13). The strains will also be screened for halotolerance (up to 1000 mM NaCl). For strain selection for mixed inoculation, tests for antagonistic interactions using spot test (16) and antibiosis test (2) to be done.

M-2 Pot experiment with single and mixed inoculations under salt stress:

Pot experiment: Plants will be cultivated in in three salt treatments (0 , 150 and 300 mM NaCl) and inoculated with bacteria (B) and/or fungi (F) selected on basis of results from G-1, M-1.

Plant physiology: Plant height and biomass (fresh and dry). Root analysis (density and branching).

Biochemical analysis: leaf CO_2/H_2O gas exchange (Li-COR 6200), chlorophyll (19), elemental analysis (e.g. total C and N), starch (10), abscisic acid (17), lipid peroxidation (5), proline (1), hydrogen peroxide content (12), antioxidant (analysis in collaboration with Faculty of Chemistry, NCU), water-soluble carbohydrates (9) and soluble proteins (4).

Endophyte colonization: Two methods microscopy staining for bacteria (18) and fungi (3) and quantitative PCR to be done.

M-3 Methods for microbial delivery in plant:

Different methods for introducing endophytes in plants include soil drenching (8), root dipping (20) and seed coating. Pot experiment with 2 bacteria and fungi and sample collection at 2 time points: ~2 weeks after inoculation and ~6 weeks of plant growth. The root and leaf samples will be analyzed for endophyte colonization using microscopy techniques and qPCR.

Expected results:

1 The PGP activity, halotolerance and interaction of two microbial groups will be deciphered as well as selection of the best microbial strains i.e. two bacteria and two fungi. **(G-1, M-1)**

2 The role of endophytic metabolites in plant development and salt stress mitigation and the effect of microbial interaction (single and mixed inoculation) on plant growth will be delineated. **(G-2, M-2)**

3 Method for inoculum delivery in plants to optimize the endophyte effect and thus paving the way towards its field application. **(G-3, M-3)**

The PhD candidate will also prepare two publications as an output to the project results.

1.4. Literature

1.Ábrahám E, et al. 2010 Methods for determination of proline in plants. In: Methods in molecular biology (Clifton, N.J.) p. 317–31.

2.Azaiez, S., et al. 2018. Biological control of the soft rot bacterium *Pectobacterium carotovorum* by *Bacillus amyloliquefaciens* strain Ar10 producing glycolipid-like compounds. Microbiol. Res. 217, pp.23-33.

3.Berthelot C, et al. 2016 Plant growth promotion, metabolite production and metal tolerance of dark septate endophytes isolated from metal-polluted poplar phytomanagement sites. FEMS Microbiol Ecol.;92.

4.Bradford, M.M., 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72(1-2), pp.248-254.

5.Cakmak, I. and Marschner, H., 1993 Effect of zinc nutritional status on activities of superoxide radical and

hydrogen peroxide scavenging enzymes in bean leaves. In Plant Nutrition—from Genetic Engineering to Field Practice (pp. 133-136). Springer, Dordrecht.

6.Chaykin, S., 1969 Assay of nicotinamide deamidase: Determination of ammonia by the indophenol reaction. Anal. Biochem., 31, pp.375-382.

7.Furtado, B.U., et al. 2019a Bacterial and fungal endophytic microbiomes of *Salicornia europaea*. AEM, 85(13), pp.e00305-19.

8.Furtado, B.U., et al. 2019b A window into fungal endophytism in *Salicornia europaea*: deciphering fungal characteristics as plant growth promoting agents. Plant Soil, 445(1), pp.577-594.

9.Hunt, M.G., et al. 2005 Near-term impacts of elevated CO2, nitrogen and fungal endophyte-infection on Lolium perenne L. growth, chemical composition and alkaloid production. Plant Cell Environ., 28 (11), pp.1345-1354.

10.Knudsen, K.E.B., 1997 Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol., 67(4), pp.319-338.

11.Koczorski, P., et al. 2021. The effects of host plant genotype and environmental conditions on fungal community composition and phosphorus solubilization in willow short rotation coppice. Front. Plant Sci., 12.

12.Loreto, F. and Velikova, V., 2001 Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol., 127(4), pp.1781-1787.

13.Nautiyal CS. 1999 An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS microbiology Letters. 170(1):265-70.

14.Patten, C. and Glick, B. 2002 Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. AEM. 68, 3795–3801.

15.Saleh, S.S. and Glick, B.R., 2001 Involvement of gacS and rpoS in enhancement of the plant growth-promoting capabilities of *Enterobacter cloacae* CAL2 and UW4. Can. J. Microbiol., 47(8), pp.698-705.

16.Schwinghamer, E.A., 1971 Antagonism between strains of *Rhizobium trifolii* in cultureSoil Biol. Biochem., 3(4), pp.355-363.

17.Sunkar, R., et al. 2003 Overexpression of a stress-inducible aldehyde dehydrogenase gene from Arabidopsis thaliana in transgenic plants improves stress tolerance. Plant J., 35(4), pp.452-464.

18.White, J. F., et al. 2019 Review: Endophytic microbes and their potential applications in crop management. Pest Manag. Sci. 75(10), 2558–2565.

19.Witham FH, et al. 1971 Experiments in plant physiology. New York (N.Y.) : Van Nostrand Reinhold.

20. Mantzoukas, S. and Eliopoulos, P.A., 2020. Endophytic entomopathogenic fungi: A valuable biological control tool against plant pests. Appl. Sci., 10(1), p.360.

1.5. Required initial knowledge and skills of the PhD candidate

- Education that should cover at least one of the following areas: molecular biology, microbiology, biology and biochemistry.
- High motivation to pursue doctoral studies and ability to independently plan and organize own experiments.
- Good ability to analyse and communicate scientific results, and to present both in oral and written form in English.
- Interest in and ability to work collaboratively within the lab group.

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

- The candidate will understand the biology of interactions under different conditions and applying the knowledge gained to be able to improve the reliability of endophyte products.
- The candidate will be trained to enhance their scientific skills in writing a project proposal for research grants, writing and submission of scientific papers
- The candidate will receive training in plant cultivation, endophyte handling and plant delivery techniques
- The candidate will be involved in learning new techniques at the faculty of chemistry as part of research collaboration.