

# BACTERIAL COMMUNITY COMPOSITION OF FREE-LIVING VERSUS ATTACHED BACTERIOPLANKTON IN THE WATER COLUMN OF THE EASTERN MEDITERRANEAN SEA: EVIDENCE OF MAJOR DIFFERENCES IN SUBSTRATE UTILIZATION BETWEEN SURFACE AND DEEP WATER BACTERIA

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## Abstract

The bacterioplankton species composition of the water column of 2 stations in the Southern and Northern Aegean Sea, respectively, was determined by terminal restriction fragment length polymorphism (T-RFLP). Generally, the number of operational taxonomic units (OTUs) remained remarkably constant throughout the water column down to 1000 m depth for both the free-living and the attached bacterial community. Only a small fraction of the OTUs detected in the free-living community was also present in the attached bacterial community. Distinct surface bound as well as specific deep water OTUs were present in both the free-living and the particle-attached bacteria.

**Keywords:** Bacteria, community composition, Mediterranean Sea, molecular analysis

Knowledge on the composition of the bacterioplankton is still in its infancy due to the lack of distinct morphological characteristics of bacterioplankton and the limitations to culture them (1). It is now well recognized that only a small fraction (usually well below 5 % of the bacterial community) can be brought in culture. Thus other methods are required to obtain information on the community composition of bacterioplankton. Molecular techniques provide the resolution required to obtain insights into the dynamics of bacterial consortia (2, 3). Terminal restriction fragment length polymorphism (T-RFLP) has been used in this study to unravel the dynamics of the community composition in the water column of 4 selected stations in the eastern Mediterranean Sea (3). Distinction has been made between the composition of the free-living versus particle-attached bacterial community in order to address the following questions: 1) are there any distinct compositional differences between the free-living and attached bacterial community? 2) Does the composition of community change with depth and if so, 3) does the number of species decline in a similar way as does the abundance of bacteria? If there is a distinct shift in the bacterioplankton community with depth what is then the potential substrate for these bacteria?

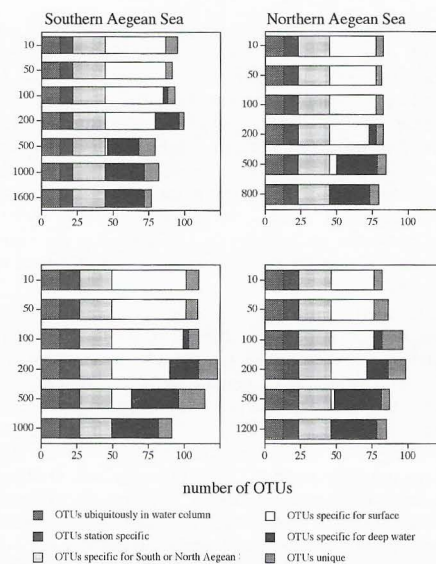
Raw seawater (RSW) was collected at 4 stations in the eastern Mediterranean Sea (24-26°E, 36-40°N) during a cruise on R/V Aegaeo in March 1998. Several depth layers were sampled with 10 liter-Niskin bottles attached to a Sea-Bird CTD profiler. Fifty ml subsamples of RSW were fixed with 0.2 µm-filtered formaldehyde (2 % v/v final concentration) and stored at 4°C for later bacterial enumeration. For molecular characterization of the bacterial communities, 3-5 liter of RSW was filtered through Whatman GF/C filter to collect 'particle-attached' bacteria. These filters were stored in sterile tubes (Greiner). The fraction of the bacterial community passing the Whatman GF/C filters was considered as the 'free-living' bacteria. These free-living bacteria were collected onto 0.2 µm Sterivex cartridges (Millipore). All filters for molecular characterization of the attached and the free-living bacteria were stored at -80°C. Nucleic acid extraction, purification and cDNA synthesis and subsequent PCR and T-RFLP analyses have been performed as described elsewhere (4).

Generally, attached and free-living operational taxonomic units (OTUs) differed considerably throughout the water column, with only ≈ 35 % for the South Aegean and ≈ 24 % for the North Aegean of all OTUs in both free-living and attached OTUs. Fig. 1 shows the distribution pattern of the (OTUs) of free-living bacteria. Three distribution categories (i.e. ubiquitously occurring, station specific and specific for either the South or the North Aegean Sea) comprised ≈ 50 % of the total number of OTUs of the free-living bacteria found in the different depth layers (Fig. 1). In contrast to the attached bacteria (Fig. 2), no increasing contribution of these three distribution categories towards greater depth was detectable when compared to the total number of OTUs. The number and the percentage of station specific OTUs was significantly lower in the free-living than in the attached bacteria (Wilcoxon, P=0.028, n=6) while no general trend was detectable in the number and percentage of OTUs specific for either the South or the North Aegean Sea. A higher number and percentage of unique OTUs was found in the free-living bacteria and a more complex deep water community than in attached bacteria. The distinct deep water community present, particularly in the free-living mode, might indicate that these bacteria are specifically adapted to the specific nutrient regime present there. The composition of the free-living deep water bacterial community appears to be as complex as the surface water bacterial community.

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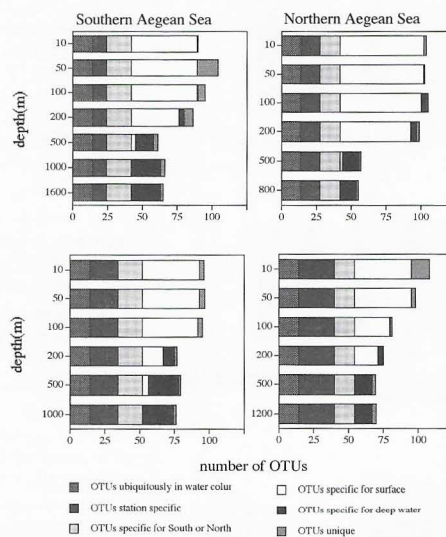
## References

- 1 - Giovannoni S.J., Britschgi T.B., Moyer C.L. and Field K.G., 1990. Genetic diversity in Sargasso Sea bacterioplankton. *Nature*, 345: 60-63.
- 2 - Acinas S.G., Anton J. and Rodriguez-Valera F., 1999. Diversity of free-living and attached bacteria in offshore western Mediterranean waters as depicted by analysis of genes encoding 16S rRNA. *Appl. Environ. Microbiol.*, 65: 514-522.
- 3 - Moeseneder, M.M., Arrieta J.M., Muyzer G. Winter C. and Herndl G.J., 1999. Optimization of terminal-restriction fragment length polymorphism analysis for complex marine bacterioplankton communities and comparison with denaturing gradient gel electrophoresis. *Appl. Environ. Microbiol.*, 65: 3518-3525.



**Fig. 1.** Analysis of the spatial distribution of OTUs for free-living bacteria using 16S rDNA and T-RFLP at 2 stations (upper and lower panel) in the southern and northern Aegean Sea.

The number of OTUs obtained from the 16S rDNA is grouped in 6 different categories of occurrence: OTUs found at every station at every depth (ubiquitously occurring); OTUs found only at a single station but throughout the water column (station specific); OTUs found at either in the South or North Aegean Sea but throughout the water column (Specific for South or North Aegean Sea); surface specific OTUs found only at one station; deep water specific OTUs found only at one station; OTUs unique for a specific depth layer.



**Fig. 2.** Analysis of the spatial distribution of OTUs for attached bacteria using 16S rDNA and T-RFLP at 2 stations (upper and lower panel) in the southern and northern Aegean Sea. Grouping into categories of occurrence was performed as described in Fig. 1.

4 - Moeseneder, M.M., Winter C. and Herndl G.J., 2001. Horizontal and vertical complexity of attached and free-living Bacteria of the eastern Mediterranean Sea determined by 16S rDNA and 16S rRNA fingerprints. *Limnol. Oceanogr.*, 46: 95-107