Endophytic bacteria associated with spontaneous legumes in arid zones of Tunisia: Genetic diversity, metabolic functionalities and potential application to mitigate the impact of climate change

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Legumes naturally adapted to harsh climate environments represent a new source of plant growth promoting (PGP) bacteria that can be used to improve crop resilience to climate change. However, the diversity and functionality of endophytic bacteria associated with endemic wild legumes in Tunisia are almost unknown. To study the taxonomic and functional diversity of these species, we conducted a study on root nodules of 15 spontaneous legumes, some of which studied for the first time, growing in three different Tunisian bioclimates (arid, semi-arid and Saharan). More than 210 strains, corresponding to 78 different phylotypes, were isolated. Sequencing of the 16S rDNA revealed the presence of rhizobial isolates belonging to the genera: *Rhizobium*, *Sinorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Neorhizobium*, *Agrobacterium*, *Phyllobacterium*, *Cupriavidus* and *Burkholderia*. Other non-rhizobial bacteria assigned to *Microbacterium*, *Pseudomonas*, *Paenibacillus*, *Starkeya*, *Kocuria* among others, were also obtained. In addition, the nucleotide similarity of housekeeping genes suggested the presence of new species in our collection. Indeed, the genomic sequencing analysis identified a new species of *Mesorhizobium* (PRJNA800673), a microsymbiont of *Retama raetam,* which is one of the most important Tunisian shrubs. Regarding PGP-screening abilities, several strains stood out for possessing several PGP traits, phosphate solubilization, indole acetic acid and siderophores production, along with high tolerance to abiotic stress (> 45°C; > 0.4M NaCl). For rhizobia, whole genome sequencing of the Saharan isolate *Sinorhizobium meliloti* IRAM:0087 (PRJNA842649) revealed the existence of several gene clusters coding for different PGP activities, rhizosphere competitiveness and stress tolerance, some of which were confirmed *in vitro* assays. Based on the evaluation of the PGP potential of non-rhizobial strains, three bacteria *P*. *salinitolerans*, *P*. *turukhanskensis* and *Starkeya* sp. were selected to improve *Pisum sativum,* a cold season crop, tolerance to a heat stress event. Our preliminary results suggest that endophytic isolates from arid legumes represent a promising resource of biofertilizers/biostimulants to increase plant resilience to heat stress. Future studies to explore the adaptative responses of these endophytes to different types of stress will allow to better understand their stress tolerance mechanisms and contribute to select strains for nature-based solutions towards the establishment of new agricultural technologies in drylands.

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