Propostas de Planos de Tese de Mestrado

Instituto de Tecnologia Química e Biológica (ITQB-UNL)

iPlantMicro Lab

Title: Back to the Future: Microbiota Associated to Lentil Ancestors to Help the Modern Varieties

Sometimes, answers to current problems can be found in traces of the past. Legumes are among the best sources of food because of their nutritional value and production performance. In this context, the Portuguese government has legumes as a preferential crop in the future in order to guarantee food security and depend less on imports. However, modern legume varieties are often seriously affected by the increasing effects of climate change, particularly drought and rising temperatures. This did not occur in the same way in the ancestral species from which modern species were bred. These species were selected based on their production characteristics and size, but not on their resistance to stress. This has led to problems with increased sensitivity in modern varieties. Fortunately, the past has come to the fore. In addition to the genetic background, the associated microbiomes can cause differences in the tolerance of these plants. Thus, using seeds from the closest ancestral species, we want to examinate the microbiota linked to lentils to assess their differences.

Detail of Internship and specific aims:

- Identify and characterize the seed microbiota of lentil ancestral species.
- Compare microbial communities between ancestral and modern lentil varieties.
- Screen beneficial microbial strains for drought tolerance enhancement.
- Evaluate the colonization ability, phenotypic effects, and drought response mechanisms induced by selected microbes.
- Investigate root exudate metabolomic profiles to understand plant-microbiome interactions.
- Develop bioinoculants to improve lentil production under drought conditions.

Tasks:

The work plan will be divided into four phases:

1. Microbiota identification and characterization: Seeds from ancestral and modern lentil varieties will be collected to analyze their microbiota. High-throughput sequencing techniques will be used to identify microbial communities associated with seeds, enabling a comparative analysis of microbial diversity and composition between ancestral and modern varieties. This will provide insight into potential microbial taxa that contribute to drought tolerance.
2. Screening and selection of beneficial microbes: Cultivable microbial strains will be isolated from the seeds of ancestral lentils. Functional screenings will be conducted to evaluate their ability to promote drought tolerance through mechanisms such as exopolysaccharide production, ACC deaminase activity, and osmoprotectant synthesis. The most promising strains will be selected for further testing in controlled conditions.

3. Drought tolerance assays: Selected microbial strains will be tested for their ability to enhance drought tolerance in modern lentil varieties. These assays will involve inoculating lentils with the beneficial strains and assessing their physiological and phenotypic responses under drought stress. Colonization ability will be tracked using fluorescent protein-tagged strains, and gene expression analyses will be conducted to examine the activation of key drought response pathways.
4. Evolutionary and metabolomic analysis: To better understand evolutionary adaptations, parallel experiments will be performed with ancestral lentils. Additionally, root exudates from both ancestral and modern varieties will be collected and analyzed using mass spectrometry to investigate metabolomic profiles under drought conditions. This analysis may involve collaboration with research institutions in Germany, providing access to advanced analytical techniques.
5. Microbiota recruitment and rhizosphere assessment: Lentil root exudates will be applied to natural soils to evaluate their effects on microbial community dynamics. Changes in the rhizosphere microbiome will be assessed using culturomics and sequencing methods to determine how lentil species and their ancestors recruit beneficial microbes. Understanding these interactions will help identify microbial consortia that could be leveraged for improving crop resilience

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									🟪	🟪