

Hydrocarbonoclastic microorganisms associated with photobioreactors-grown microalgae *Pavlova lutheri* and *Nannochloropsis oculata*

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Background

Marine photosynthetic microalgae produce oleophilic compounds, such as alkanes, long-chain fatty acids, and alcohols and were reported to host naturally occurring hydrocarbon-degrading specialist bacteria [1-3]. However, little is known about the taxa of microalgae that can host such bacteria, composition of microalgae-associated microbial consortia and the responses of these consortia to the petroleum.

Results

This study assessed structures of microbial communities, particularly those with hydrocarbon degradation abilities for both aliphatic and aromatic hydrocarbons associated with two photobioreactors-grown cultures of ubiquitous microalgae, *Pavlova lutheri* and *Nannochloropsis oculata* (Fig.1), using culture-independent (barcoded SSU rRNA amplicon sequencing Illumina MiSeq platform) and cultivation approaches.

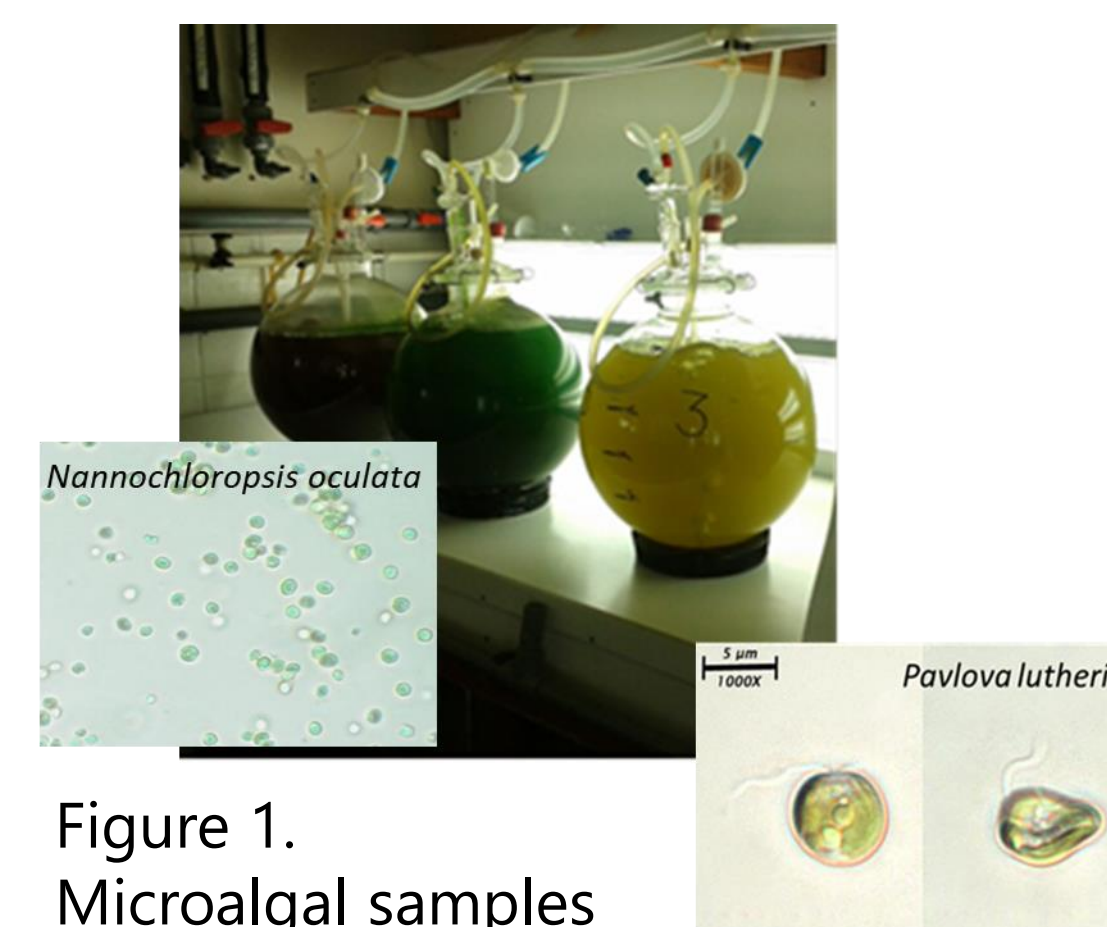


Figure 1. Microalgal samples

Barcoded SSU rRNA amplicon sequencing (Illumina MiSeq platform)

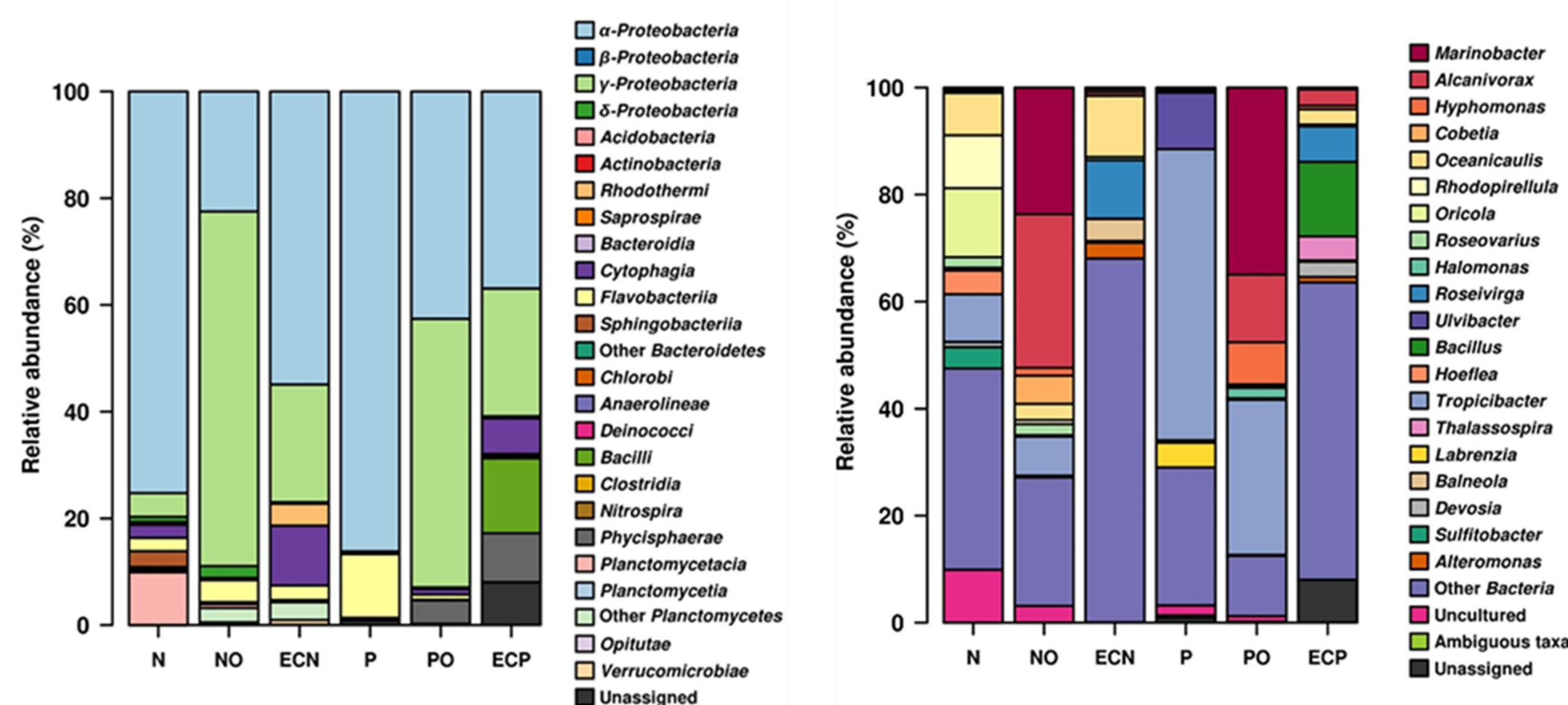


Figure 2. Bacterial community composition and structure at class-(A) and genus-levels (B). N, *N. oculata*; NO, enrichment of *N. oculata* with crude oil; ECN, control of *N. oculata* culture without crude oil; P, the culture of *P. lutheri*; PO, enrichment of *P. lutheri* culture with crude oil; ECP, control of *P. lutheri* culture without crude oil.

The addition of crude oil resulted in stark changes in both microalgal cultures. More than 25% of the total reads in crude oil enrichments were derived from members of genera *Alcanivorax* and *Marinobacter*, which were detected in less than 0.5 % reads in bioreactor communities (Fig.2).

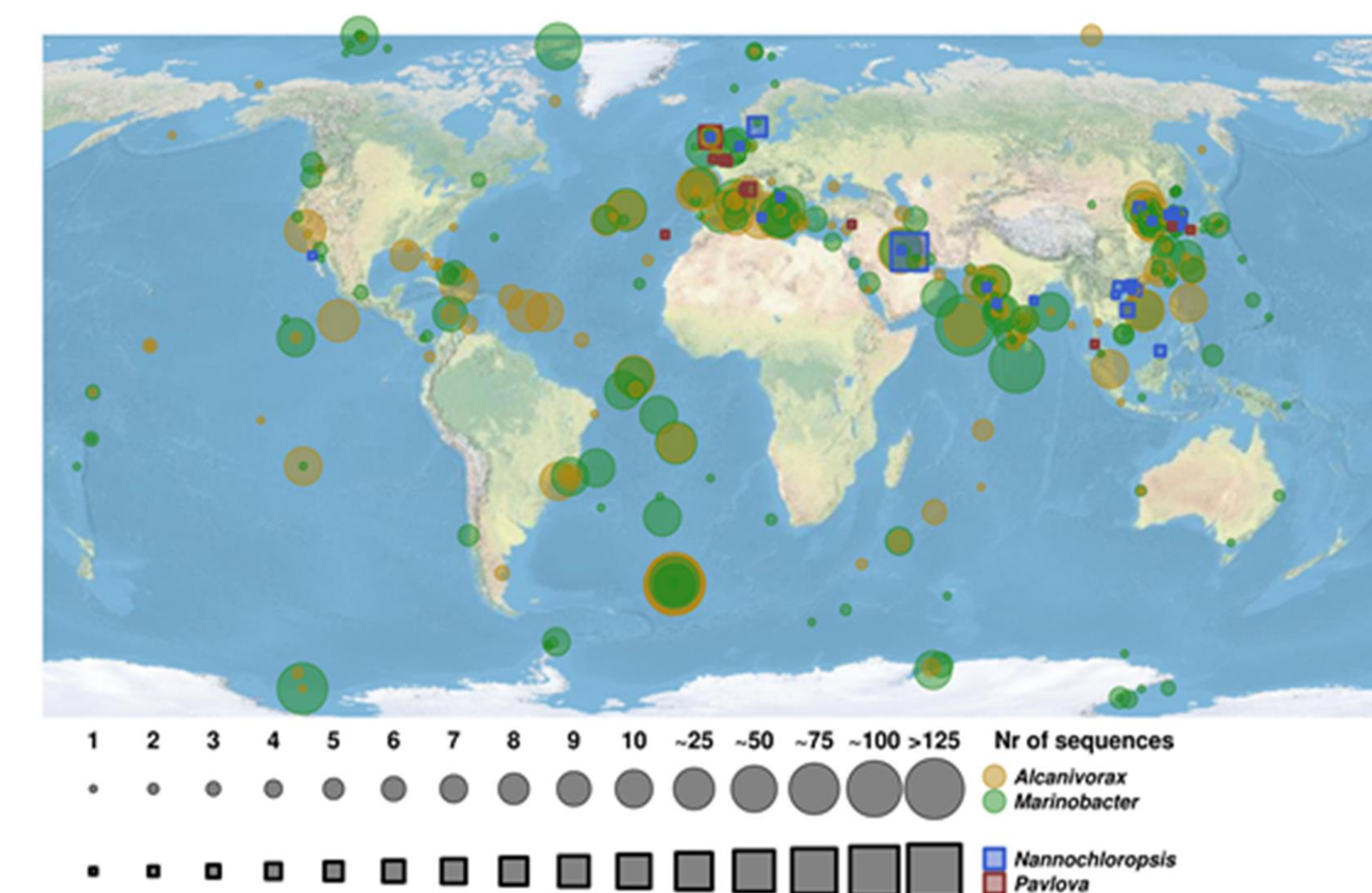


Figure 3. A map of distribution areas of *Nannochloropsis* (blue)/ *Pavlova* (red) species and *Alcanivorax* (yellow)/*Marinobacter* (green) species.

Geographic distribution of hydrocarbonoclastic bacteria and microalgal hosts

Analysis of geographical locations of hydrocarbon-degrading bacterial strains and microalgal species demonstrated that *Alcanivorax* and *Marinobacter* species co-occurred and/or matched with microalgae *P. lutheri* and *N. oculata* isolation sites in most cases (Fig.3).

Diversity of microalgae-associated hydrocarbon-degrading bacterial isolates

Using a cultivation approach, 48 bacterial non-redundant strains were isolated and identified to belong to the genera *Alcanivorax*, *Marinobacter*, *Thalassospira*, *Hyphomonas*, *Halomonas*, *Marinovum*, *Roseovarius*, *Oleibacter* (Fig.4).

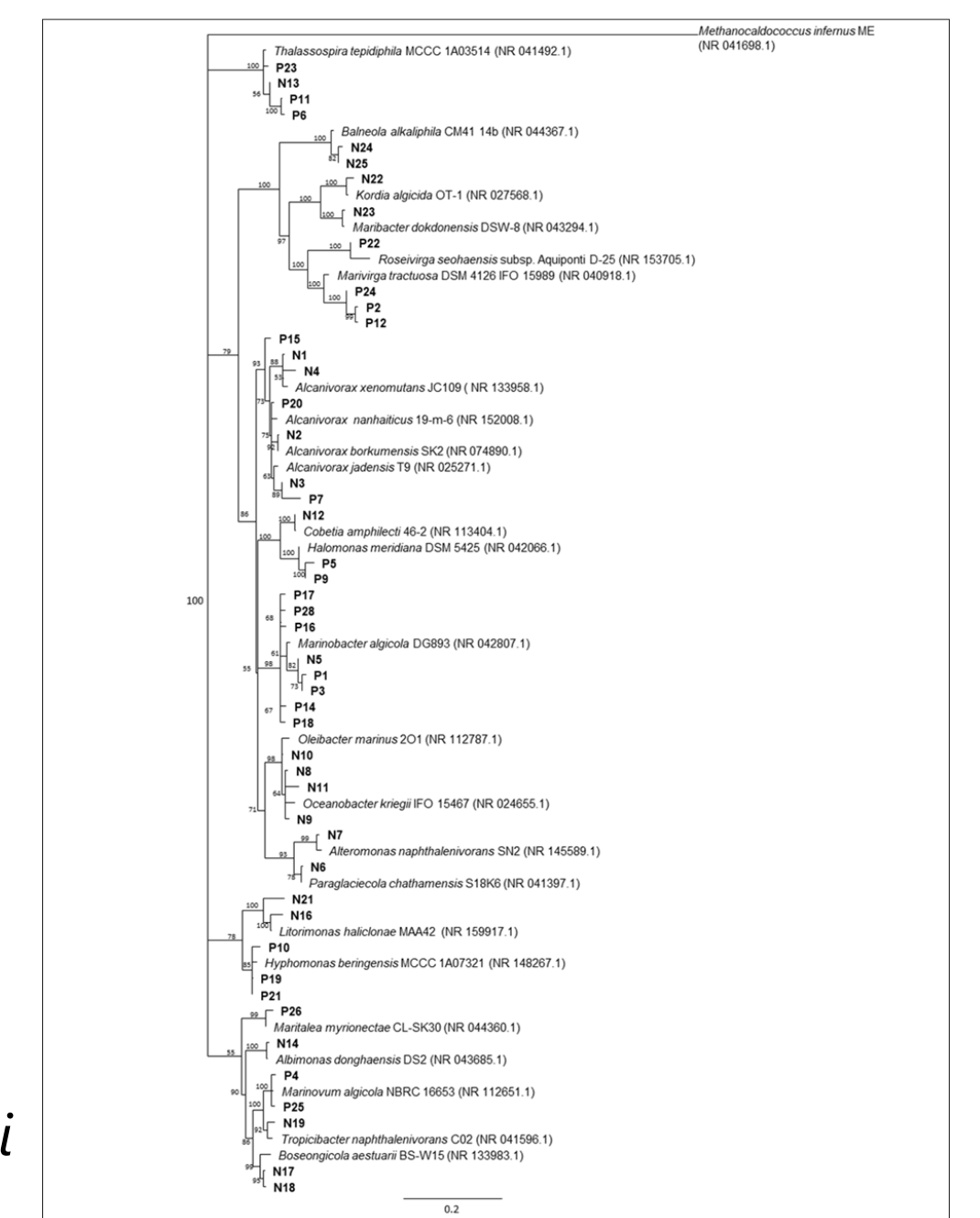


Figure 4. The bacterial strains isolated from enrichment cultures of *N. oculata* and *P. lutheri*

Conclusion

Microalgal species, *Pavlova lutheri* and *Nannochloropsis oculata*, hosted diverse microbial communities. As a response to the crude oil supplementation, there was a strong selection towards 'classical' hydrocarbon-degrading bacteria of genera *Alcanivorax* and *Marinobacter*, previously commonly attributed to technogenic oil spills and natural seeps. The results of this study provided the strong experimental evidence for linking microalgae *Pavlova lutheri* and *Nannochloropsis oculata* with hydrocarbon-degrading specialist bacteria, such as *Alcanivorax* and *Marinobacter*, the renowned hydrocarbonoclastic bacteria, explained their ubiquity in marine environments and showed that microalgae *Pavlova lutheri* and *Nannochloropsis oculata* represent natural sources of diverse groups of hydrocarbon-degrading bacteria.

References

- Thompson et al., 2017. Environ Microbiol 19 (7), 2843-2861
- Green et al., 2004. FEMS Microbiol Ecol. 47(3), 345-357
- Seymour et al., 2017. Nat Microbiol .2, 17065

Acknowledgments

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