COMPARISON OF MICROBIAL COMMUNITY COMPOSITION FROM DIFFERENT SEDIMENTS OF THE EASTERN MEDITERRANEAN SEA USING T-RFLP, DGGE AND PLFA ANALYSIS

Paraskevi N. Polymenakou [1, 3 *], Stefan Bertilsson [2], Anastasios Tselepides [1], Euripides G. Stephanou [3]

[1] Institute of Marine Biology of Crete, Old American Base of Gournes, Heraklion Crete, Greece - * polymen@imbc.gr; ttse@imbc.gr
[2] Department of Limnology, Evolutionary Biology Center, Uppsala University, Uppsala, Sweden - stebe@ebc.uu.se
[3] ECPL, Chemistry Department, University of Crete, Heraklion Crete, Greece - stephanou@chemistry.uoc.gr

Abstract

The distribution of complex marine bacterial communities in different sediments of the Eastern Mediterranean Sea was investigated by denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP) and phospholipid-linked fatty acid (PLFA) fingerprinting methods. Dendrograms derived from DGGE, T-RFLP and PLFA profiles were significantly different. The DGGE derived dendrogram groups the North (Thermaikos Gulf) and South (Cretan Sea, Ionian Sea, Levantine Sea) sampling sites, whereas T-RFLP analysis indicates differences between shallow and deep sea sediments. However, PLFA analysis placed the shallow Therm30 station from the North within the deep Southern cluster of stations.

Keywords: DGGE, T-RFLP, PLFA, Eastern Mediterranean Sea, sediments

The eastern Mediterranean Basin is considered to be one of the most oligotrophic regions in the world with an overall nutrient deficit (1). The areas under investigation (Thermaikos Gulf, Cretan, South Ionian and Levantine Sea) are characterized by persistent geomorphological, hydrographic and meteorological features (1-3), which affect the distribution of nutrients and organic matter production in the euphotic zone and eventually its propagation to the ocean floor to fuel benthic communities (4).

Within the benthic community, bacteria are significant due to their key role in regulating the biogeochemical cycles of the major organic elements (carbon, nitrogen, oxygen and sulfur). The role of sediments in regulating microbial community composition is poorly known. Hence, sediment samples from a variety of environments (Thermaikos Gulf, Cretan Sea, Levantine Sea and the deep South Ionian Sea) were chosen for the analysis of microbial communities by performing three different culture independent fingerprinting techniques (i.e. the DGGE, T-RFLP based on 16S rRNA amplification and PLFA analyses).

The dendrogram derived from the DGGE analysis displayed two distinct clusters (Fig. 1a). The first cluster contained all the samples from Thermaikos Gulf and was grouped far from the other cluster, indicating that the largest shift in community composition occurred with sampling location. High similarity was obtained between the Thermaikos Gulf stations (77.78 – 95.24 % similarity) indicating that this region contained very similar bacterial populations which differ significantly from the sampling sites in the South. These stations were found internally similar (40 – 85.41 % similarity).

However, the T-RFLP fingerprinting technique produced a different set of clusters compared to the DGGE method (Fig. 1b). The first cluster contained all the shallow stations from Thermaikos Gulf and Cretan Sea. The samples from Thermaikos Gulf show high similarity (52.63 – 68.42 %); however, station Therm30 is clustered together with station Creta-2 indicating the similarity of bacterial composition between these geographically distinct sampling sites.

The total PLFA content in the sediments averaged 95.70 – 2293.93 ng/g dry weight of sediment and it is almost three times lower compared to other environments probably due to the low organic carbon content (5, 6). The highest similarity value was recorded from Thermaikos Gulf (94.84% similarity between stations Therm27 and Therm38) and the lowest between the South Ionian Sea and Thermaikos Gulf (30.98% similarity between stations S.Ionian-B and Therm17).

DGGE and T-RFLP analyses demonstrated that the community structure changed with sampling location and depth, respectively. DGGE profiles were simpler than those of T-RFLP, indicating the slightly higher resolution of the T-RFLP fingerprinting technique. However, PLFA analysis shows a completely different profile. The complexity of the bottom geomorphology and the local hydrological features of the different sampling sites, can explain the structural shifts of the microbial community. Therefore, depending on the questions posed and the complexity of the environment under investigation, a number of different fingerprinting techniques must be applied to assess the whole or viable bacterial community composition.

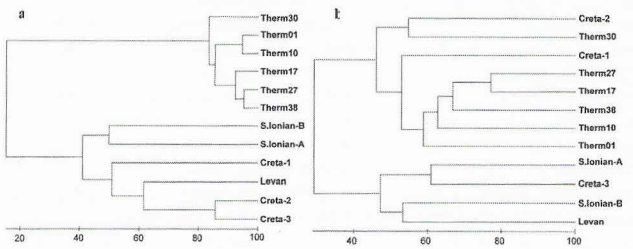

Fig. 1. Cluster analysis dendrograms from the different surface sediment samples taken in the Eastern Mediterranean Sea based on comparison of a) DGGE patterns b) T-RFLP patterns.

References

1 - Tselepides A., Zervakis V., Polychronaki T., Danovaro R., and Chronis G., 2000a. Distribution of nutrients and particulate organic matter in relation to the prevailing hydrographic features of the Cretan Sea (NE Mediterranean). *Prog. Oceanogr.*, 46: 113-142.

2 - Lascaratos A., Roether W., Nittis K., and Klein B., 1999. Recent changes in deep water formation and spreading in the eastern Mediterranean Sea: a review. *Prog. Oceanogr.*, 44: 5-36.

3 - Balopoulos E.Th., Theocharis A., Kontoyiannis H., Varnavas S., Voutsinou-Taliadouri F., Iona A., Souvermezoglou A., Ignatiades L., Gotsis-Skretas O., Pavlidou A., 1999. Major advances in the oceanography of the southern Aegean Sea-Cretan Straits system (Eastern Mediterranean). *Prog. Oceanogr.*, 44: 109-130.

4 - Tselepides A., Polychronaki T., Marrale D., Akoumianaki I., Dell'Anno A., Pusceddu A., and Danovaro R., 2000b. Organic matter composition of the continental shelf and bathyal sediments of the Cretan Sea. *Prog. Oceanogr.*, 46: 311-344.

5 - Guezennec J., and Fiala-Medioni A., 1996. Bacterial abundance and diversity in the Barbados Trench determined by phospholipid analysis. *FEMS Microbiol. Ecol.*, 19: 83-93.

6 - Bowman J.P., McCammon S.A., Gibson J.A.E., Robertson L., and Nichols P.D., 2003. Prokaryotic metabolic activity and community structure in Antarctic continental shelf sediments. *Appl. Environ. Microbiol.*, 69: 2448-2462.