Propostas de Planos de Tese de Mestrado

Instituto de Tecnologia Química e Biológica (ITQB-UNL)

iPlantMicro Lab

Title: Superfood or Superbacteria? Microbiota behind the high nutritional value seeds of local varieties of fenugreek (*Trigonella foenum-graecum*) and grass pea (*Lathyrus sativus*)

In recent years, the concept of 'superfoods' has gained attention due to their potential nutritional and health benefits. While some of these claims are marketing-driven, others reflect a revival of traditional crops with unique properties. Local varieties of fenugreek (*Trigonella foenum-graecum*) and grass pea (*Lathyrus sativus*) have been recognized for their high nutritional value, including a balanced nutrient composition, antioxidant content, and the presence of essential micronutrients. These crops, long neglected due to shifts in agricultural priorities, present an opportunity to enhance human diets while promoting sustainable farming practices. However, their productivity and nutritional potential may be further improved by leveraging the microbiota associated with their seeds, which can play a crucial role in plant health and performance. This study seeks to explore the beneficial microbial populations associated with these seeds, assess their potential as inoculants, and evaluate their effects on plant growth and seed quality under varying environmental conditions.

Detail of Internship and specific aims:

- Identify and characterize the seed-associated microbiota of local fenugreek and grass pea varieties.
- Compare microbial community structures between different landraces from Portugal, Spain, and India.
- Screen cultivable microbial strains for plant growth-promoting traits and stress resilience.
- Assess the ability of selected strains to colonize plants and enhance growth under both irrigated and drought conditions.
- Evaluate the impact of microbial inoculation on the nutritional value of harvested seeds.
- Explore potential field trials to validate the applicability of microbial inoculants in agricultural settings.

Tasks:

The work plan will be divided into four phases:

1. Microbiota identification and characterization: Seeds of fenugreek and grass pea will be collected from different geographic regions (Portugal, Spain, and India) and analyzed to determine their microbiota composition. High-throughput sequencing techniques will be employed to characterize the bacterial populations associated

with these seeds. Comparative analysis will be conducted to assess differences in microbial diversity across landraces and determine potential correlations with seed nutritional content.

2. Screening and selection of beneficial microbes: Cultivable strains from the seeds will be isolated and subjected to functional screening for beneficial traits, such as nitrogen fixation, phosphate solubilization, and production of plant growth-promoting metabolites. Additionally, strains will be tested for their ability to enhance stress tolerance, particularly under drought conditions. The most promising candidates will be selected for further testing and functional characterization.
3. Plant inoculation and phenotypic evaluation: Selected microbial strains will be applied to fenugreek and grass pea plants under controlled greenhouse conditions. Phenotypic analyses will include growth parameters, root architecture, and drought resilience. The colonization ability of the microbes will be tracked using fluorescent protein-tagged strains to assess their establishment within plant tissues and their interaction with host plants.
4. Nutritional and metabolomic analysis: To determine the effects of microbial inoculation on seed quality, harvested seeds will be analyzed for key nutritional components, including protein content, essential micronutrients, and antioxidant levels. Metabolomic profiling will be performed using mass spectrometry to detect changes in biochemical pathways influenced by microbial inoculation.
5. Potential field trials and economic impact: Depending on resource availability, field trials may be conducted to validate laboratory findings under real agricultural conditions. These trials will help assess the feasibility of using microbial inoculants to enhance crop productivity and seed quality. Additionally, the potential socio-economic benefits of promoting these underutilized crops in rural areas will be explored, including their role in supporting local farmers and sustainable agricultural practices.

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. Pérez-Jaramillo, J. E., Carrión, V. J., Bosse, M., Ferrão, L. F. V., de Hollander, M., & Raaijmakers, J. M. (2017). Linking rhizosphere microbiome composition of wild and domesticated *Phaseolus vulgaris* to genotypic and root phenotypic traits. ISME Journal, 11(10), 2244-2257.
2. Gil, T., Romão, I. R., Gomes, J. D. C., Vergara-Diaz, O., de Carvalho, L. A. L., Sousa, A., Kasa, F., Teixeira, R., Mateus, S., Katamadze, A., Pinheiro, D. G., Vicente, R., & Vílchez, J. I. (2024). Comparing native and non-native seed-isolated strains for drought resilience in maize (*Zea mays* L.). Plant Stress, 12, Article 100462.
3. Niza-Costa, M., Rodríguez-dos Santos, A. S., Rebelo-Romão, I., Ferrer, M. V., Sequero López, C., & Vílchez, J. I. (2022). Geographically disperse, culturable seed-associated microbiota in forage plants of alfalfa (*Medicago sativa* L.) and pitch clover (*Bituminaria bituminosa* L.): Characterization of beneficial inherited strains as plant stress-tolerance enhancers. Biology, 11(12), 1838.

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										
Task 5										
Thesis										