

MICROSCOPIC EUKARYOTES ARE ALIVE AND WELL IN MARINE OIL-POLLUTED ENVIRONMENTS

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Abstract

The dynamics of eukaryotic groups were studied in experimental oil-polluted marine microcosms (EC- COMMODE-VK-CT2002-00077). PCR amplification and sequencing of 18S rRNA gene combined with microscopic counts revealed that ubiquitous microscopic eukaryotes such as *Paraphysomonas foraminifera* were effectively grazing of hydrocarbonoclastic bacterial cells.

Keywords : Biodiversity, Food Webs, Plankton, Petroleum, Pollution.

The advent of molecular approaches brought a wealth of knowledge in the study of microbial diversity [1] and inoculated this field with more novel methodologies. The lack of eukaryote-focused studies in oil-pollution investigations is even more apparent, in contrast with the prokaryotes [4]. In order to investigate the dynamics of the dominant eukaryotic groups, we applied PCR amplification and sequencing of 18S rRNA genes in oil-polluted microcosms along with conventional cell counts (epifluorescence microscopy) in the framework of the EC-COMMODE project which aimed to increase our understanding of the dynamics of natural and anthropogenic remediation of crude oil-polluted systems. The 100 l capacity microcosms contained seawater from chronically polluted (P) and non-polluted (NP) oligotrophic sites of the Aegean Sea implemented with crude oil, crude oil + nutrients and crude oil + emulsifier. The analysis also included the relevant blank treatments (i.e. no oil or nutrients added). The microcosms were incubated at 21 °C for 10 days and sampled daily. In all cases, most of the heterotrophic flagellates (HNF) population (>90%) was killed after the addition of crude oil. A few resistant/opportunistic species exponentially grew after 96 hours on average, decimating the elevated bacterial concentrations. At the peak of the HNF abundance, DNA was extracted from 1-2 l of water and was used for the construction of 18S rRNA gene libraries. For each treatment 20-52 non-chimeric phylotypes were analyzed. GenBank numbers of the retrieved sequences are AY789780-AY789790 and DQ781322-DQ781336.

suggesting that this species was selected against others after crude oil addition by being able to graze the hydrocarbonoclastic bacteria. Indeed, the *P. foraminifera* abundance peaked one or two days after the peak of the bacterial cells. This ubiquitous phagotrophic genus has a widespread distribution in the world oceans and reproduce rapidly in presence of photosynthetic, heterotrophic bacteria and nanoflagellates, which they decimate, suggest that they are voracious feeders on prey almost as big as themselves, ca. 3.5 x 2.5 mm [2,3]. Other dominant phylotypes in the crude oil NP and P microcosms were the commonly occurring bacterivores *Pseudobodo tremulans* and the choanoflagellid *Monosiga brevicolis*, and a few novel phylotypes. Regarding bioremediation, our current findings suggest the occurrence of a microbial food web that includes intense bacterial grazing by protists and this aspect should be taken into account in the fate and activity of hydrocarbonoclastic bacteria in oil polluted marine systems.

References

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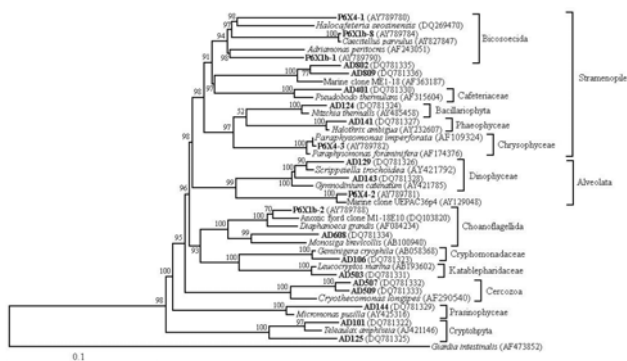


Fig. 1. NJ phylogenetic tree constructed with 1000 bootstrap replicates of 18S rRNA genes retrieved from oil-polluted marine microcosms (in bold).

Redundancy analysis and phylotype coverage was performed according to [5] by using the S_{Chao} and S_{ACE} indices. The retrieved phylotypes belonged to a wide range of taxa of Stramenopile, Alveolata, Choanoflagellida, Cryptomonadaceae, Katablepharidaceae, Cercozoa, Prasinophyceae and Cryptophyta (Fig. 1). The diversity of both N and P treatments was always higher to those containing crude oil ones. This was also indicated by the low phylotype coverage (21.6 -41.1%) in the former cases. The opposite occurred in the crude oil NP and P treatments, irrespective of the presence of nutrients or emulsifiers, where coverage reached 89.9% due to the dominance of certain phylotypes. A phylotype with 99% similarity to *Paraphysomonas foraminifera* dominated in all crude oil NP and P treatments. This phylotype appeared in the blank treatments as well,