Propostas de Planos de Tese de Mestrado

Instituto de Tecnologia Química e Biológica (ITQB-UNL)

iPlantMicro Lab

Title: Deciphering the role of *myo*-inositol in legume nodulation and microbiome recruitment under drought stress

The beneficial interaction of legumes with soil microbiota is one of the best studied. Most of these interactions take place in specialized structures called nodules, where both Rhizobia and other species are housed. These interactions improve the availability of nutrients and even improve the response to pathogens. However, many legumes reduce or even avoid forming these structures in the face of prolonged periods of high temperatures or drought. Unfortunately, these events are becoming more frequent, especially in climates similar to those of the Mediterranean basin, conditioning the production of these species. It has been observed that *myo*-inositol is a compound involved in nodulation through its catabolism, however, its role in the process is not clear. This compound is a regular part of root exudates and has been described as attracting beneficial microbiota, but its production and exudate can be conditioned by environmental stresses. The study of the effects of an exogenous treatment could facilitate future improvements in stress management, guaranteeing the production of legumes in periods of severe stress.

Detail of Internship and specific aims:

- Investigate *myo*-Inositol's Role in Nodule Formation: Determine the significance of *myo*-inositol in the process of legume nodulation, particularly focusing on its influence on nodule formation and development.
- Study *myo*-Inositol's Impact under Drought Conditions: Explore how drought stress affects the production and availability of *myo*-inositol in the rhizosphere, and its subsequent implications on legume nodulation events.
- Characterize Rhizosphere Microbiota Dynamics: Analyze the composition and diversity of rhizosphere microbiota in response to varying *myo*-inositol levels and drought stress conditions, aiming to understand the interplay between root exudates and microbial communities.
- Evaluate Nodulation Efficiency: Assess the efficiency of nodulation in *Medicago* sp. plants under different *myo*-inositol concentrations and drought stress levels, quantifying nodule formation parameters such as number, size, and distribution.
- Advance Knowledge in Plant-Microbiota Interactions: Contribute to advancing knowledge in the field of plant biology and agroecosystems by generating new insights into the role of *myo*-inositol and rhizosphere microbiota in legume nodulation processes, with implications for sustainable crop production and global food security.

Tasks:

The work plan will be divided into four phases:

1. Task 1: Experimental Setup and Pilot Studies. Design and set up preliminary experiments to optimize growth conditions for *Medicago sp.* and establish methodologies for studying *myo*-inositol levels and nodulation events. Conduct pilot studies to test the feasibility of experimental procedures and troubleshoot any potential issues in root exudate analysis (chromatographic profiling) and impactful concentrations of *myo*-inositol over soil microbiota (culturomics). Studies with drought will be included. Analyze collected data using appropriate statistical methods to assess the impact of *myo*-inositol and drought stress on nodulation events and legume growth.
2. Taks 2: Influence on Microbiota. To evaluate the effects of *myo*-inositol we will use soil key strains, including growth curve, as well as beneficial traits in microbiota as biofilm production, auxin production, antioxidant production, proline production, and nutrient-related traits such as nitrogen fixation, sulfur oxidation, siderophore production, and the solubilization of P, K, Ca, Si, Mn, and Zn. This evaluation will include the use of *myo*-inositol as only C source, and prepare some evaluations about transcriptomic changes.
3. *Taks 3: Effects on Full-Scale System*. Set up large-scale experimental plots or growth chambers for *Medicago sp.* cultivation, incorporating drought stress treatments and controls. Apply external treatment of *myo*-inositol under stressing conditions will include sampling of plant tissues and collection of rhizosphere samples at key stages of nodulation. Physiologically characterize plant response to the several treatments, assessing its impact on photosynthesis. Collect data of transcriptomics, physiological and plant development, rhizosphere microbiota composition, and nodule formation parameters. Moreover, we will prepare similar tests with the strains from Task 2 that showed better performance in the use and consumption of myo-inositol, as auxiliary treatment to enhance nodule production under drought conditions.
4. Task 4: Data Interpretation, Reporting, and Dissemination. Interpret the results of data analysis to draw conclusions regarding *myo*-inositol's role in legume nodulation under drought conditions. Prepare scientific papers for publication in peer-reviewed journals, presenting the research findings and implications. Present research findings at conferences, seminars, and workshops to disseminate knowledge and engage with the scientific community. Prepare and defend a comprehensive thesis summarizing the project's objectives, methodologies, results, and conclusions.

Techniques:

- Culturomic and (eventually) metagenomic analysis
- PCR and electrophoresis
- RNA extraction and transcriptomic analysis
- Untargeted metabolomics
- *In vitro* biochemical assays
- Basic bioinformatics

Place: iPlantMicro Lab, Instituto de Tecnologia Química e Biológica (ITQB, Oeiras, Portugal.

Duration: 9 months to 1 year

Number of students: 1 highly motivated student that wishes to pursuit a career in research. Proficiency in English is desirable.

Contacts and more information: Dr. Juan Ignacio Vilchez (nacho.vilchez@itqb.unl.pt)

Bibliography and suggested reading:

1. A functional *myo*-inositol dehydrogenase gene is required for efficient nitrogen fixation and competitiveness of *Sinorhizobium fredii* USDA191 to nodulate soybean (*Glycine max* [L.] Merr.) - Jiang et al., 2001, Journal of Bacteriology.
2. Investigation of *myo*-inositol catabolism in *Rhizobium leguminosarum* bv. *viciae* and its effect on nodulation competitiveness - Fry, Wood and Poole, 2001, Mol Plant Microbe Interact.
3. Inositol catabolism, a key pathway in *Sinorhizobium meliloti* for competitive host nodulation – Kohler et al., 2010, Appl Environ Microbiol.
4. DNA demethylases are required for *myo*-inositol-mediated mutualism between plants and beneficial rhizobacteria - Vilchez et al., 2020, Plant Nature.
5. Plant *myo*-inositol transport influences bacterial colonization phenotypes – O'Banin et al., 2023, Current Biology.

Timeline:

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	Month 7	Month 8	Month 9	Month 10
Task 1										
Task 2										
Task 3										
Task 4										
Thesis										