

# PATTERNS OF BACTERIOPLANKTON DIVERSITY IN RECREATIONAL MARINAS IN THE ISLAND OF MALLORCA (BALEARIC ISLANDS) AND ITS RELATION TO HYDROCARBON POLLUTION

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## Abstract

The analysis of bacterioplankton in surface seawater from different recreational marinas evidenced common patterns in the diversity of some bacterial groups, i.e. *Alphaproteobacteria*. In particular, certain phylotypes of the *Roseobacter* clade predominated in these environments. Although this clade seemed to be stimulated by experimental diesel-oil pollution, their apparent success in polluted environments, such as marinas, is most likely due to a combination of factors and not only to hydrocarbon pollution.

**Keywords:** *Bacteria, Balear Islands, Biodiversity, Pollution, Coastal Waters*

Studies on bacterioplankton diversity in man-made, artificial habitats, such as ports and marinas are scarce (1-3). Besides, we do not know which are the key factors determining changes in bacterioplankton diversity in areas adjacent to ports (2), nor the importance that these changes might have in the overall functionality of the coastal ecosystem. The increasing pressure of nautical tourism justifies the importance of improving our knowledge about the microbiota of these environments. Preliminary studies done by our group in Mallorca (Balearic Islands) indicated that there might be common patterns in the bacterioplankton diversity in recreational marinas. Therefore, our first aim was to demonstrate that such patterns in diversity really existed. We also hypothesised that at least some of the components of the bacterioplankton in marinas were selected by the stress caused by hydrocarbon pollution and we made two laboratory experiments to address this issue.

Surface water samples from seven marinas around the Island of Mallorca were taken from the shore throughout a year period (2008-2009). For molecular characterisation of diversity, DNA was extracted from 0.22 µm filters through which 2 litres of pre-filtered water (5 µm) was filtered. Bacterial diversity studies were based on terminal restriction fragment length polymorphism analysis (T-RFLP) of 16S rRNA genes with primers for *Bacteria* and for *Alphaproteobacteria*, and by the generation of clone libraries as described previously (2-3). For the experimental pollution experiments, surface water samples from a pristine coastal location (39°29'05"N, 2°28'16"E) were taken at two time points, late winter and summer 2007. Each time, four laboratory microcosms were prepared (45 l of water): two controls and two treated with low concentrations (30 mg l<sup>-1</sup>) of diesel oil. Diversity analysis was based in the amplification of ribosomal RNA by reverse transcription and PCR (RT-PCR). Changes in diversity were followed for a period of 89 h.

Our study in surface water from different recreational marinas confirmed that there were common trends in the composition of bacterial communities. Fig. 1 shows a non-metric multidimensional plot representing the similarity of T-RFLP profiles for *Alphaproteobacteria* obtained for the different marinas. Different marinas harboured similar alphaproteobacterial populations.

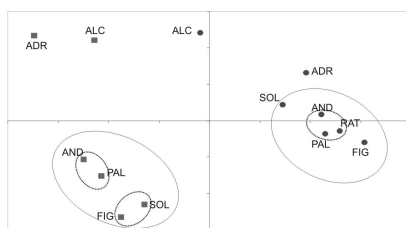


Fig. 1. NMDS plot of the similarities of T-RFLP profiles for 16S rDNA of *Alphaproteobacteria* from different recreational marinas in Mallorca Island. Marinas are identified by a three-letter code. Sampling data: September 2008, squares; March 2009, circles. Solid lines encircle profiles with similarities >70% (Bray-Curtis coefficient) and dashed lines those with similarities >80%. Stress value, 0.108

We also observed a temporal dynamics in the diversity of these populations; samples from different times separated well in relation to the vertical axis, and the dynamics were the same in different marinas. There were phylotypes, (defined at 99% sequence similarity cut-off), particularly in the *Roseobacter* clade of the *Alphaproteobacteria*, which predominated in all the marinas. In the pristine waters used for microcosm experiments the *Roseobacter* clade

constituted a minor component of the bacterioplankton. However, this group increased in abundance in response to diesel addition, and contributed, among others, to the rapid changes (within hours) in bacterial composition observed after diesel addition (Fig. 2), particularly in summer. Although we have evidences that some isolates of this group can grow on hydrocarbons we think that their apparent success as main components of the bacterioplankton of recreational marinas is the result of a combination of factors, and not only to hydrocarbon pollution.

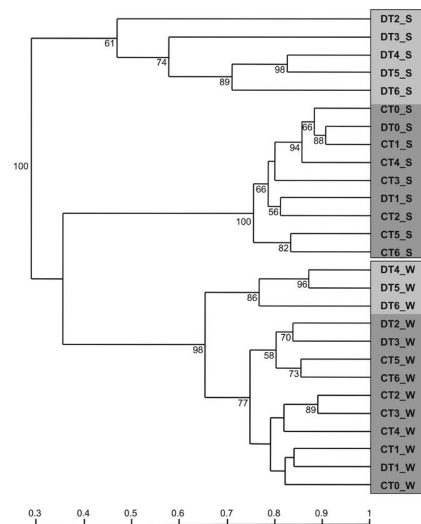


Fig. 2. UPGMA dendrogram of the T-RFLP profiles for metabolically-active bacterioplankton (16S rRNA) in control (label C) and diesel-treated microcosms (label D). Winter samples, W; summer samples, S. Sampling times are: T0, 0h; T1, 3h; T2, 17h; T3, 27h; T4, 41h; T5, 65h and T6, 89h. Numbers next to the nodes correspond to Bootstrap values (1000 replicates) >50%. Scale bar represents similarity (Bray-Curtis coefficient)

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