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Functional and genotypic diversity of pea (*Pisum sativum* L.) microsymbionts in several geographical sites in Tunisia: Selection of inoculant strains for biofertilizer formulation

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Pea (*Pisum sativum* L.) is a leguminous plant that is cultivated for its nutritional value and advantageous effects on soil fertility when used as a preceding crop. Its symbiotic nitrogen fixation and phosphorus solubilization properties thanks to the association with rhizospheric bacteria make it crucial component in cereal-based cropping systems. In Tunisia, the upscaling of pea cultivation faces numerous challenges, including low yields attributed to soil fertility depletion and the low abundance or ineffectiveness of specific rhizobia for achieving optimal pea nodulation.

The current study aims to assess the diversity and plant growth promoting traits of pea endophytic bacteria in order to select effective inoculant strains. For this purpose, 166 bacterial strains were isolated from root nodules of pea plants, collected from 46 regions in Tunisia. The strains were subjected to thorough *in vitro* assays, involving morphological, functional, and genetic characterization.

The results demonstrated that 153 strains were tested Gram-negative and 13 strains Gram-positive. Among the Gram-negative isolates, 44 strains induced nodule formation on pea plants of the 'Lincoln' variety, but mostly produced low nodule number and biomass and poor plant growth. The assessment of phosphorus solubilization among the whole isolates collection revealed a highly significant difference in the halo diameter formed on Pikovskaya medium and the phosphorus solubilization index. One hundred thirteen isolates were capable of solubilizing inorganic phosphorus.

The analysis of functional diversity of pea microsymbionts showed that *Rhizobium* strains (Oued Bj0.16, BjD, KalAM, MzBrg, Jbn, Morg15, Jed3, Sbit1, and Sb4), that were originated respectively from Beja, Kalaat Andalous, Menzel Bourguiba, Jbeniana, Morneg, Jedaida, Sbitla, and Sbiba sites, presented the highest efficiency in regards of nitrogen fixation. Furthermore, the strain Oued Bj0.16 demonstrated a moderate ability to solubilize phosphorus whereas the strain SoliL stands

as the most efficient phosphorus-solubilizing bacteria (PSB). Concerning non-nodulating bacteria, BsM, Mat3L, MzelTM, and Mok4 were identified as a highly efficient PSB. In addition, the Gram-positive strain TebkL, originated from Beja, was identified as the most efficient P-solubilizer. The 16S gene sequencing revealed that pea nodular microsymbionts were attributed to 6 different genera: *Rhizobium* sp., *Rhizobium leguminosarum*, *Parabulkolderia fungorum*, *Pantoea* sp., *Pseudomonas fluorescens*, *Pseudomonas baetica*, *Bacillus subtilis*, *Paenibacillus polymyxa*, and *Rhizobium nepotum*. The exploration of pea microsymbionts diversity demonstrated extensive functional and genetic variations associated with the isolates origin.

In a nutshell, the application of single or mix of these beneficial bacteria as inoculant is an eco-friendly option that provides nitrogen and phosphorus to the crops and gets rid of chemical fertilizer, thereby promoting plant growth and preserving the environment in a Mediterranean context.