Short Communication Journal of Applied Microbiology and Biochemistry 2020
Vol.4 No.4

Applied Microbiology 2016- Bacterial diversity and biogeochemical analysis of sediments in Eastern Mediterranean Sea - Ilknur Tuncer - Dokuz Eylul University – Turkey

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Abstract
With the various number of studies in relationship among bacterial community with the environmental parameters composition in sediments of Eastern Mediterranean include bacterial biomass, macromolecule concentration and cultivation independent studies. Cultivation related methods, on the opposite hand, are important for further studies like production of secondary metabolites and identification of new species. In the current study, totally 19 stations with 0-1235 m depths were took as samples from sediments of Eastern Mediterranean Sea. The grain size and carbon, nitrogen, phosphorus contents of sediment samples were analyzed. Bacterial isolation is done using 7 different types of sediment processing methods and 7 isolation media prepared with sterile seawater and then incubation at 20-28 °C up to two months. 16S rRNA gene sequences of 185 strains were deposited into NCBI GenBank database and phylogenetic analysis was performed with 1000 bootstrap neighbor-joining method. Hierarchical cluster analysis was used to compare bacterial community composition. The shallowest sediments affected by continuous terrestrial and anthropogenic inputs had the highest phylogenetic diversity in higher taxa. The deep and oligotrophic stations in North and South Aegean had higher diversity in lower taxa.

Cultivation-independent methods are mostly used to study bacterial diversity in deep sediments due to the largest part of the microbial community formed by non-cultivable ones. In recent studies based on the comparative analysis of 16S rRNA clone libraries, it was reported that the dominance in deep sediments was constitute the Gammaproteobacteria in Northeastern Pacific Ocean and eastern South Atlantic Ocean, Acidobacteria in Eastern Mediterranean Sea (EMS). Cultivation-based studies, on the opposite hand, are generally preferred for further studies such as production of secondary metabolites and determination of physiological characteristics needed in especially identification of new species .According to 16S rRNA gene sequences, the Gammaproteobacteria with Halomonas the foremost frequent genus then Firmicutes were mainly identified from deep sediments of eastern South Atlantic Ocean whereas mainly Firmicutes with Bacillus the most frequent genus and then Actinobacteria from deep sediments of the EMS. Since there is a limited number of studies on the relationship between environmental parameters together with geographical differences and therefore the bacterial community composition supported bacterial biomass, nucleic acid concentration, cultivation independent studies in the sediments of EMS which is one of the most oligotrophic regions in the world, much more research including the phylogenetic diversity are needed to understand the biogeochemical variability in EMS. Therefore, the purpose of the present study is to isolate and phyogenetically analyze bacteria from sediments of EMS with cultivation-based methods and also to research biogeochemical parameters of EMS with regional variability.2. Material and Methods

2.1. Sediment Sampling and Analysis

The Eastern Mediterranean Sea is one of the most oligotrophic regions in the world, in addition to the rise of oligotrophy with longitudinal gradient in Mediterranean. One of EMS basins is the Aegean Sea (AS) separated by the Cyclades plateau into the North Aegean (NA) and the South Aegean (SA) which display different hydrographic and tropic characteristics thanks to the influence of Black Sea (BS) and Levantine Sea (LS), respectively. While relatively shallow NA receives the nutrient-rich surface waters from the BS through Marmara Sea, SA is nutrient depleted.

In the present study, sediment samples were taken from stations A, B and C in EMS for both bacterial Isolation and sediment analysis. The sampling was performed from stations A (5 stations in the inner, 2 in the middle and 2 in the outer bays) at intertidal zone of Izmir Bay which is one of the largest bays and under stress of anthropogenic activities like tourism-derived, industrial, agricultural and nautical Activities in AS. On the other hand, the research vessel RV/K Piri Reis (Dokuz Eylul University, Turkey) was used for sampling at stations B (totally 5 stations) between Lesvos Island and Karaburun of NA and also at stations C1–C10. While the stations C1–C3 were located at the upper most NA, especially C1 near to the mouth of Dardanelle Strait carrying BS water passing through Marmara Sea to AS, the stations C6–C8 were at the lower most SA where influenced by LS waters. Then, the sediment samples were collected into sterile plastic bags, 40 ml glass containers and

This work is partly presented at 3rd World Congress and Expo on Applied Microbiology November 07-09, 2016 Dubai, UAE

sterile plastic cores for different processes and kept at –20ºC till the analysis.The particle size of the sediments was determined by the sieve analysis and the hydrometer method for the larger and the finer particles, respectively according to standard test method for particle size analysis of soils D 422-63 issued by American Society for Testing and Materials.

2.2. Bacterial Isolation

Isolation of bacteria was achieved using seven different sediment processing methods and 7 isolation media prepared with sterile seawater. The isolation media consisted of the following: M1, 18 g agar, 10 g starch, 4 g yeast extract, 2 g peptone, 1 liter sterile seawater; M2, 18 g agar, 1 g starch, 0.4 g yeast extract, 0.2 g peptone, 1 liter sterile seawater; M3, 18 g agar, 2.5 g starch, 1 g yeast extract, 0.5 g peptone, 750 ml sterile seawater, 250 ml distilled water; M4, 18 g agar, 1 liter sterile seawater; M5, 18 g agar, 750 ml sterile seawater, 250 ml distilled water; M6 (DifcoTM marine agar), 55 gr medium, 1 liter distilled water; M7 30 (DifcoTM actinomycete isolation agar, modified), 22 g medium, 5 ml glycerol, 500 ml sterile sea water and 500 ml distilled water. The isolation media M1 and M7 were used with or without six different antibiotics ascycloheximide (100 µg/ml), nystatin (50 µg/ml), polymixin B sulfate (5 µg/ml), rifampin (5 µg/ml), kanamycin sulfate (5 µg/ml), novobiocin (25 µg/ml). Seven different sediment processing methods were performed. In the first processing method (a), 10ml wet sediment sample were dried overnight then 0.5 g dry sediment was aseptically spread in circularfashion onto the agar media. In the dry spot method (b) dry sediment was crazy sterile sponge and putclockwise on the agar media. In the third method (c), 1 ml wet sediment was diluted with sterile seawater (1:4) and then heated for 6 min at 55°C. After vortexing for 30 s, 75–100 µl was spread aseptically onto agar-based isolation media. In the fourth method (d), wet sediment was heated for 15 min at 70°Cand then spread aseptically on the agar surface in a circular fashion. In the fifth method (e) [20], wet sediment was kept for 30 sec under UV and then spread aseptically in a circular fashion onto the agar media.

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