

Genomic analysis of *Sinorhizobium meliloti* IRAMC:0087 a halotolerant rhizobium isolated from the Tunisian desert

Roukaya Ben Gaied¹, Imed Sbissi¹, Clarisse Brígido², Mohamed Tarhouni¹

¹Arid Lands Institute of Médenine, Pastoral Ecology Laboratory University of Gabés- Tunisia

²MED – Mediterranean Institute for Agriculture, Environment and Development & CHANGE – Global Change and Sustainability Institute, Institute for Advanced Studies and Research, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal.

E-mail: roukaya.bengaied@gmail.com



Introduction

Arid, semi-arid and Saharan lands represent 33% of the Earth's surface with harsh environmental conditions. These territories are characterized by a thin vegetation cover spatially dispersed due to water limitation, nutrient deficiency and soil salinity [1]. As a consequence, an increased interest has been demonstrated for the rhizospheric microbial community which offers a biological reservoir for bio-fertilizers agents to improve crop productivity.

Objective

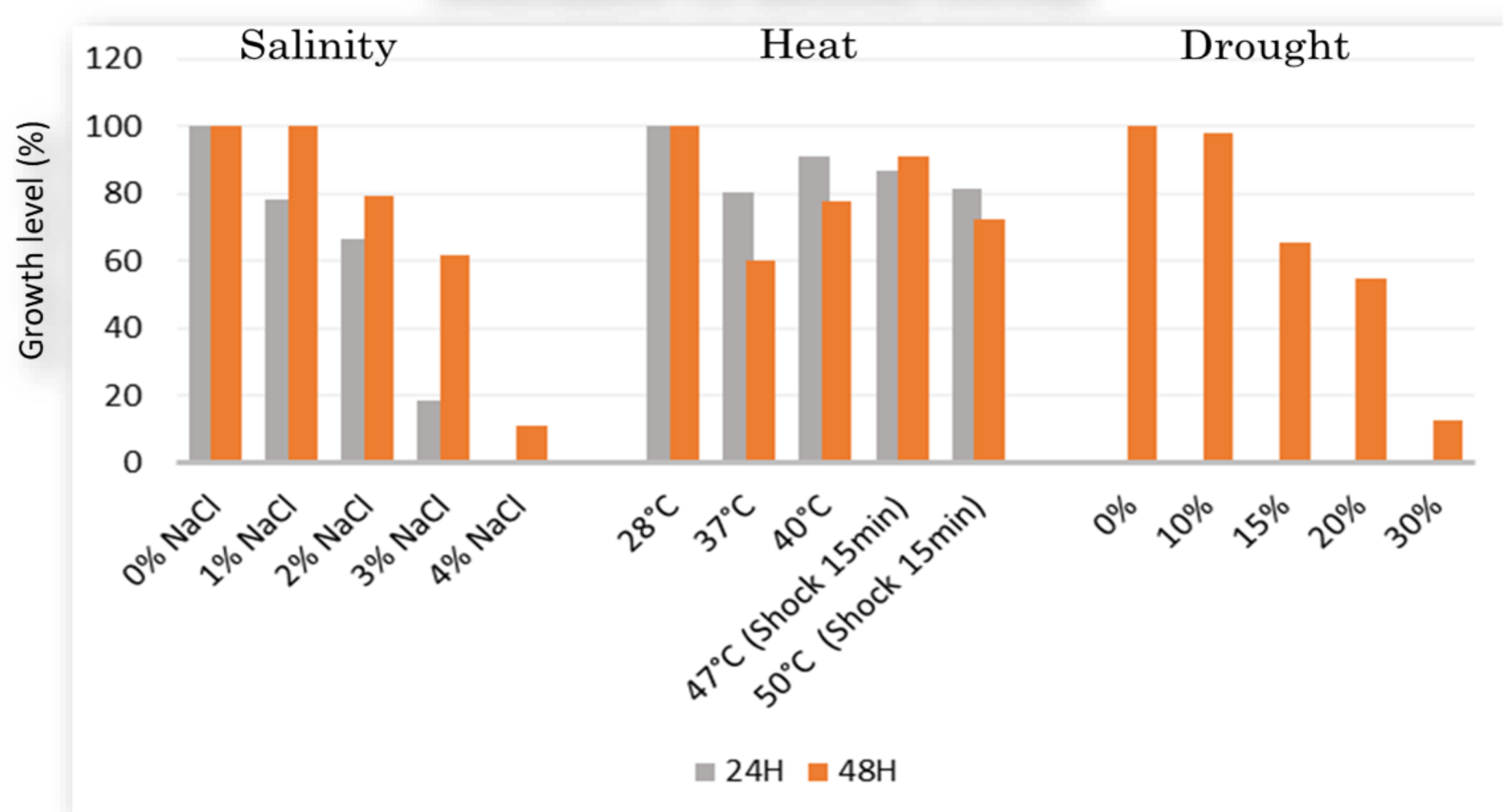
The main goal of this study was to explore the phenotypic and genomic features of the *Sinorhizobium meliloti* IRAMC: 0087.

Results and discussion

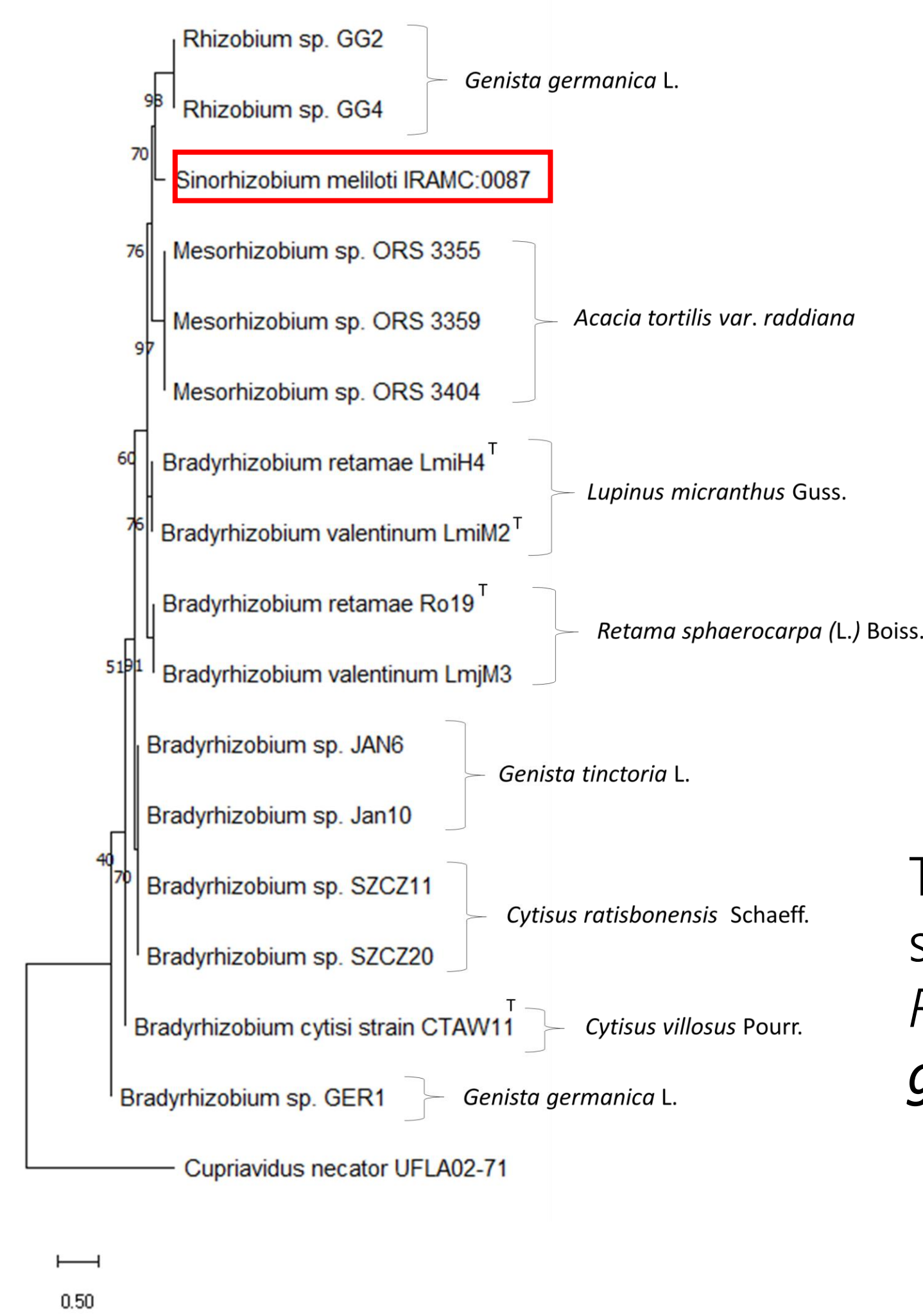
IRAMC: 0087 strain was isolated from root nodules of a Saharan shrub *Calobota saharae* growing in Southern Tunisia under extreme weather conditions. Molecular analysis of the rDNA 16S gene and housekeeping genes revealed 100% of homology with the type strain *Sinorhizobium meliloti* 2011, a microsymbiont of *Medicago sativa*.

Phenotypic and symbiotic characterization

Tolerance to abiotic stress



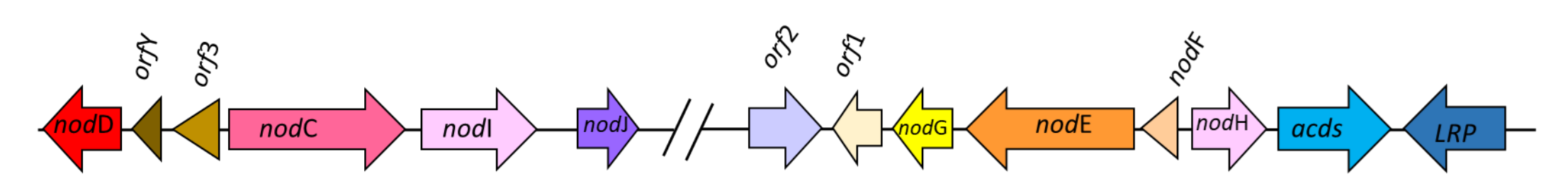
IRAMC: 0087 has shown a high tolerance to different abiotic stresses, being more tolerant to heat shock than salinity or drought. Nevertheless, this strain could grow above 65% under 3% of NaCl and up to 54% in the presence of 20% PEG6000. IRAMC: 0087 presented a high tolerance to both continuous and shock heat stress.



Effective nodules

Plant growth trial revealed the ability of IRAMC: 0087 to nodulate *Acacia tortilis var. raddiana*, a plant-tree adapted to extreme climatic conditions.

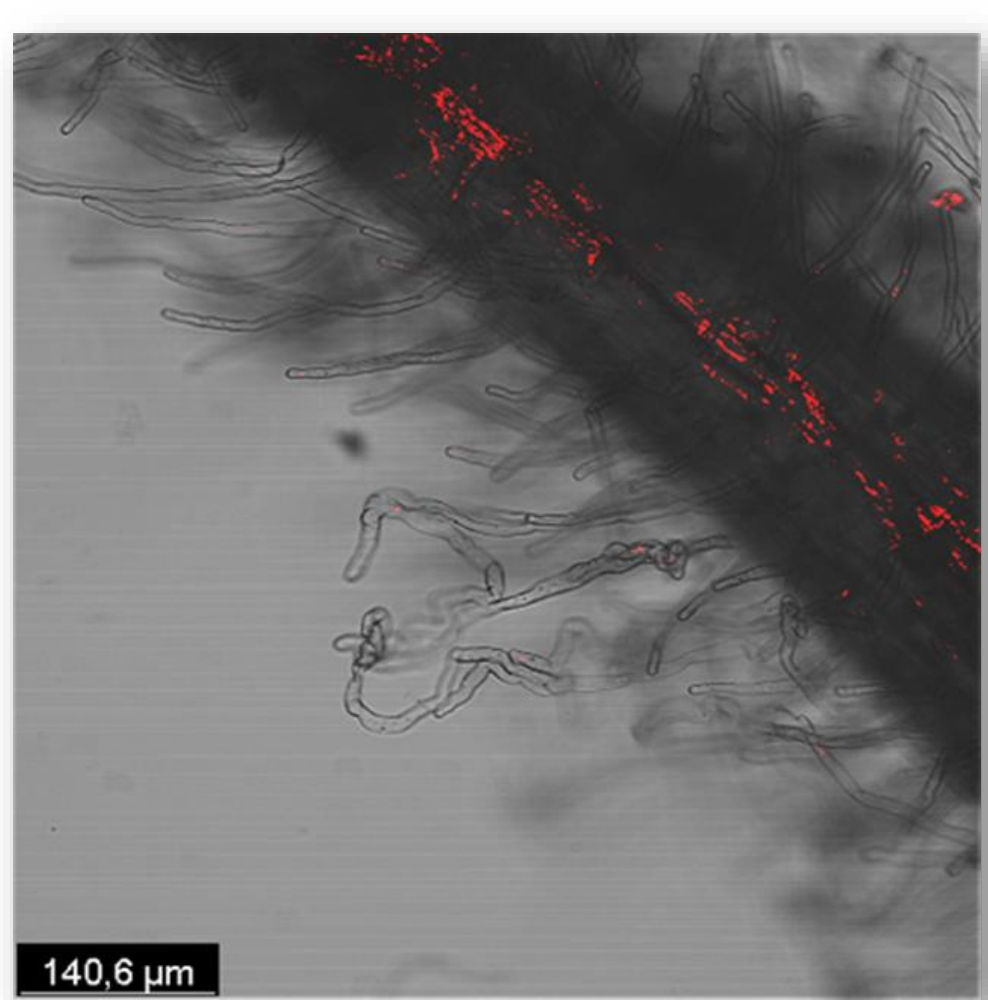
The Neighbor joining phylogenetic tree based on *nodC* gene sequences shows that IRAMC: 0087 is closely related with *Rhizobium sp.* strains isolated from root nodules of *Genista germanica* L.



A: Nodulation cluster
B: Nitrogen fixation cluster

WGS analysis revealed the existence of the symbiotic island, containing the genes coding for nodulation process and nitrogen fixation together with the *acdS* gene.

Endophytic characterization



Endophytic behaviour in non-host plants was revealed by confocal microscopy analysis.

Red-tagged strain IRAMC: 0087 in the roots of *Trifolium subterraneum* plants

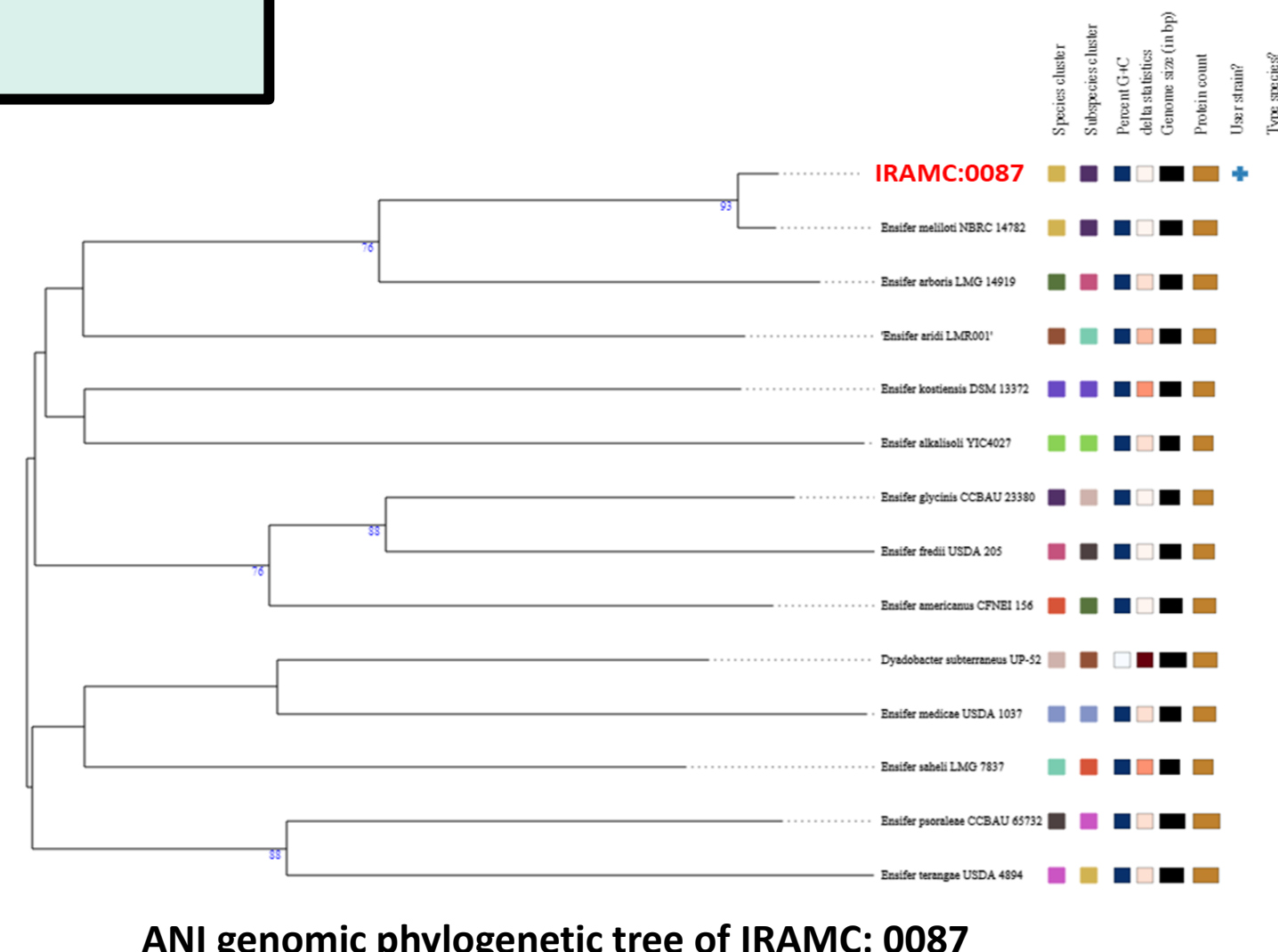
WGS analysis revealed the existence of the genes or gene clusters encoding for different plant growth promoting activities and rhizosphere colonization processes, some of which confirmed *in vitro*.

PGP Activities and rhizo-competence		IRAMC: 0087
Phyto-hormones	ACC deaminase	●
	IAA synthesis	●
	Cytokines biosynthesis	●
	Jasmonates	●
Phosphorus solubilization	Abscisic acid biosynthesis	X
	Pyroloquinoline-quinone	●
	Phosphonatase	●
	Phytase	X
Siderophores production	Pyochelin	●
	Aerobactin	X
	Achromobactin	X
	Pyovertin	X
Antimicrobial activity	Methionethiol	●
	Hydrogen cyanide	X
	Alkaline protease	X
	Lipase	X
Rhizo-competence and plant tissues colonization	chitinase	X
	Phenazine-1-carboxamide	●
	Respiratory nitrate reductase	●
	Chemotaxis	●
	2,3-butanediol metabolism and biosynthesis	●
Acetone metabolism and biosynthesis	●	

Genomic comparative study

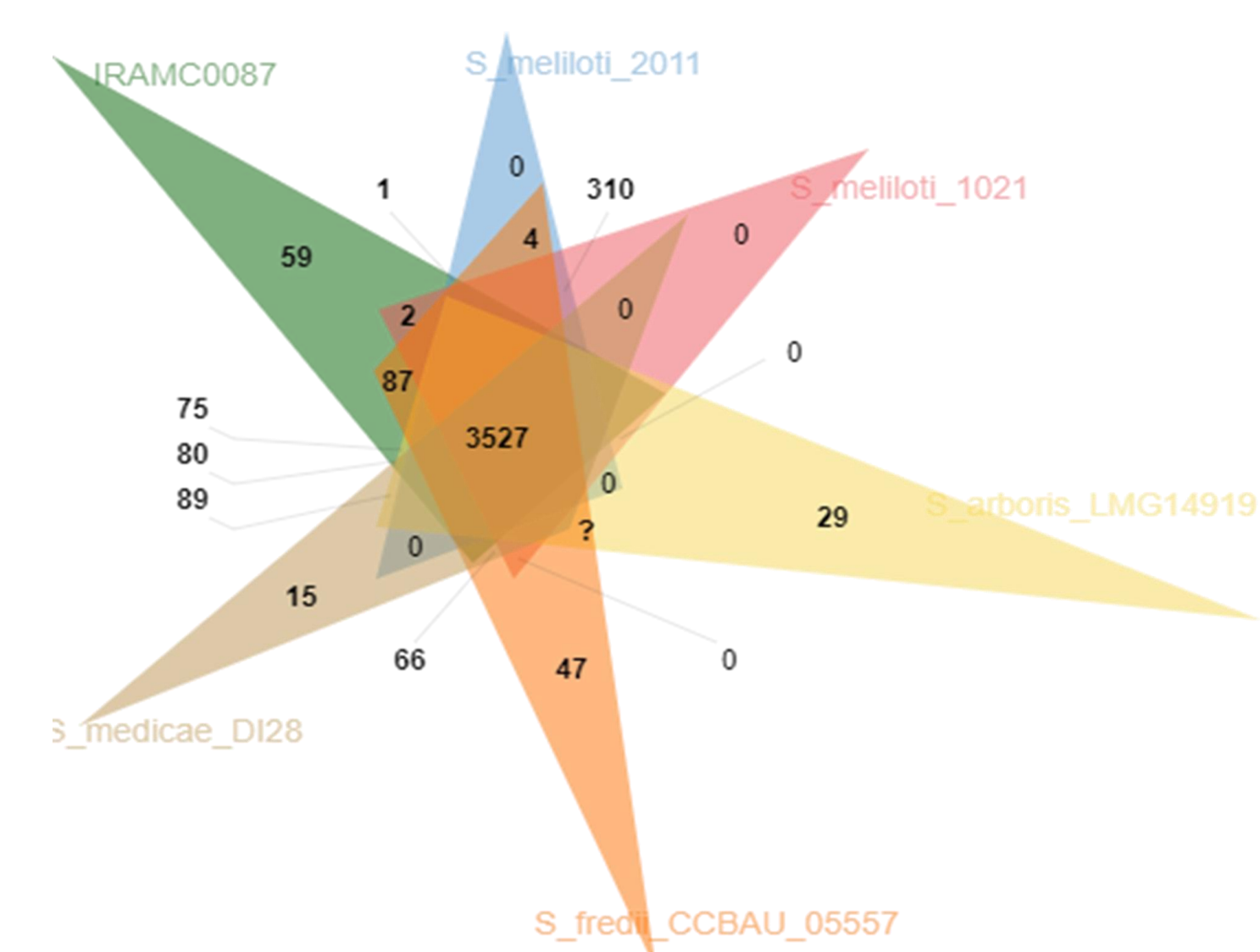
Strain	<i>Sinorhizobium meliloti</i> IRAMC:0087	<i>Sinorhizobium meliloti</i> NBRC 14782 ^T
Genome size	7,265,739 bp	6,693,185 bp
G+C	61.94%	62.2%
Protein coding genes	7536	6696
Number of RNAs	55	63

Average Nucleotide Identity (ANI) revealed a similarity of 93% between the type strain *S. meliloti* 2011 and the IRAMC: 0087 isolate. These two strains shared 5302 ortholog gene clusters.



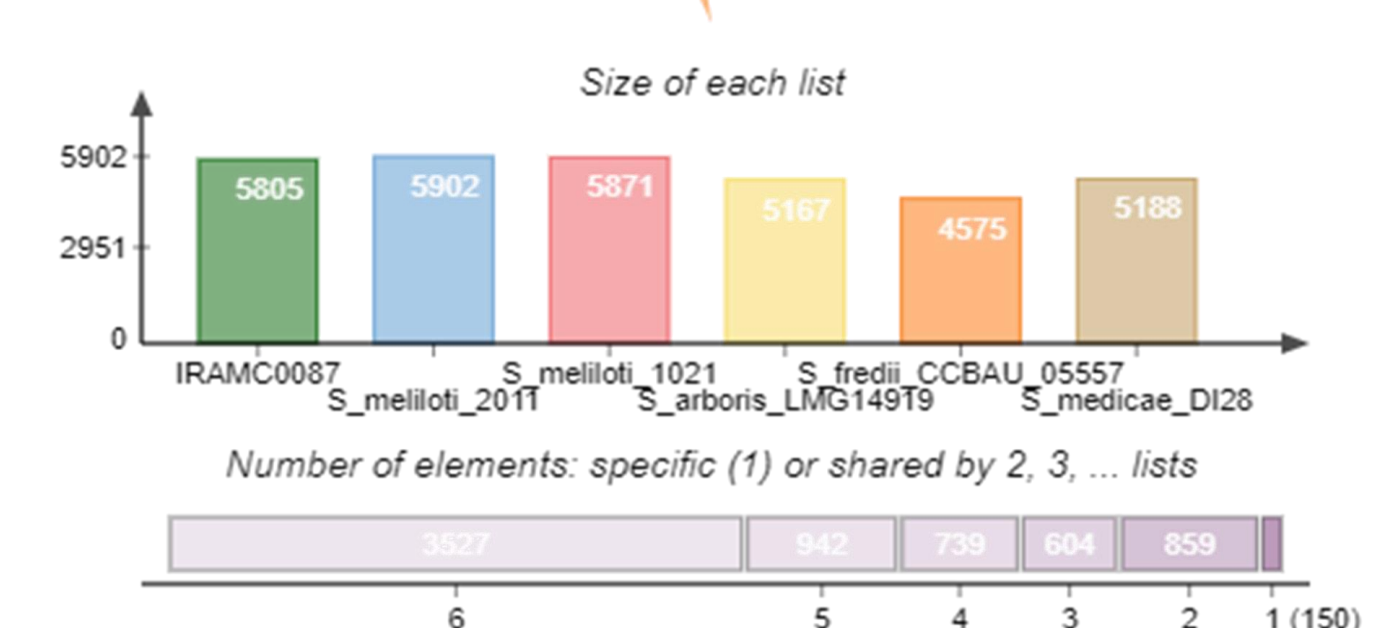
IRAMC: 0087 contains more protein encoding genes than *S. meliloti* 2011, with 7536 genes in which the major differences lie on the number of functionally unclassified genes.

IRAMC: 0087 contains 3527 ancestral clusters and 59 specific gene clusters among which over 100 proteins are classified under hypothetical proteins or with unknown function.



Conclusions

IRAMC:0087 is an interesting strain as it exhibits an endophytic and symbiotic behavior with hosts adapted to extreme climatic conditions. Comparative genomic analyses with other rhizobial strains have the potential to reveal novel factors mediating symbiosis under those conditions.



Acknowledgements:

This work was conducted under the PhD mobility fellowship supported by the University of Gabés (FSG) and the Ministry of Higher Education and Scientific researches Tunisia. CB acknowledges a Auxiliary Researcher contract (CEECIND/00093/2018) from FCT. This work was supported by the Project POCI-01-0145-FEDER-016810 (PTDC/AGR-PRO/2978/2014).

Reference

[1]- 1. Cherlet, M., Hutchinson, C., Reynolds, J., Hill, J., Sommer, S., and von Maltitz, G. (2018). World Atlas of Desertification. Luxembourg: Publication Office of the European Union.