Biofertilizers: Unraveling the *in vivo* potential of PGPB traits

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Root-Benefit
Beneficial root-associated micro-organisms for sustainable agriculture

Scope and Objectives

Soil degradation is a worldwide concern that results mostly from anthropogenic activities such as industry and the use of non-sustainable farming practices, like the overuse of agrochemicals. This problem severely impairs the quality and productivity of crops.

The use of formulations **(biofertilizers)** including living microorganisms, such as **phosphate-solubilizing bacteria** and **non-symbiotic nitrogen-fixing bacteria**, has attracted distinct attention due to their potential to help ameliorate soil functions while enhancing crops' growth under stress conditions.

However, **bacteria selection** is often **based exclusively on *in vitro* traits**, which coupled with a **lack of long-term quality control and cell viability assessment**, has resulted in **biofertilizer's low efficiency under field application.**

Formulations should be tailored, as far as possible, to climatic conditions, and the plant to be inoculated. **A single formulation may not be suitable for such diverse and complex ecosystems.**

This work (ongoing) aims to add value to this area of knowledge by validating, *in vivo* (greenhouse), the performance of different bacterial strains that have demonstrated the best ability to solubilize phosphorus and fix nitrogen in laboratory tests.

Methods

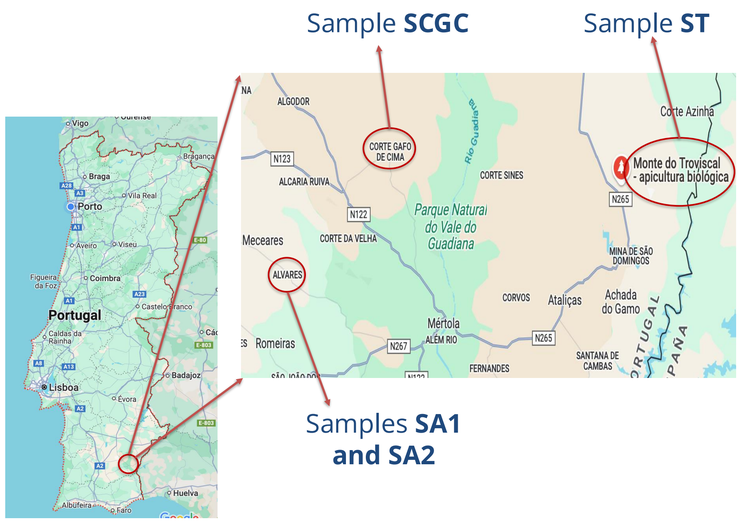
Soil Sampling- Mértola, Alentejo, Portugal

Four composite samples were collected from three different locations:

Alvares — Two fields: one sown with triticale and the other sown with oat.

Corte Gafo de Cima — Sheep grazing field with no specifically defined vegetation.

Troviscal — Field sown with oat and lupin in the soil stand-by season.

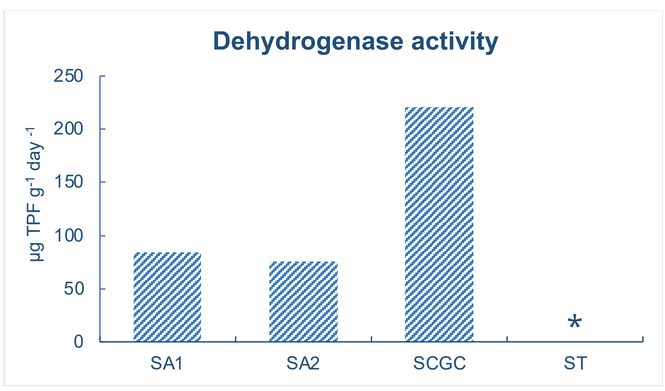


After soil physicochemical, biochemical characterization and microbial functional diversity assessment, **54 representative bacterial strains were isolated and P solubilization and N_2 fixation abilities** were verified.

Results

Soil Biochemical Characterization

Soil dehydrogenase enzymatic activity enables to infer about the viable microbial community present in the soil.

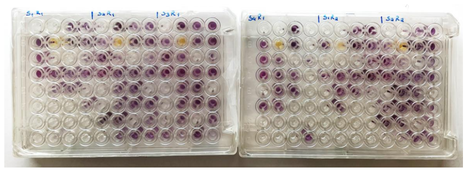


*below the limit of quantification

Soil Microbial Functional Diversity

Assessment of the physiological profile of the microbial community through the ability to oxidize different C substrates in a 96-well commercial plate.

Biolog® EcoPlate™

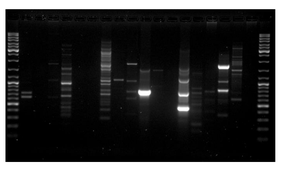


	Richness / NUS	Shannon's Diversity Index (H')	Shannon's Evennes Index (E)
SA1	16.17 ± 2.47	2.74 ± 0.17	0.99 ± 0.01
SA2	24.17 ± 0.76	3.13 ± 0.01	0.98 ± 0.01
SCGC	22.5 ± 1.0	3.06 ± 0.04	0.98 ± 0.01
ST	BLQ	BLQ	BLQ

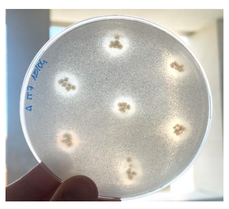
BLQ: below the limit of quantification

Bacteria isolation, and PGP traits assessment

54 representative strains (BOX-PCR fingerprinting analysis through BioNumerics software)

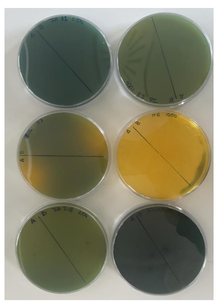


BioNumerics



P solubilization test

≈ 30% of the isolates solubilized $Ca_3(PO_4)_2$ (with higher and lower solubilization indexes)



N_2 fixation test

≈ 54.5% of the isolates exhibited nitrogen fixation (qualitative analysis)

Ongoing Work

- ✓ Several PGPB traits are being tested: **osmotic stress resistance, siderophores, lytic enzymes, and EPS production.**
- ✓ Selection of top ten best-performing bacterial strains.
- ✓ **PGPB testing in a pot experiment** using soil from Mértola's region and two different crops (wheat and oat).
- ✓ Plants will be analyzed for biomass, elongation, and nutritional composition.
- ✓ Rhizospheric soil samples will be collected at sowing, after bacterial inoculation, and at the end of the experiment for microbial community analysis and physicochemical characterization.

Acknowledgements

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