

16S rDNA ANALYSIS OF MICROBIAL COMMUNITIES IN DANUBE DELTA REGION OF THE BLACK SEA

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Danube's delta in the Black Sea is natural environment where river and seawater mix forming a unique ecosystem. Danube is the second biggest river in Europe and is an active transport vehicle. Bacteria are brought here from all the Europe with the river flow and get into marine conditions. Bacterial communities from the Danube River and its tributaries have been largely described (Winter *et al.*, 2006). In this work we aimed to study bacterial diversity in the Black Sea water as well the impact of Danube river flow at their communities' composition. We provide a comparative analysis of our samples to public data and analyze if the studied area is characterized by typical marine or river microbial inhabitants.

Three surface water samples were collected during July 2014 in the region of Danube delta (45.196000; 29.476000) and passed through 0.22 µm filters. Genomic DNA was extracted from each filter using the PowerWater DNA Isolation kit according to manufacturer's protocol. 16S rDNA genes were amplified in triplicate using dual-indexing primer pair 515F and 806R for library construction and further sequencing on Illumina Miseq platform. Raw sequences were processed and quality controlled. Obtained trimmed 59958 sequences followed downstream analysis in QIIME pipeline (Caporaso *et al.*, 2010).

In the result there were picked 931 distinct OTUs. The absolute majority of sequences were affiliated with phylum Proteobacteria (91.1%). The rest belonged to phyla Bacteroidetes (4.5%), Actinobacteria (1.5%), Verrucomicrobia (1,2%), Cyanobacteria (0,9%) and others. These indicators greatly differ from our previous investigations of other Black Sea regions, where Cyanobacteria representatives were either dominating or comparable with Proteobacteria. Investigated water sample was enriched with Betaproteobacteria and specifically *Dechloromonas* genus (26.1%). This genus has been observed previously in lots of aquatic sediment habitats and recently was isolated from Potomic River sediment (Salinero *et al.*, 2009). However it has never been described before for this region of the Black Sea. *Dechloromonas* are capable of oxidizing aromatic compounds. The other numerous representatives from Betaproteobacteria class were members of *Comomonadaceae* family (8.9%) and specifically *Hydrogenophaga* genus among it (4.2%). This genus of hydrogen-oxidizing bacteria includes former 4 *Pseudomonas* spp., and has never been detected before for the studied marine area.

The other dominating class Gammaproteobacteria was greatly represented by genera *Pseudomonas* (16.1%) and *Acinetobacter* (9.9%). Alphaproteobacteria

class was mainly represented by Rhodobacteraceae (6%) and Sphingomonadaeae families (4%) families.

Spartobacteria genus that belongs to Verrucomicrobia phylum composed 0.8%. This bacterium has been described in our previous studies of estuarine microbial diversity (Bobrova *et al.*, 2015) and is considered to be ubiquitous in water and sediment columns, playing important in biogeochemical cycle of carbon.

These results show that low salinity and powerful river flow into the sea are the main factors in forming microbial communities in studied area of the Black Sea. The unexpected finding of aromatic compounds' oxidizing bacteria points at complexity of biochemical cycles occurring in this water. Thus, the region is very interesting for further investigations, such as metabolic microbial pathways, seasonal changes in community structure and functional gene profiles.

References

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