Characterization and Biotechnological Potential of Marine Bacteria from the Sado Estuary, Portugal

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Introduction & Aim

Marine ecosystems host diverse **bacterial communities** that play a crucial role in **marine ecology** and biogeochemical processes, including organic matter decomposition, nutrient recycling, and bioremediation. The **harsh environment** in marine conditions (e.g., temperature shifts, light variations, osmotic pressure, limited nutrients, and competition) influences and shapes these microorganisms. This fosters **distinctive genetic and phenotypic adaptations** to stress, ultimately giving rise to **novel biosynthetic pathways** for **unique bioactive compounds**.

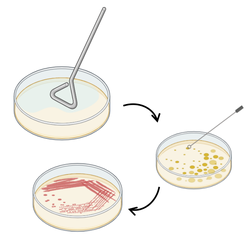
Marine microorganisms also evolved several **mechanisms to interact with each other**, with many bacteria capable of establishing **complex relations with other bacteria and eukaryotes** such as **microalgae**. In mutualistic relations **bacteria can uptake metabolites** from microalgae **phycosphere** while recycling and providing important nutrients such as vitamins, nitrogen, phosphorous, and sulphur. Moreover, marine **bacteria can also produce and secrete info chemicals** such as phytohormones (indole 3- acetic acid), quorum-sensing molecules, among others, which actively **impact microalgae physiology.**

The **unique properties** of beneficial marine bacteria are **key** for the development of **biopharmaceutical and biotechnological applications**, and to boost biotechnological microalgae production and downstream processes.

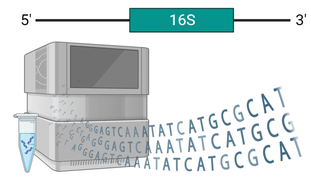
In this work, the **phenotypic and genotypic characterization** of fourteen marine bacteria isolated from seawater collected in diverse locations across the Sado estuary in Portugal is presented. In addition, the isolated bacteria were tested for their ability to **promote the growth and the accumulation of valuable compounds** in the microalgae, ***Phaeodactylum tricornutum .***

Methods

Water collection from several points in the Sado Estuary

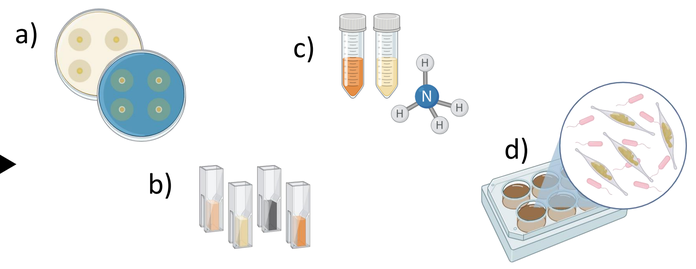


Seawater inoculation and streaking were done until pure colonies were achieved



DNA extraction, identification via 16S rRNA and full genome sequencing

Genome mining for enzymes and compounds with biotechnological potential



Phenotypic screening for: a) extracellular lytic enzyme production; b) indolic compounds biosynthesis; c) ammonia production; d) microalgae growth and pigment enhancement

Results

Genomes highlights

- NFXS35 has a photosynthetic system
- All isolates present the ability to metabolize several vitamins and cofactors
  B1, B2, B5, B6, B7, B12, K2, NAD, CoA, CoQ10, heme, etc…
- NFXS3, NFXS7, NFXS13 and NFXS35 have pathways for carotenoids production
- NFXS9, NFXS11 have a type VI secretion system, often related to interaction with eukaryotic hosts

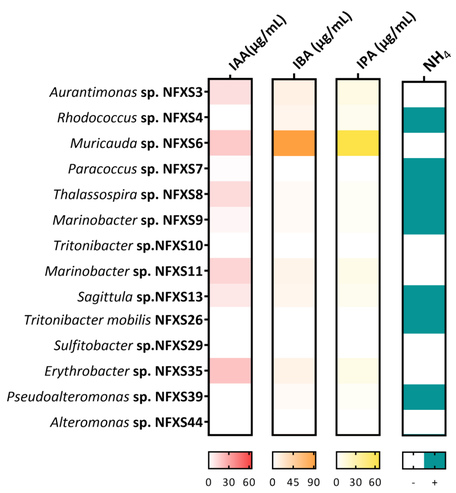
Production of extracellular lytic enzymes in high salinity media

	DEGRADATION HALO DIAMETER IN CM								
	Protein	Lipids		Carbohydrates					
Isolate	Skimmed milk	Tributyrin	Sunflower oil	Chitin	Alginate	Cellulose	Starch	Pectin	Agar-agar
Muricauda sp. NFXS6	1.0	1.7	1.0	1.4	1.4	1.4	0.0	0.0	0.0
Paracoccus sp. NFXS7	1.1	0.7	1.6	0.6	0.0	0.5	0.0	0.6	0.0
Marinobacter sp. NFXS9	2.4	1.8	1.2	2.2	1.7	1.7	1.0	1.9	0.0
Tritonibacter sp. NFXS10	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Marinobacter sp. NFXS11	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0
Sagittula sp. NFXS13	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0	0.0
Tritonibacter mobilis NFXS26	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0
Sulfitobacter sp. NFXS29	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
Erythrobacter sp. NFXS35	0.6	0.0	0.8	0.0	0.7	0.0	0.0	0.0	0.0
Pseudoalteromonas sp. NFXS39	0.4	0.4	2.6	0.2	0.0	0.0	0.0	0.2	0.0
Alteromonas sp. NFXS44	0.0	0.3	2.0	0.0	0.0	0.0	0.0	0.2	0.0

Thirteen isolates were capable of degrading at least one substrate, and none was capable of degrading agar-agar;

Isolate NFXS9 was able to degrade nine of the ten substrates and presented the highest inhibitions halos.

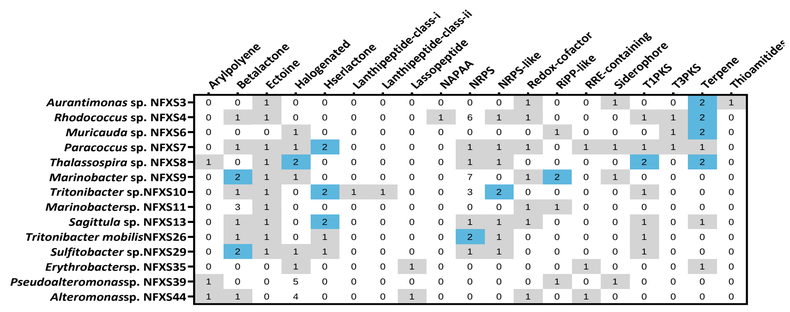
Biosynthesis of indolic compounds and ammonia



Eight isolates were capable of producing at least one of the three indolic compounds analysed (indole-3 acetic acid, IAA; indole-3-butyric acid, IBA; and indole-3-butyric acid, IPA). Being NFXS6 the biggest indolic compounds producers;

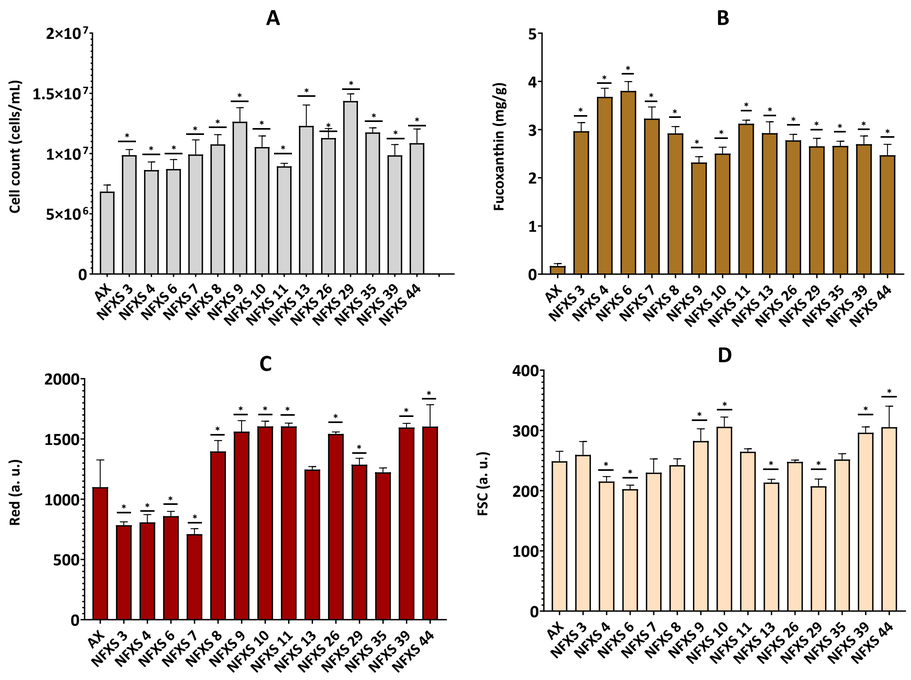
Nine bacterial isolates were capable of producing ammonia.

Secondary metabolite biosynthetic gene clusters



When mining the genome, it was possible to detect nineteen clusters regarding the biosynthesis of secondary compounds, namely siderophores that can be associated with microalgae interactions.

Phaeodactylum tricornutum co-cultures



All the bacterial isolates showed a notable capacity to increase *P. tricornutum* cell count (**A**) and fucoxanthin production (**B**);

Several bacteria were able to modify *P. tricornutum* chlorophyll production, hereby quantified via RED autofluorescence **(C)**;

Regarding cell size **(D**) variable effects were observed. Four isolates increased cell size (NFXS9, NFXS10, NFXS39 and NFX44), while four lowered (NFXS4, NFXS6, NFXS13 and NFXS29). The remaining isolates did not induce any significant effect on *P. tricornutum* cell size.

Conclusions

This study provides a **deep analysis of Portuguese marine bacteria** isolates and their **biotechnological potential**.

Bacterial genomic analysis revealed the presence of **unique genes** involved in the production of secondary metabolites of **biotechnological potential**.

The bacterial isolates **produced functional and stable extracellular lytic enzymes** under high salt conditions, interesting for application in **downstream processes** in **marine industries**.

Several genes related to the biosynthesis of **microalgae growth-promoting** compounds were found in bacterial genomes

The bacterial isolates **promoted *P. tricornutum* growth** and may be used to **boos**t *P. tricornutum*-based **biotechnological processes** such as industrial **fucoxanthin production**.

Acknowledgements

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