Environmental drivers shape the fungal holobiont of the cultivated red seaweed *Gracilaria chilensis* revealed by full-length ITS PacBio sequencing

Comunicación Oral

Ecología microbiana y microbioma

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Gracilaria chilensis is the most important cultivated red seaweed in Chile and a key resource for agar production and coastal aquaculture. However, its production has stagnated over recent decades due to increasing disease incidence, epiphytism, and environmental stress, highlighting the need for innovative strategies to improve crop health and resilience. While bacterial communities have received increasing attention, the fungal component of the *Gracilaria* holobiont remains virtually unexplored despite fungi being recognized as important mediators of nutrient cycling, stress tolerance, host protection, and disease in marine ecosystems. Characterizing the seaweed mycobiome is therefore an essential step toward understanding its ecological role and potential applications in sustainable aquaculture. Here, we characterized the mycobiome of *G. chilensis* from natural and aquaculture populations across marine and brackish habitats in southern Chile using PacBio full-length ITS sequencing and assessed its relationship with local physicochemical and nutrient conditions to explore the environmental context underlying fungal community variation.

After quality filtering, denoising, and chimera removal, 3,669 amplicon sequence variants (ASVs) were recovered from 56 samples. Taxonomic assignment against UNITE, complemented by BLAST-based curation, showed a community dominated by Ascomycota and Basidiomycota together with a substantial representation of poorly

resolved fungal lineages, including Rozellomycota-like taxa and Fungi incertae sedis. Fungal community composition differed among marine and brackish habitats and between natural and aquaculture-derived *Gracilaria.* Community variation occurred across marked physicochemical gradients, with temperature, salinity, and pH significantly associated with fungal community ordination. Nutrient conditions also covaried with community structure, particularly phosphate, nitrate, and nitrite.

Our findings reveal that the *G. chilensis* mycobiome is structured across contrasting coastal habitats and cultivation origins, while also uncovering a substantial reservoir of poorly characterized fungal diversity. Distinguishing the fungal communities associated with natural and farmed populations provides an ecological baseline for identifying fungal associations potentially linked to host acclimatization, health, and resilience. This knowledge also establishes a foundation for identifying beneficial fungal partners and exploring their potential contribution to host performance under cultivation. Incorporating this largely overlooked component of the *Gracilaria* holobiont could ultimately support microbiome-informed strategies aimed at improving the sustainability and productivity of *Gracilaria* aquaculture.

Palabras Clave

(1) Holobionte, (2) fungal community, (3) Gracilaria chilensis, (4) full-length ITS PacBio.

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