

Selection and optimization of autochthonous bacterial strains isolated from Port of Leixões for bioremediation of crude oil and maritime fuels

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Introduction

Oil spills?

- ✓ Catastrophic events
- ✓ May occur during exploration, production, refining, transport and storage of petroleum
- ✓ Require immediate, simple effective and eco-friendly actions



Bioremediation with autochthonous microorganisms can be an advantageous solution

Aim



Selection and identification of **autochthonous hydrocarbon-degrading** bacteria from seawater of Port of Leixões



Test the efficiency of some strains to **degrade petroleum hydrocarbons** and other **maritime fuels**



Growth **optimization** tests with different carbon sources, alternative to hydrocarbons

Material and methods

Selection of hydrocarbon degrading bacteria

- ✓ 145 bacterial strains isolated from seawater collected from the Port of Leixões
- ✓ Enriched with:

Crude oil (CO)

Turbine oil (TO)

Diesel oil (DO)

- ✓ Cultivated in 3 culture media: M1, MA and BH

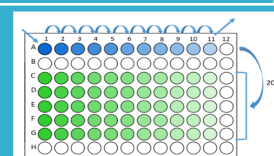
Bacterial identification

- ✓ DNA extraction
- ✓ 16S rRNA gene amplification
- ✓ Phylogenetic identification
- ✓ 6 strains of *Pseudomonas marincola* species collected from seawater enriched with 3 oils and grown in 3 media

	Oil	Media
BP5	Crude oil	MA
BP24	Crude oil	BH
BP45	Crude oil	M1
BTO19	Turbine oil	BH
BTO22	Turbine oil	BH
BDO24	Diesel oil	M1

Abundance of hydrocarbon-degraders by the Most Probable Number method (MPN)

- ✓ 15 days incubation
- ✓ 3 oils
- ✓ Initial OD (600 nm) of 0,1.



Growth screening tests

- ✓ Tested in 96 well-plates
- ✓ Four different carbon sources (10g/L) in minimum media

Sodium acetate

Glycerol

Glucose

Peptone

- ✓ Initial OD (600 nm) of 0,05.

Results

Bacterial identification

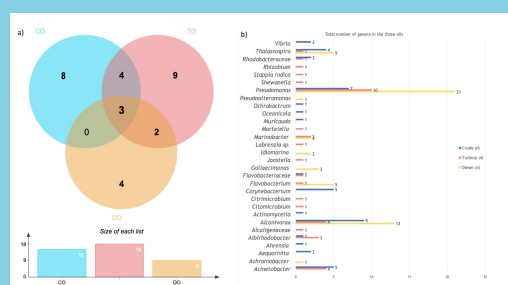


Fig. 1. a) Venn diagram representing the common genera collected from seawater enriched with the three oils; b) histogram representing the total number of genera collected from seawater enriched with the three oils.

- ✓ The genera in common between the 3 oils enrichments: *Alcanivorax*, *Pseudomonas* and *Thalassospira*.
- ✓ The most numerous strains found in the three oils enrichments were *Pseudomonas* (7 in CO, 12 in TO, 21 in DO).

Hydrocarbon degrading potential of the *Pseudomonas marincola* strains

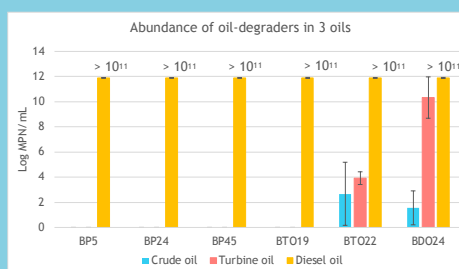


Fig. 2. Abundance of hydrocarbon-degrading bacteria, for the *Pseudomonas marincola* strains, collected from seawater enriched with three oils, by the MPN method.

- ✓ All the bacterial strain showed high potential to degrade diesel oil ($>10^{11}$ MPN/mL), while BTO22 and BDO24 showed same ability also on crude oil (10^4 , 10^2 MPN/mL) and turbine oil (10^4 , 10^{11} MPN/mL).

Growth screening test of the *Pseudomonas marincola* strains

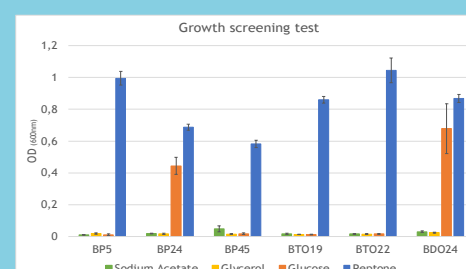


Fig. 3. OD (600nm) of the *Pseudomonas marincola* strains after 48 hours growth with sodium acetate, glycerol, glucose and peptone, in 96 well-plates.

- ✓ Strains showed ability to grow on peptone, with optical density higher than 0.6. Only BP24 and BDO24 grew also on glucose (with an OD respectively of 0.4 and 0.7).

Conclusions

- ✓ Known oil degrading bacterial genera were identified in the water collected from the Port of Leixões after an enrichment with crude oil, turbine oil and diesel oil. *Alcanivorax*, *Pseudomonas* and *Thalassospira* were common between the three oil enrichments.
- ✓ The bacterial strains belonging to the species *Pseudomonas marincola*, collected after the three enrichment, showed higher potential to degrade diesel oil and preferred peptone as sole carbon source, alternative to hydrocarbons.
- ✓ BTO22 and BDO24 were the most promising oil degrading strains in this study, and they might be good candidate for future application as a bioremediation product in spills of diesel and turbine oil.