

Community structure, bioactivities and secondary metabolite production of bacteria associated with a temperate octocoral



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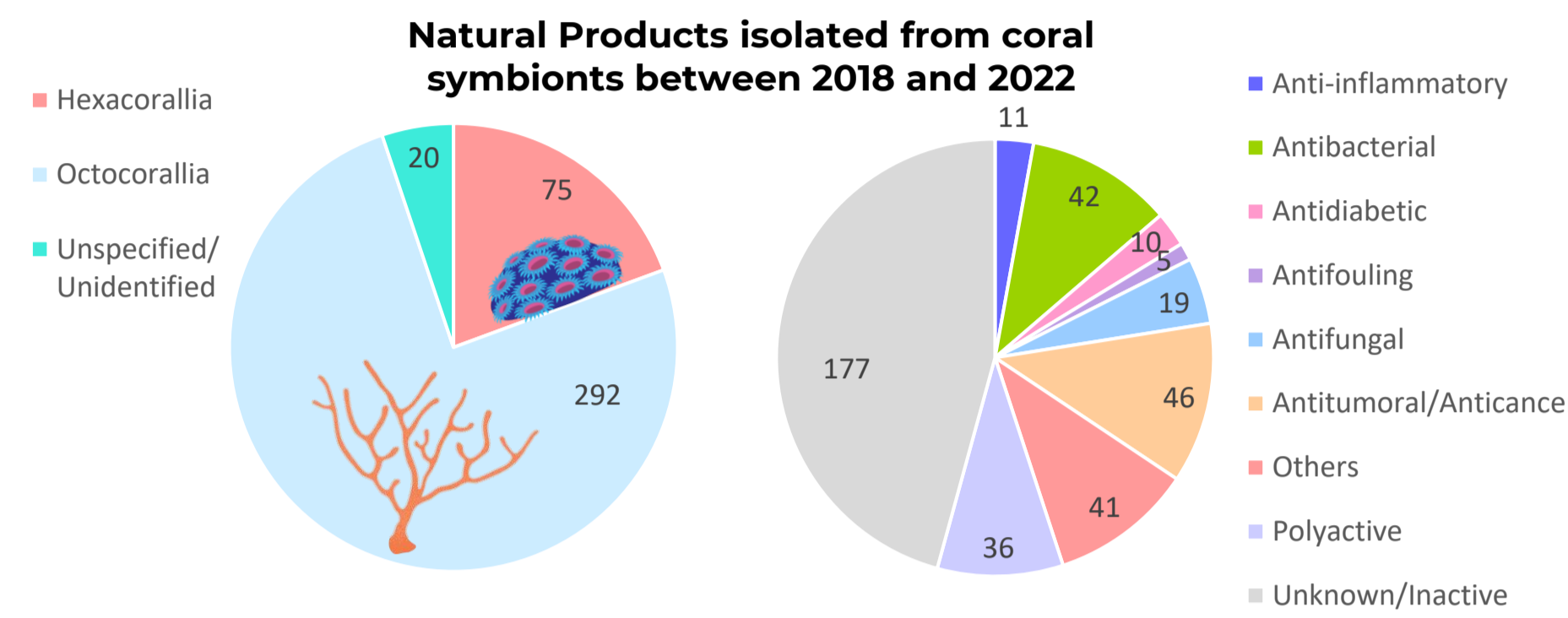
IDEAS TO IMPACT

MIT Portugal
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Motivation and background: The coral microbiome and its biotechnological potential



- Marine invertebrate animals such as corals host diverse microbial communities.
- Corals and their microbiota rely on chemical defence and signalling for protection, competition and for physiological processes.
- Octocoral-associated bacteria and fungi are prolific natural product producers.



The profile of the natural products reported is seemingly influenced by **sampling bias** and **cultivation bias**:

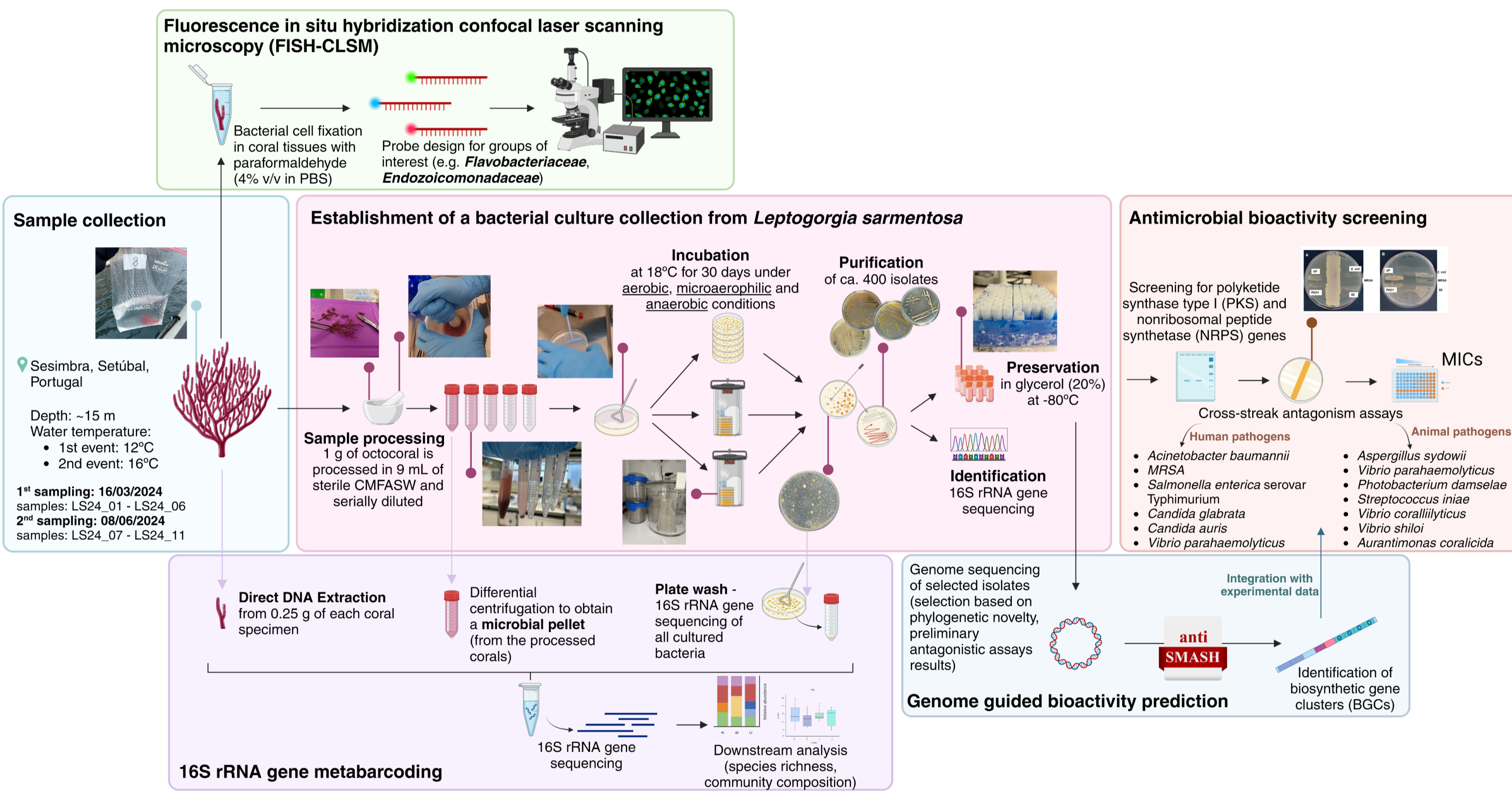
- Location:** strong investment in tropical regions;
- Type of coral investigated:** Mostly octocorals, which might be easier to process;
- Well-studied, easy-to-cultivate microbes** prevail e.g., *Streptomyces* spp., *Aspergillus* spp.,
- Some **bioactivities**, e.g., antibacterial, are more represented than others.

Objectives

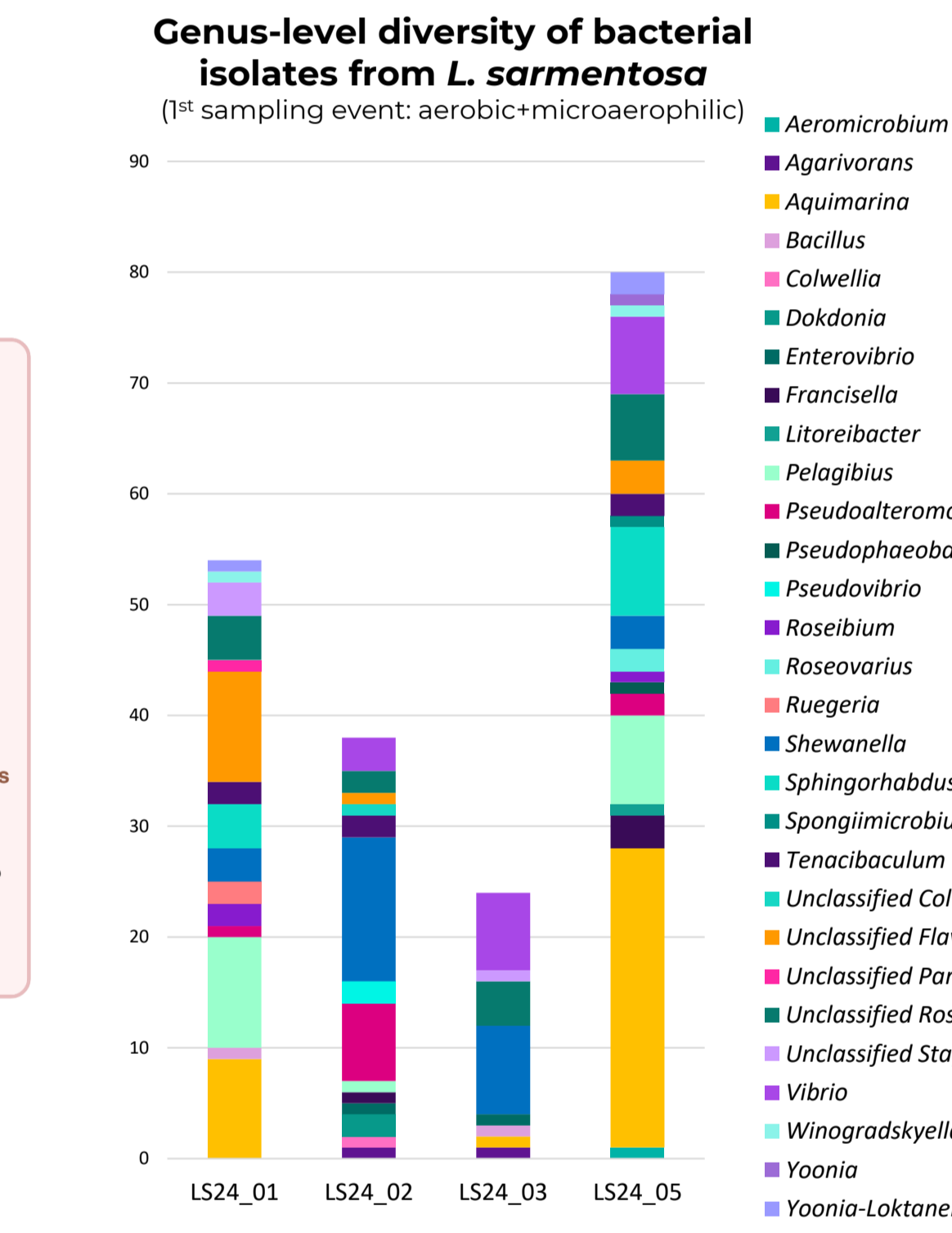
- This project aims to analyse the cultivability of the microbiome of the understudied temperate octocoral *Leptogorgia sarmentosa*, while addressing the great plate anomaly by using innovative strategies to cultivate previously undiscovered microbes. Additionally, it seeks to contribute to the preservation of the octocoral microbiome through methods such as biobanking.
- Moreover, it addresses the world's pressing challenge to discover new natural products for human health, agriculture, and aquaculture sectors.

The *Leptogorgia sarmentosa* bacterial culture collection

Experimental Approach:



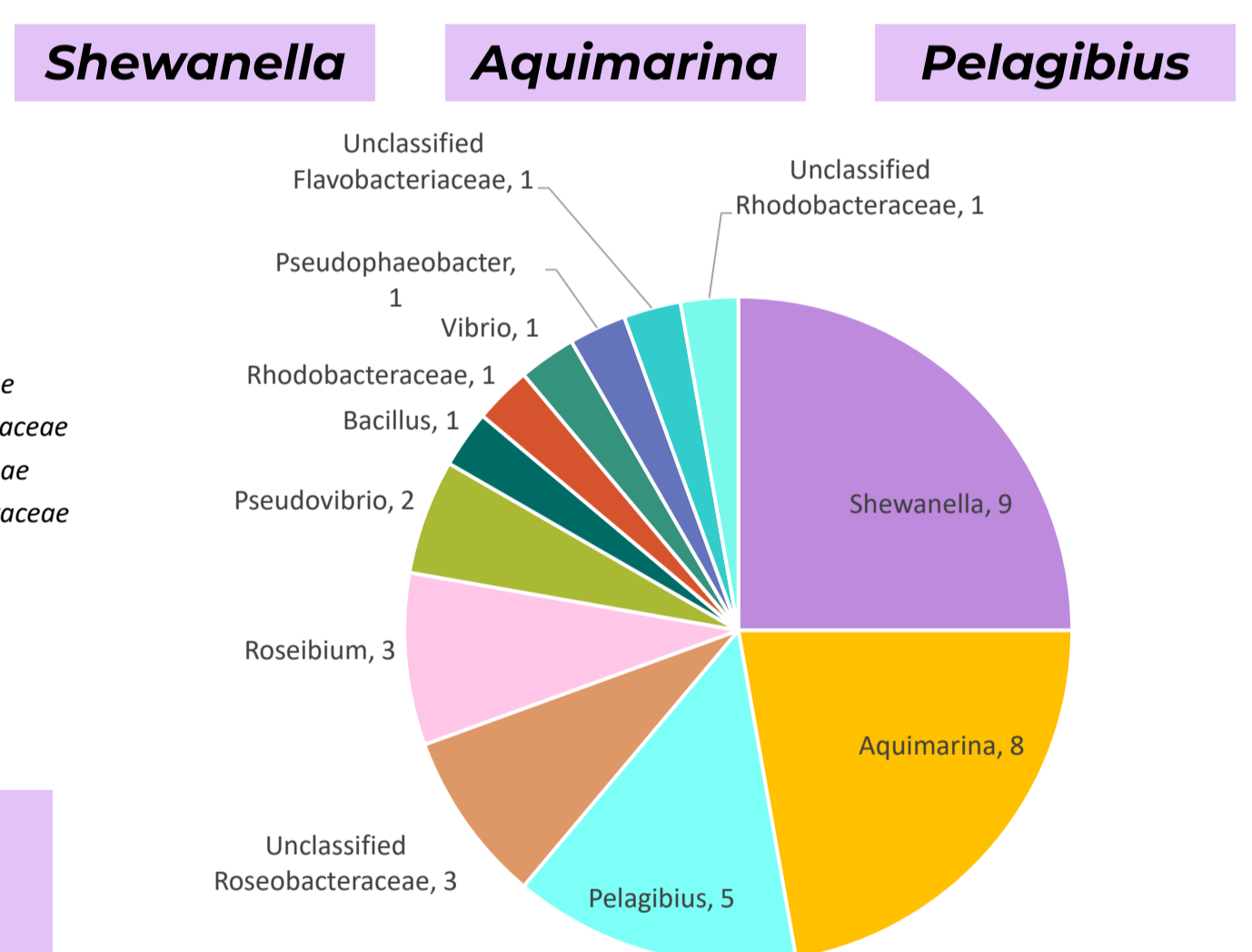
First Results:



Phylogenetic Screening with PKS- and NRPS-specific primers:

36 positive results for the type I PKS gene out of 135 strains screened so far.

Most represented genera carrying type I polyketide synthase encoding genes:



Optimization of chemical extraction protocols and preparation bioactive extracts

Increase bioactivity and unravel new activities through application of the OSMAC principle.

Strains with strong positive results in antagonistic tests will be selected and grown at larger scale in liquid medium.

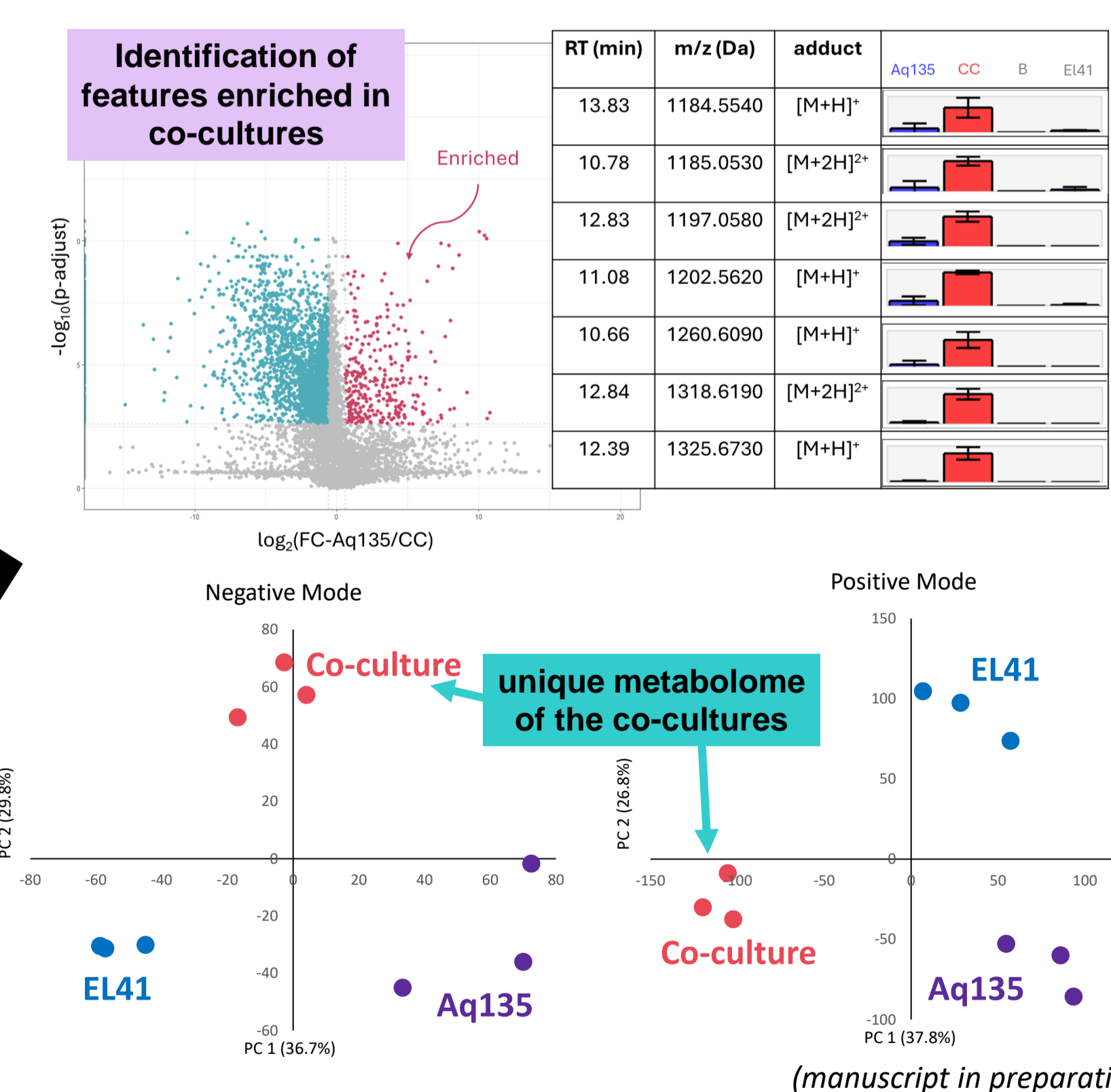
Liquid-Liquid and/or Solid Phase Extraction (SPE) of secondary metabolites, using e.g., HLB (Oasis®) cartridges (broad compound range).

To improve bioactivities, multiple strategies may be employed: **OSMAC principle**

Aquimarina aquimarini Aq135 was selected for co-cultivation with *Vibrio* sp. EL41, a sympatric bacterium, with the aim of enhancing secondary metabolite production.

Development of co-culture protocols:

First Results: Metabolomic Profiling



Metabolomic profiling of the selected strains and identification of metabolites.

Characterization of active extracts by liquid chromatography-mass spectrometry (UPLC-HR-MS, Orbitrap).

Identification of known metabolites → GNPS spectral libraries and Compound Discoverer™ software with integrated metabolic pathway identification capabilities based on multiple spectral libraries and databases.

Molecular network and multivariate analyses will be used to explore relationships between compounds and metabolomic extracts.

Link the metabolomic information to the SM-BGC profiles of the genomes of active isolates.

Conclusion and Outlook

- The *Leptogorgia sarmentosa* culture collection has successfully preserved approximately 400 bacterial isolates, representing a wide range of taxa, including those known for their versatile secondary metabolism, such as the genus *Aquimarina*.
- Phylogenetic screenings have identified several promising bacteria harbouring PKS type I genes, and further studies, including the use of co-cultures, have demonstrated encouraging results in enhancing the bioactivity of a selected strain.
- Future work will reveal the diversity of the cultivated bacteria through comparison with 16S gene amplicon sequencing, and will further explore the bioactive potential of this collection.

References: Couceiro JF, Costa R, Keller-Costa T. Chapter 16 - Beyond Restoration: Coral Microbiome Biotechnology in Coral Reef Microbiome. 1st ed. Springer. Edited by Peixoto, R. and Voolstra, C. (in press) Couceiro JF, Fernandes AM, Keller-Costa T, Costa R. Exploring the potential of co-cultures to enhance secondary metabolites production in the marine bacterium *Aquimarina aquimarini* Aq135. (in preparation)