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European blueberry microbiome for new biofertilizers development

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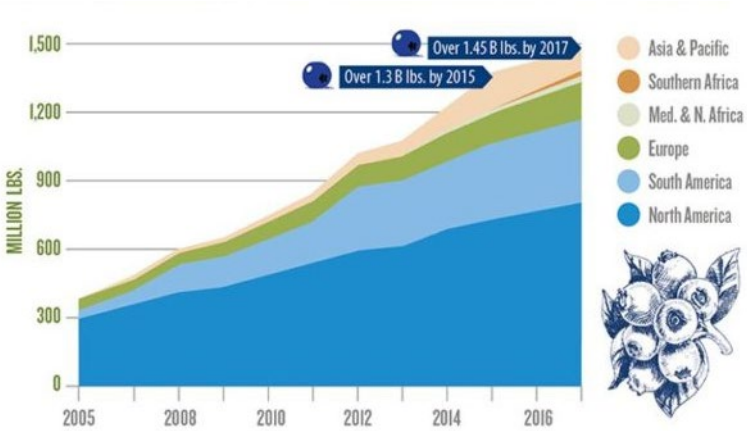
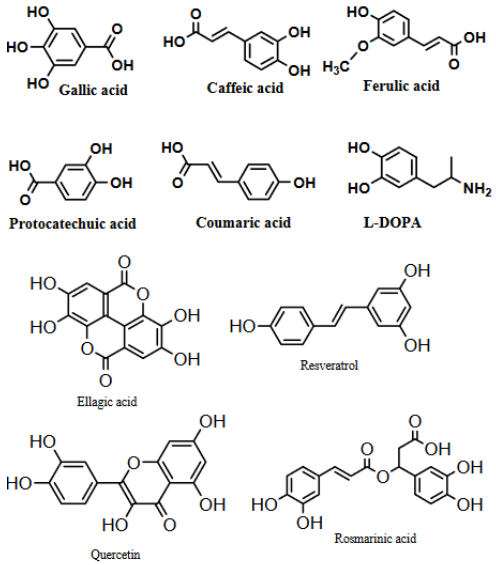
Berries and health

High content in antioxidant compounds: flavonoids, flavonones, estilbens, etc
Dietary fiber and low sugar concentration
Positive effect in human health



- Cardioprotector
- Reduction of oxidative stress
- Reduction of neurodegenerative illness risk
- Antibiotic effect for urinary track



Vaccinium: botanical and crop features

- Two autoctonous species in Portugal
  - *Vaccinium myrtillus*
  - *Vaccinium uliginosum*

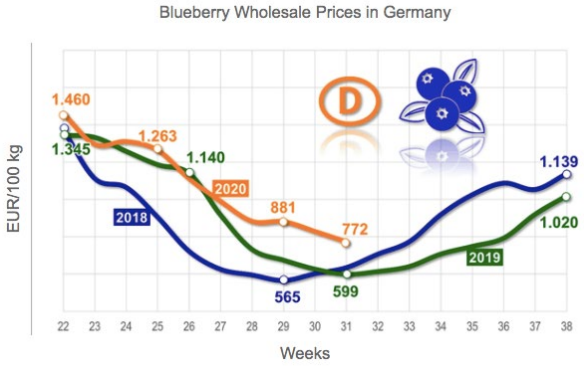
Acid soils(<6,5) and drained, high precipitation

Light root system consisting of rhizomes and lateral roots.

- One cultivated species
  - *Vaccinium corymbosum*

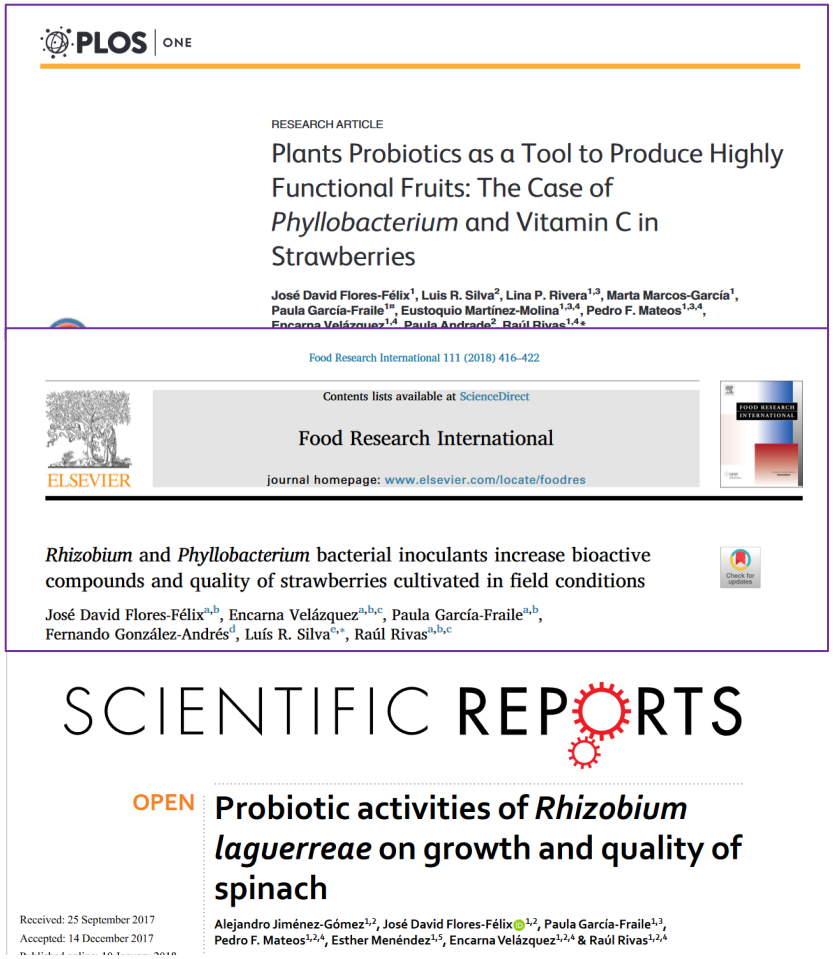
Similar requirements to *V. myrtillus*

Greater size (up to 3 meters) and multiple cultivars



Atractive crop due to it prize and adaptation to acid soil, common in Portugal.

Biofertilizers and phenolics compounds



PLOS ONE

RESEARCH ARTICLE

Plants Probiotics as a Tool to Produce Highly Functional Fruits: The Case of *Phyllobacterium* and Vitamin C in Strawberries

José David Flores-Félix[1], Luis R. Silva[2], Lina P. Rivera[1,3], Marta Marcos-García[1], Paula García-Fraile[1¤], Eustoquio Martínez-Molina[1,3,4], Pedro F. Mateos[1,3,4], Encarna Velázquez[1,4], Paula Andrade[2], Raúl Rivas[1,4]*

Food Research International 111 (2018) 416–422

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Food Research International

journal homepage: www.elsevier.com/locate/foodres

Rhizobium and *Phyllobacterium* bacterial inoculants increase bioactive compounds and quality of strawberries cultivated in field conditions

José David Flores-Félix[a,b], Encarna Velázquez[a,b,c], Paula García-Fraile[a,b], Fernando González-Andrés[d], Luís R. Silva[e,*], Raúl Rivas[a,b,c]

SCIENTIFIC REPORTS

OPEN

Probiotic activities of *Rhizobium laguerreae* on growth and quality of spinach

Received: 25 September 2017
Accepted: 14 December 2017

Alejandro Jiménez-Gómez[1,2], José David Flores-Félix[1,2], Paula García-Fraile[1,3], Pedro F. Mateos[1,2,4], Esther Menéndez[1,5], Encarna Velázquez[1,2,4] & Raúl Rivas[1,2,4]

Improve the quality of the production in different crops:

- Strawberry
- Lettuce
- Spinach

Different plant probiotics

- *Rhizobium*
- *Phyllobacterium*

foods

MDPI

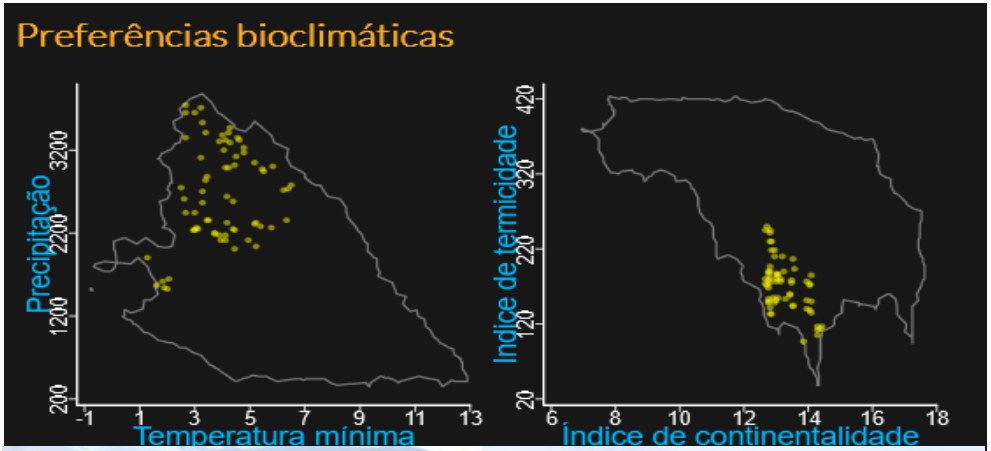
Article

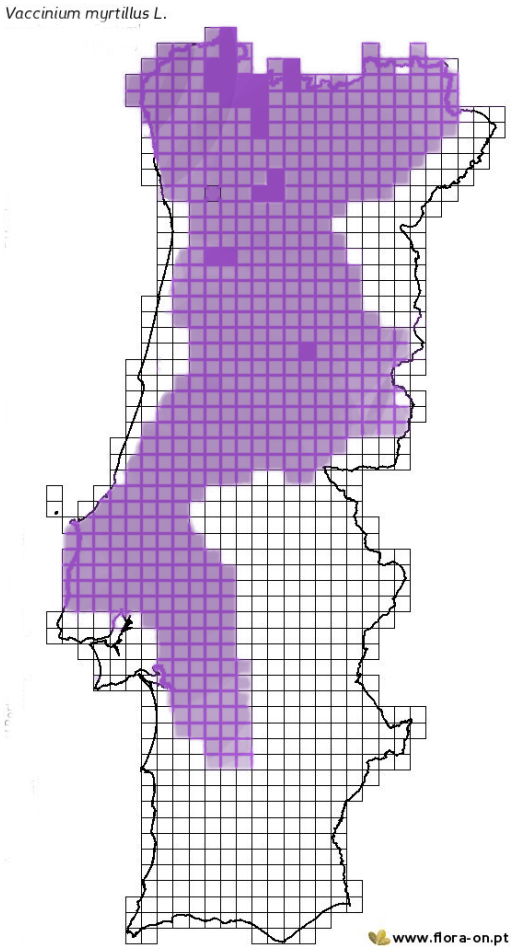
Rhizobium laguerreae Improves Productivity and Phenolic Compound Content of Lettuce (*Lactuca sativa* L.) under Saline Stress Conditions

Miguel Ayuso-Calles [1,2], Ignacio García-Estévez [3], Alejandro Jiménez-Gómez [1,2,*], José D. Flores-Félix [1,2], M. Teresa Escribano-Bailón [3] and Raúl Rivas [1,2,4]

Project BIOLACVAC

- **Ignorance of the blueberry microbiota**
- **Design of new fertilization strategies.**
- **Transcriptomic analysis of phenolic compounds biosynthesis**



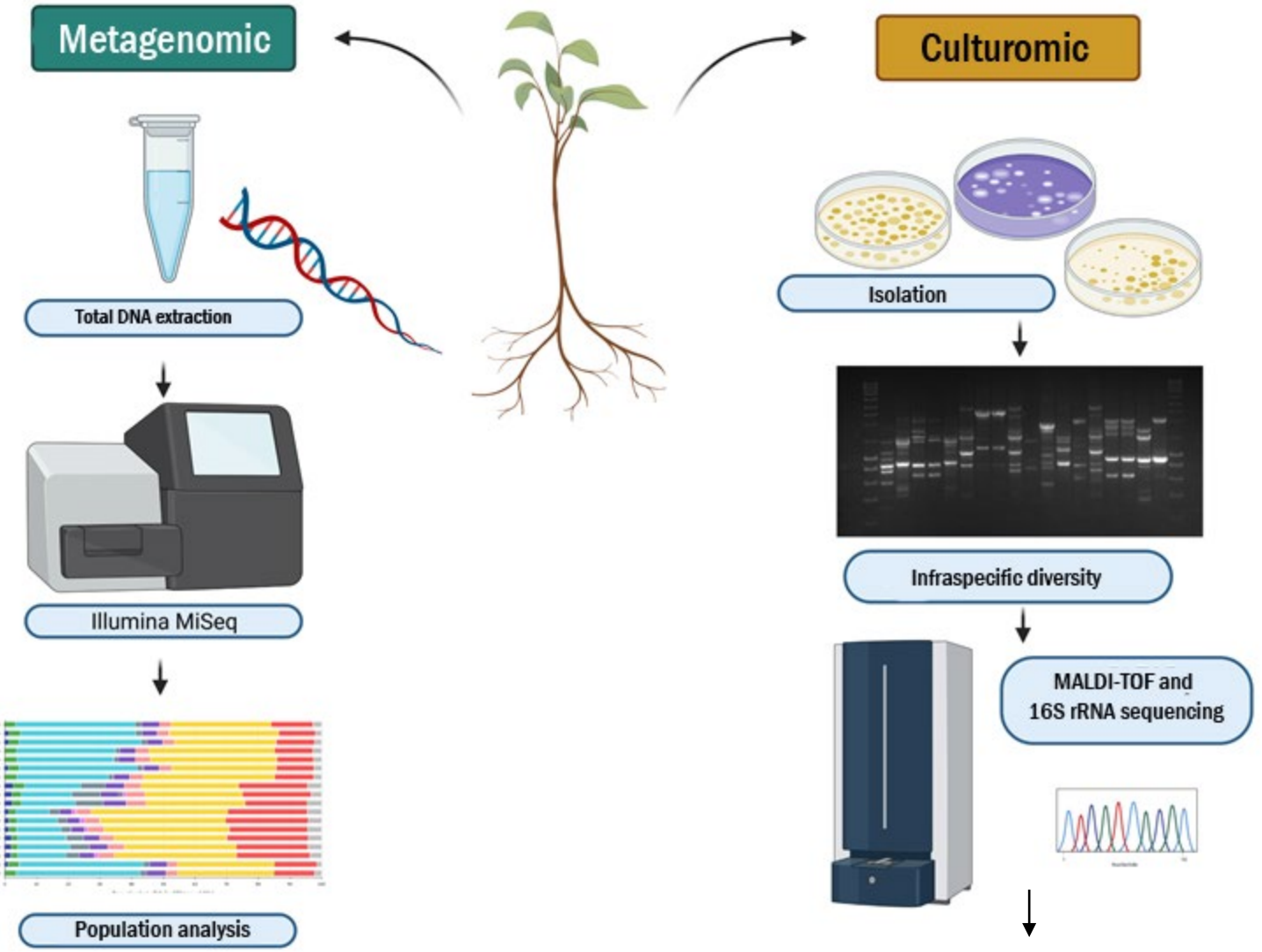


In dark violet, distribution of wild population of blueberry, in violet, distribution of blueberry crops.

Isolation sites

	Serra da Estrela	Serra da Freita	Serra do Marão
Bioclimatic stage	Supratemperate superior	Supratemperate inferior	Supratemperate inferior
Orientation	Northwest	West	Southwest
Biotope	Peatbog	Riparian understory	Degraded scrub
Ombrotermic	Ultra/humid superior	Hiper-humid superior	Hiper-humid superior
Altitude (m)	1700	1100	1200
Medium precipitation (mm)	1900	2350	2700
Maximum Tra (ºC)	15,3	19	18,3
Minimum Tra (ºC)	1,2	3,5	2,7

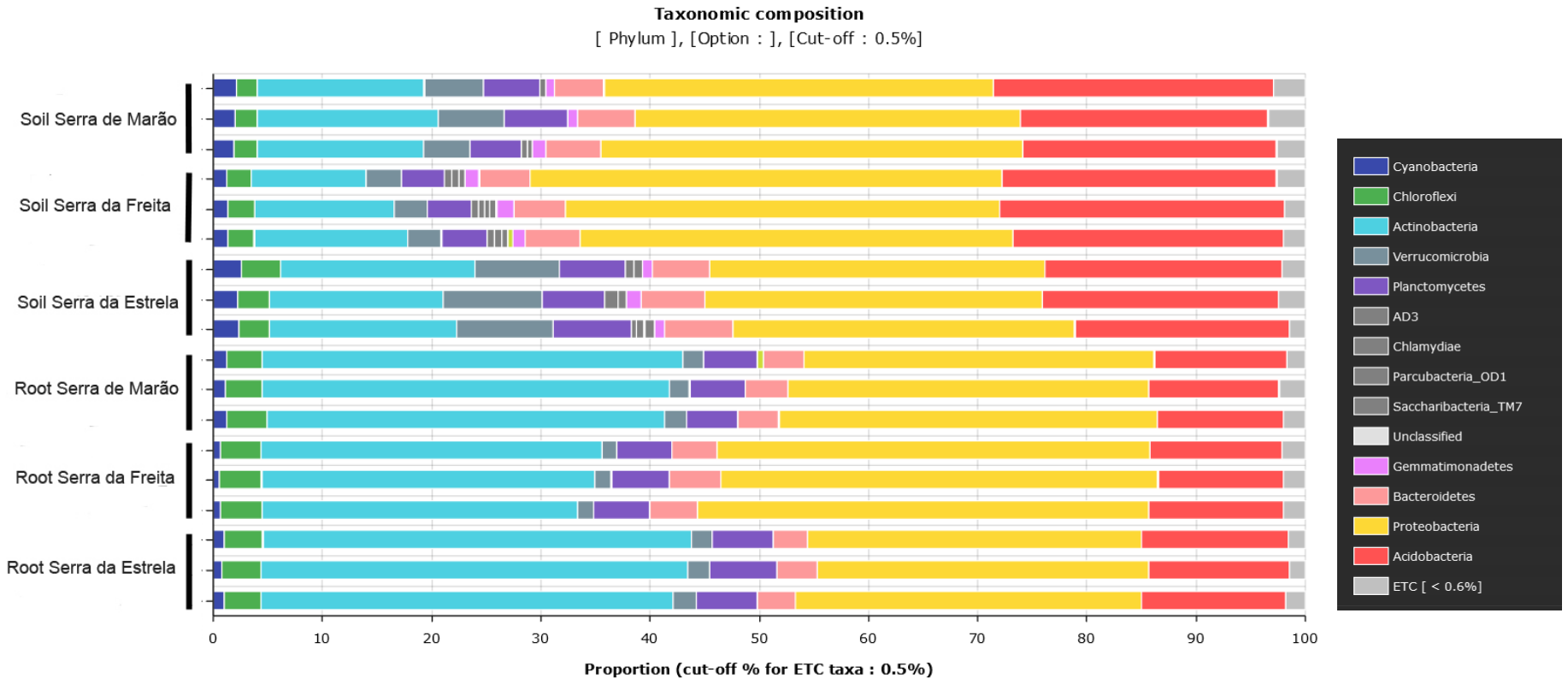
Methodology



Metagenomic analysis

Rhizospheric population dominated by Proteobacteria y Acidobacteria, althought endospheric are governed Actinobacteria y Proteobacteria.

Similar structure of endophityc population. Relationship with plant selection.



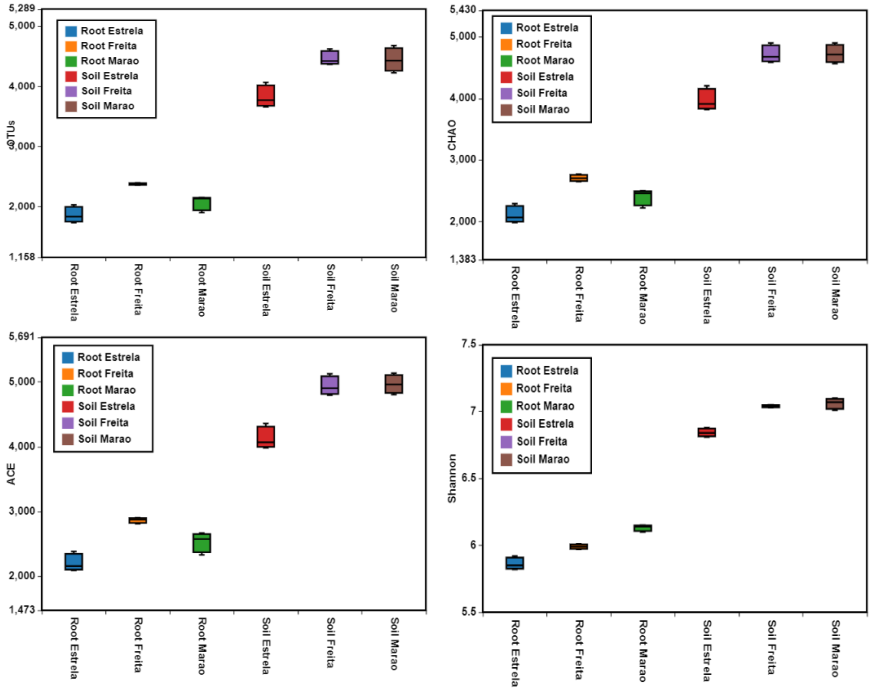
Alpha and Beta diversity

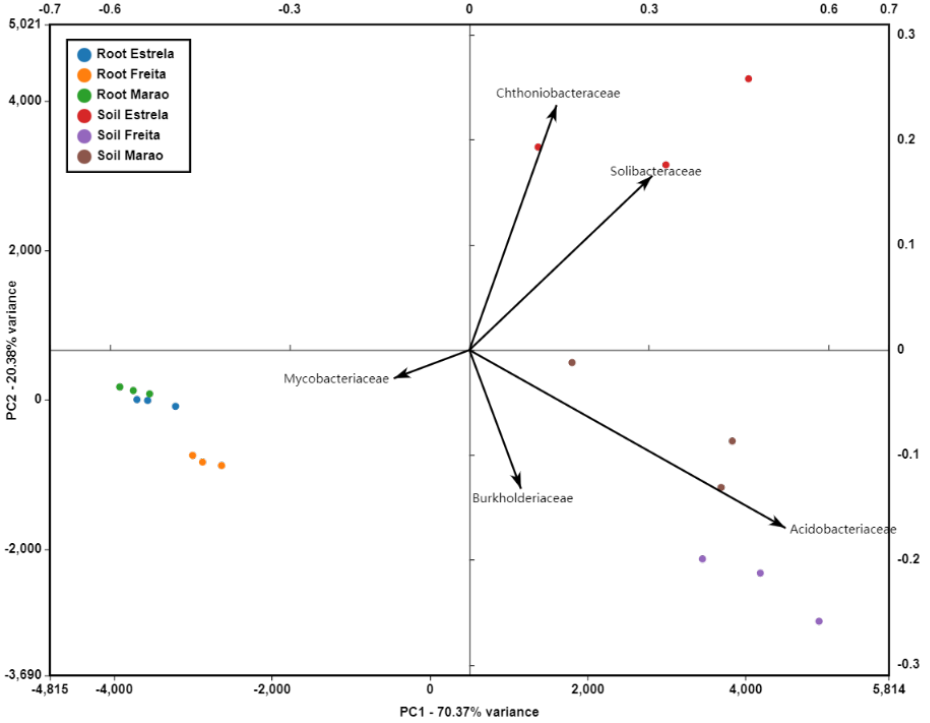
Serra da Estrella is the site with lower diversity.

The populations of Serra de Marão and Serra da Freita present similar values in the rhizospheric populations, but significantly lower in S. da Estrela in the endospheric populations (p<0,001).

Important differences in the abundance of some families, such as Acidobacteriaceae or Chtoniobacteriaceae.

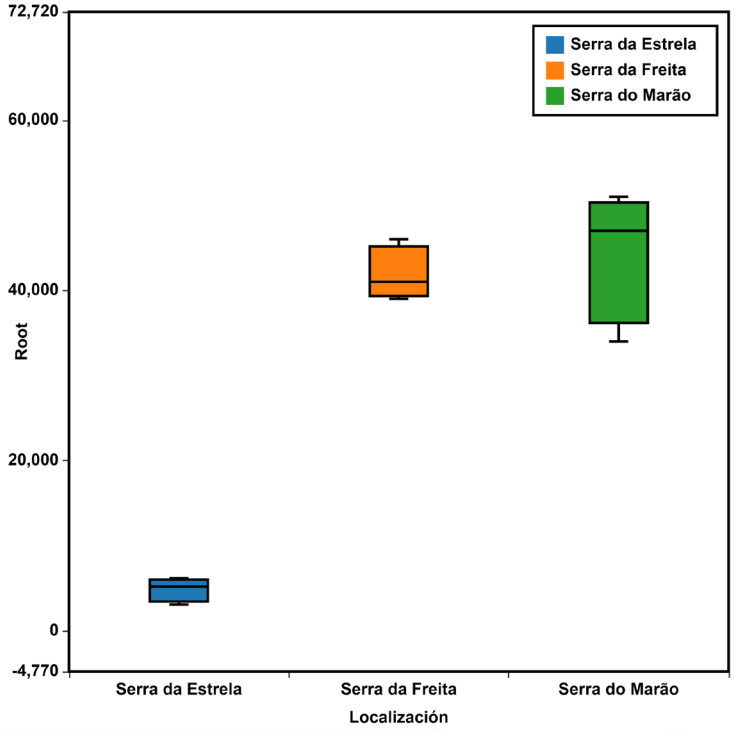
Endophytical metagenomes show a high similarity, unlike rhizospheric metagenomes, where S. da Estrela is the most divergent.

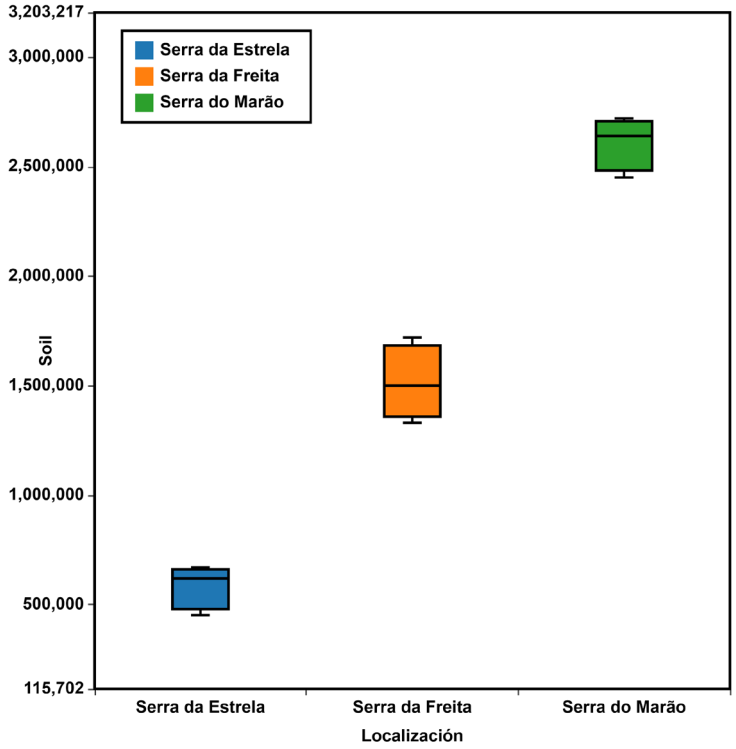




Culturomic approach

- Employment of different media (TSA, MRS, YMA, Jensen Free Nitrogen medium)
- Around 300 strain by site (183 rizosphere-135 root)
- Estimation of culturable populations (TSA)



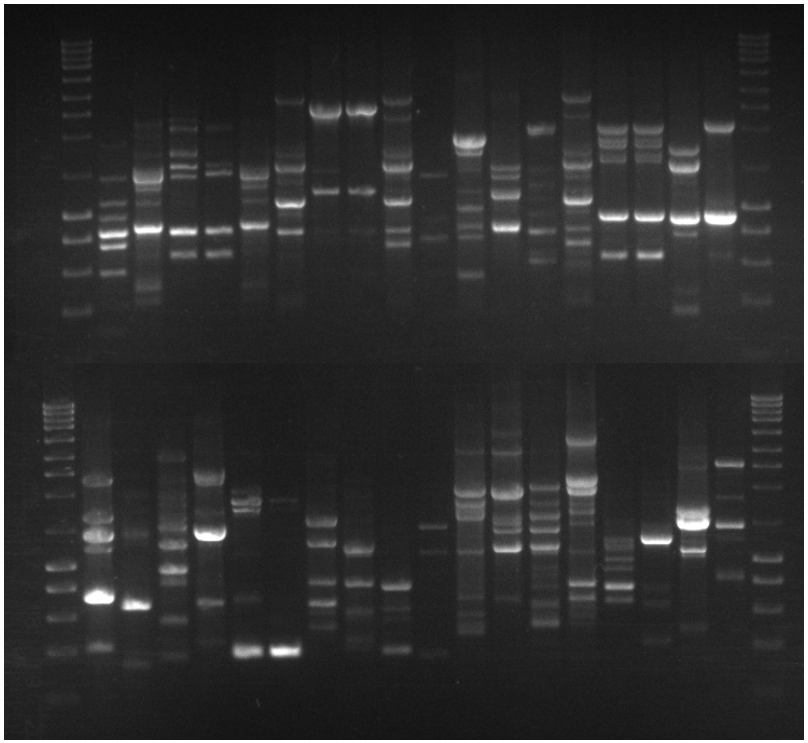


Infraespecific diversity analysis

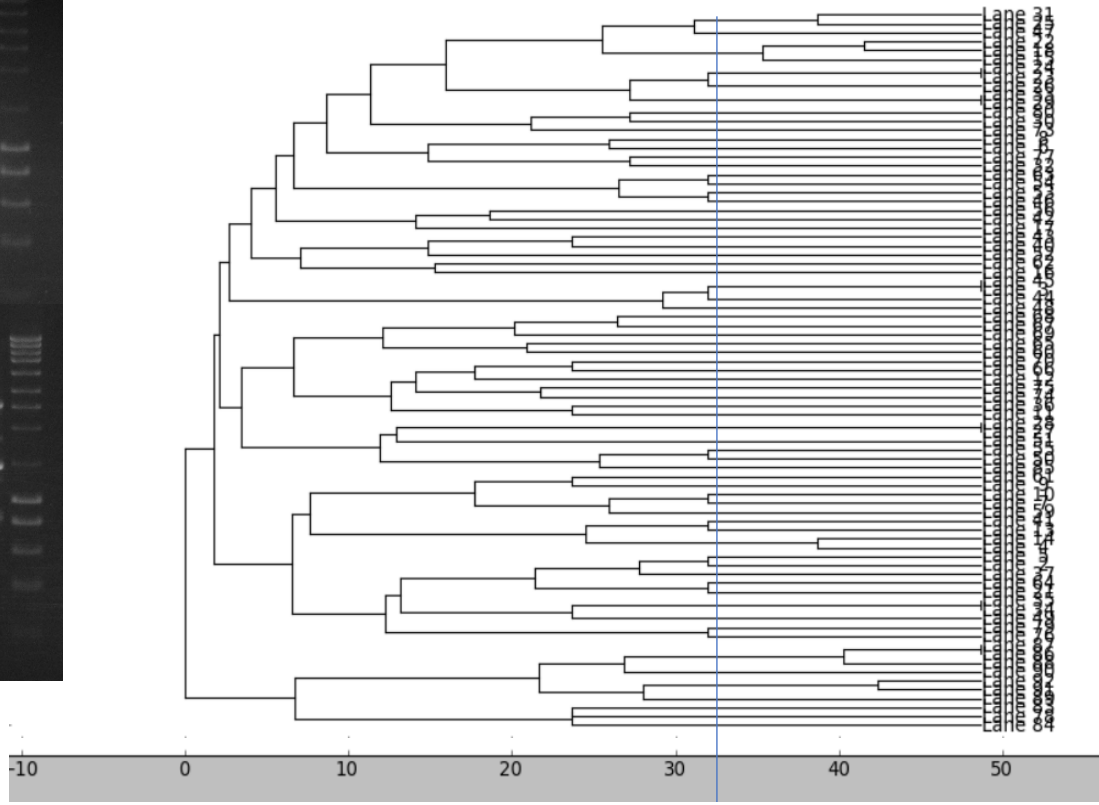
M13-RAPD (Random Amplified Polimorphic DNA).

Analysis by site and origin

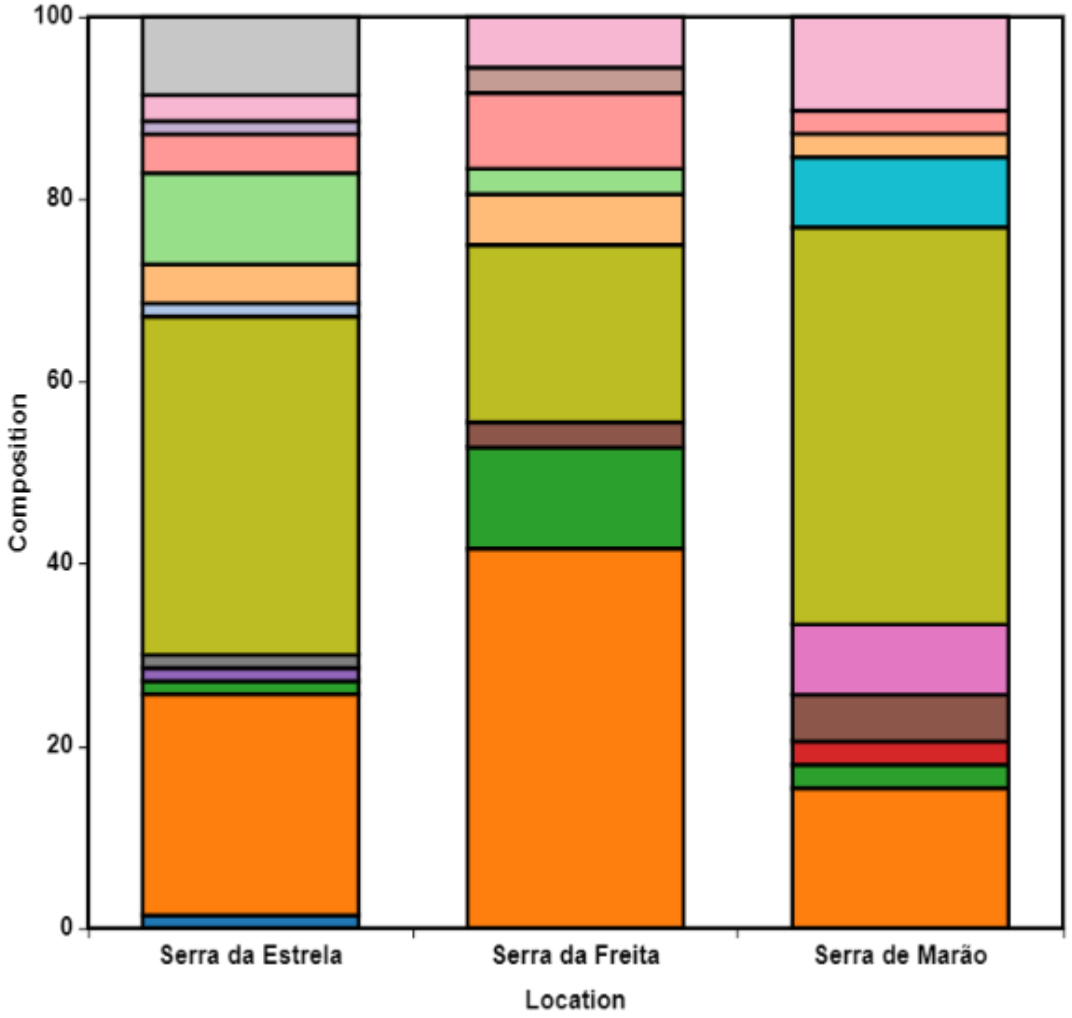
245 independiente groups



UPGMA method for clustering



Identification by MALDI-TOF



27% of strain were identified by MALDI-TOF (lower score than 1,699)

Nuclear culturome and other variable families

Relationship with the biotope and the medium precipitation of the isolation site.

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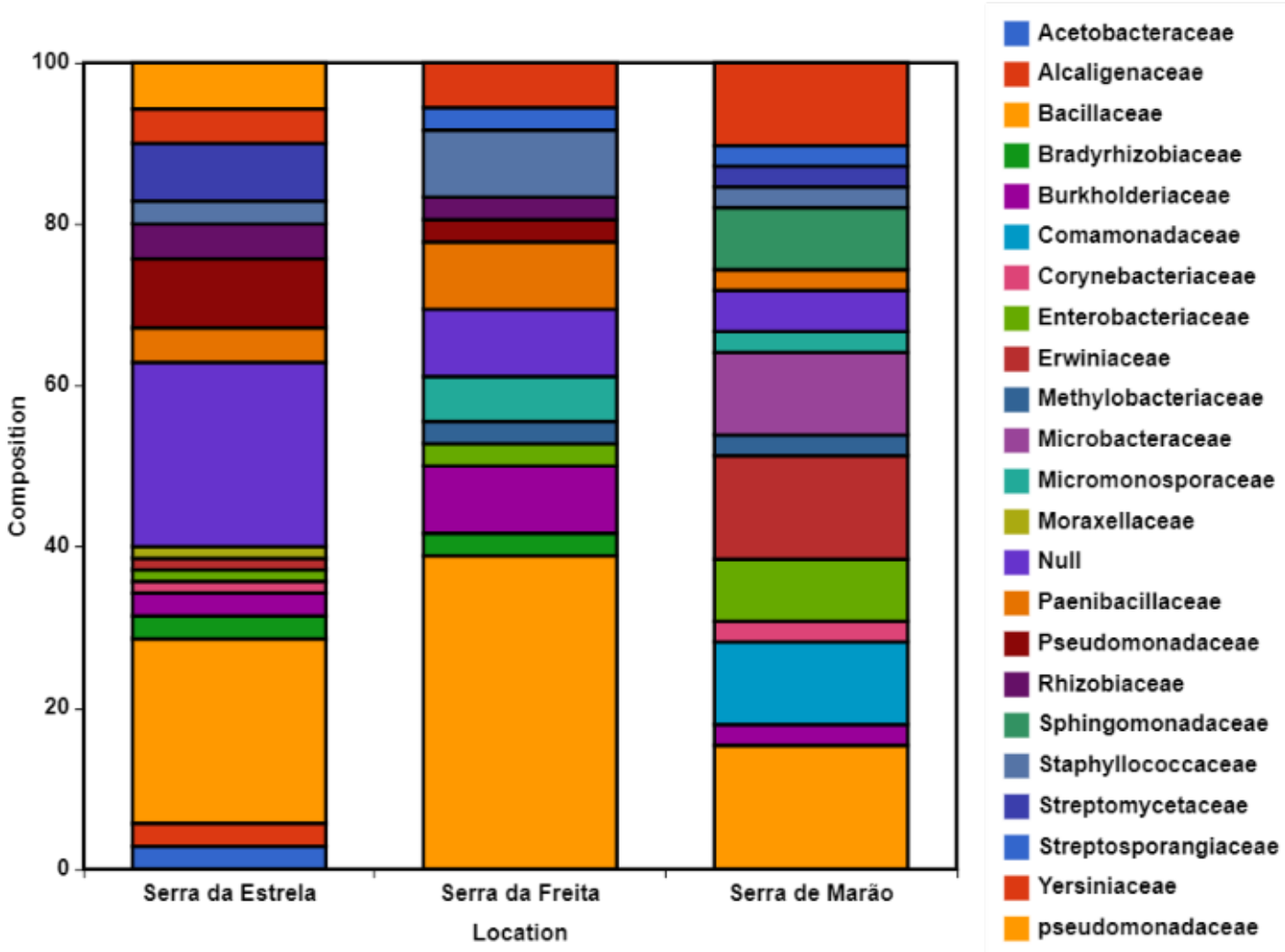
Identification by 16S sequencing



27% of strain were identified by MALDI-TOF (lower score than 1,699)

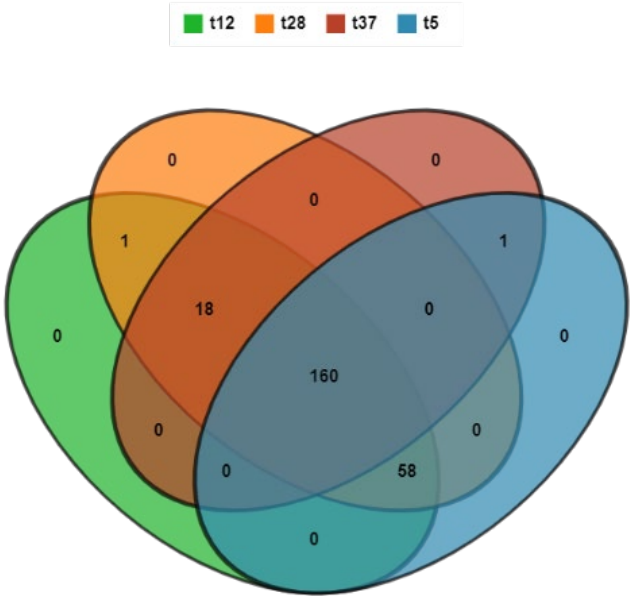
Nuclear culturome and other variable families

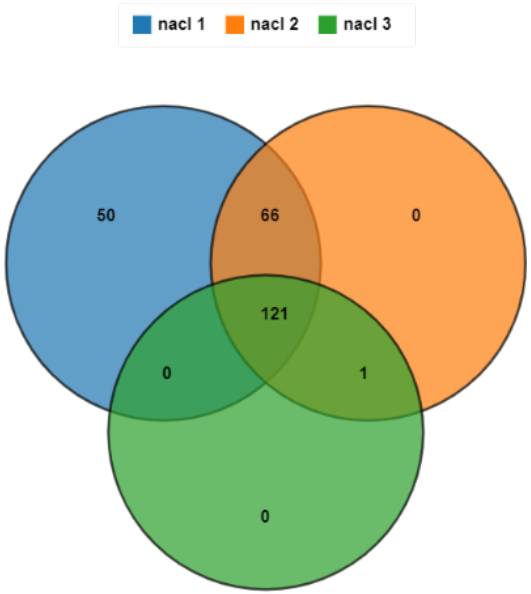
Relationship with the biotope and the medium precipitation of the isolation site.

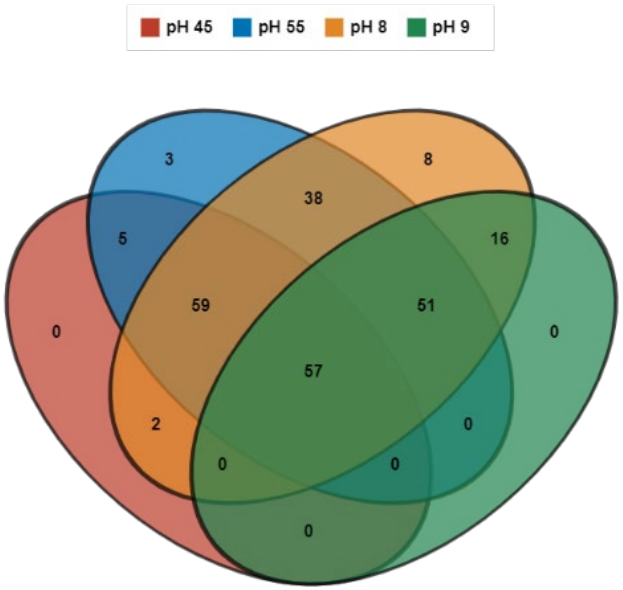
Phenotypic characterization of the isolated strains

Psycrotolerants, medium salinity tolerances and growth in pH 5,5 conditions

Correlation between origin and characteristics.



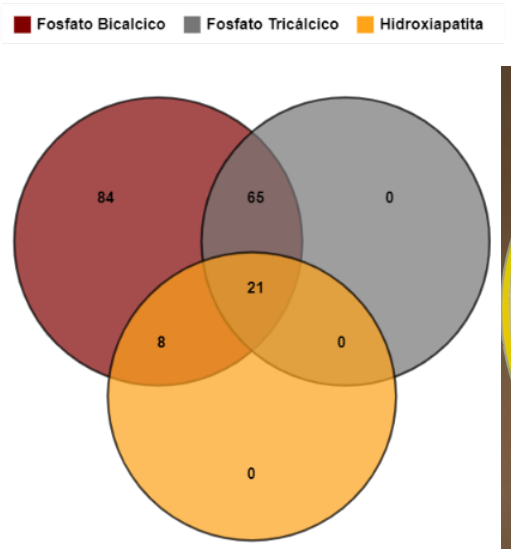


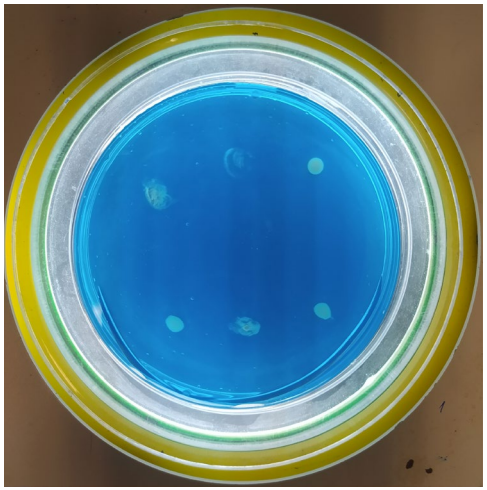


PGPR mechanisms

Phosphate solubilization: 75%-bicalcium, 35%-Tricalcium, 12%-hidroxiapatite

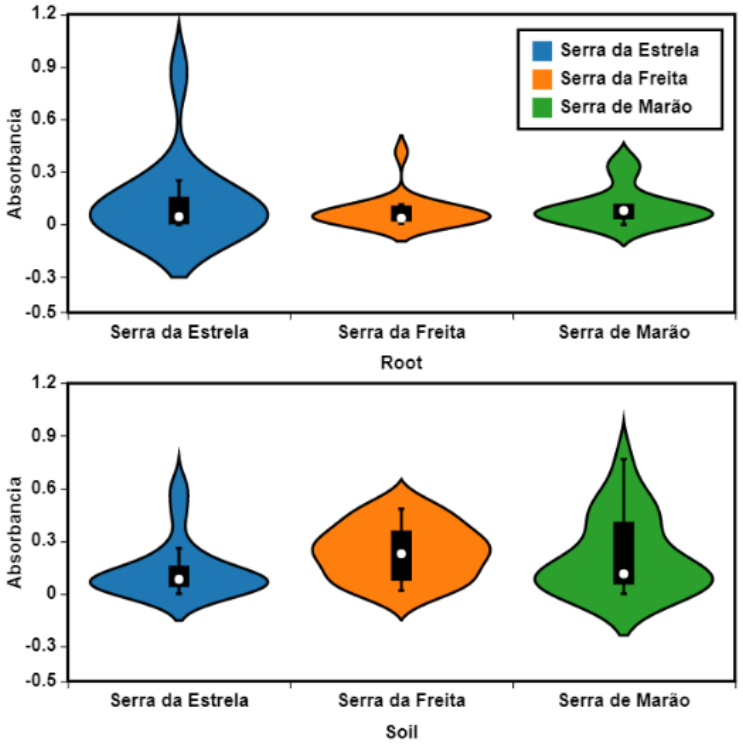
Low siderophore production: 17% - not abundant characteristic





Most of isolates produced IAA

Rhizospheric bacteria produced higher amounts of this phytohormone.



Conclusions

- Modulation of endophytic populations by blueberry
- Influence of climate on rhizospheric populations of wild blueberry in Portugal.
- Nucleus culturome with dominance of Proteobacteria and Firmicutes.
- Populations adapted to acidity and low temperatures.

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Thank you very much

Marie Skłodowska-Curie IF grant Nº 101003373

MARIE CURIE ACTIONS

BIOLACVAC