

## HIGHLIGHTING OF SOME PHYSIOLOGICAL BACTERIAL GROUPS ISOLATED FROM DANUBE DELTA BIOSPHERE RESERVE, AREA ROȘU, ROȘULEȚ AND PUIU LAKES

Doina Maria Cîrstea<sup>1</sup>, Mugur Ștefănescu<sup>1,\*</sup>

<sup>1</sup> Institute of Biology Bucharest, Spl. Independentei no. 296, sect. 6, Bucharest, Romania

### Abstract

*Industrialization and the socio-economic conditions, as well as the human standards changing from the last few decades, led to increased pollution degree including the discharge area of the Danube River in the Black Sea. Therefore it becomes essential the ecosystems assessment, in order to know their status. In this case the evaluation can be made also by studying the area microbiota. In this study it was followed main bacterial groups from Roșu, Roșuleț and Puiu lakes, part of the Danube Delta Biosphere Reservation, located in the proximity of the Sulina harbor. Our researches indicated the presence of some microbial groups, like the anaerobic heterotrophic bacteria, anaerobic facultative bacteria, heterotrophic aerobic bacteria, denitrifying bacteria and sulfate-reducing bacteria. These microorganisms, presents both in the water mass and in the benthic layer, of the investigated area, are not only statistical references, but a potential indicator strains and tests sources for laboratory biotechnological experimental level of the potential of these microorganisms.*

*Keywords: bacterial strains, microorganisms, potential biotechnological.*

### 1. INTRODUCTION

The advanced researches that study the flowing and stagnant waters together with the benthos of the Danube Delta in order to preserve the flora and fauna, as well as the economic potential, comprising the river transport, tourism and sociodemographic factors, aim equally on the physico-chemical and biological analysis of circulating (Kolarević et al., 2011), stagnant or low flow circulating waters, such as those forming the channels and lakes adjacent to the Sulina branch and port (Păceșilă et al., 2008).

During September 2016, the ecological monitoring studies were accompanied by sampling of both the water mass at different depths and the mud bed at the water bottom. The samples were subjected to qualitative analyzes to identify the main physiological groups of microorganisms, as well as quantitative analyzes for assessing the density of bacterial cells per volume unit.

The results obtained are only indicative for the bacterial biodiversity, requiring further researches by analyzing the samples taken at regular intervals, seasonal or caused by certain natural or anthropogenic disturbances that could occur, so that a more precise assessment of the distribution of the local microbiota could be performed, and also its contribution to the biodegradation of degradable products present or carried by the river waters, the correlation and the influence on the development of the characteristic ichthyofauna and the potential involvement of some bacterial

populations in the complex decomposition process, and possibly the reduction of some contaminating compounds or pollutants by active bioremediation procedures.

## 2. MATERIALS AND METHODS

Water and sediment samples were taken from the Red, Rosuleț, Puiu and Mandra lakes, as well as from the Împutița and Busurca channels, indicated by ecological scientists, the water ride being made with the Sulina Ecological Station's boat, belonging to the Bucharest Biology Institute. The water depth at the sampling points ranged between 1.17 and 2.70 m, and the solid samples consisted of sediment cores.

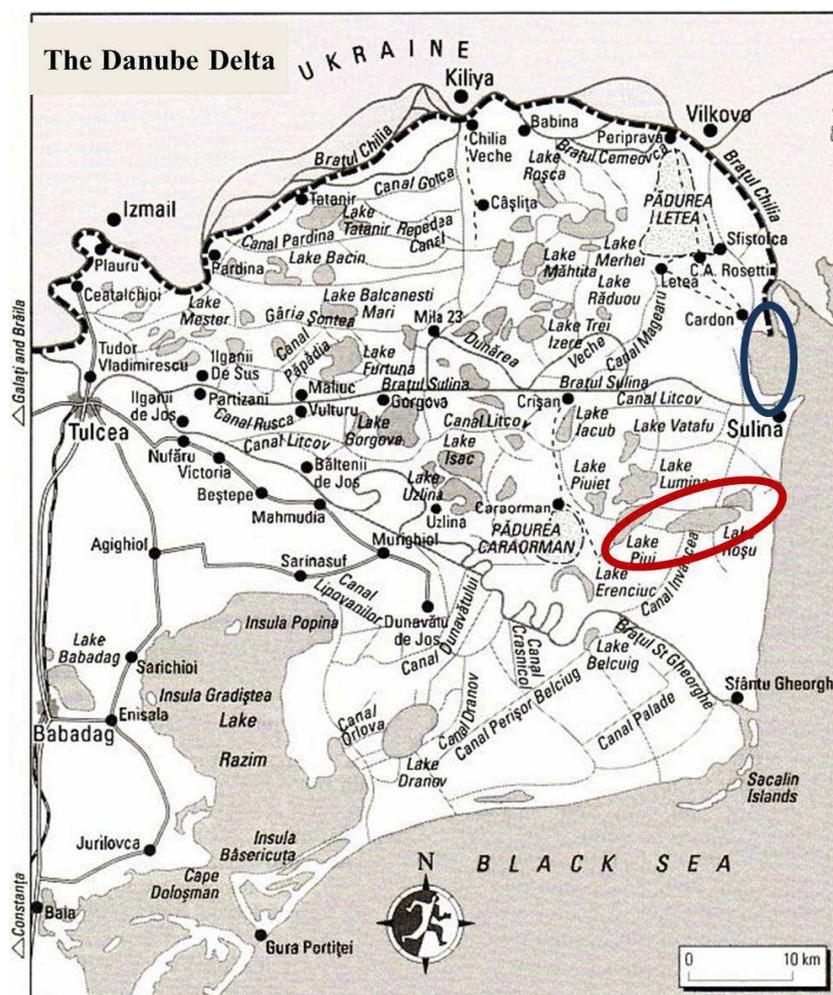


Figure 1. The Danube Delta map (<http://www.caraiman.home.ro/delta/danubedelta2.htm>)

The microbiological analyzes were carried out in the microbiology laboratory of the Bucharest Institute of Biology by the research team for remediation biotechnologies of waters and soils potentially contaminated with anthropogenic products .

Serial dilutions of samples were seeded in selective media in triplicates, by tube/ dilution/ sample repeats, which were incubated at 28 °C for varying lengths of time to allow bacterial growth (Postgate, 1966; 1984; Rodină, 1972; Lazăr et al., 2005).

Growth rate was assessed according to McReady comparative method.

### 3. RESULTS AND DISCUSSIONS

The performed analyzes are a first signaling of microbial diversity at the points of ecological interest.

Tables 1 and 2 present the results of the identification of the main bacterial groups present in the Danubian water and the sediment at the level where Danube starts formation of its delta.

Only the bacterial cultures that showed cell growth on the used selective media were mentioned.

*Table 1. Quantitative estimation of some physiological groups of microorganisms present in water samples collected in the proximity of Sulina harbor, using McCready table for establishing the most probable number*

Selective culture media/Physiological groups of bacteria	No. of cells/ml sample				
	Channel between Roşu and Puiu lakes	Mândra lack water	Roşu lake	Roşuleţ lake entry (Împuţiţa channel)	Busurca channel
	1.5 m deep	1.7 m deep	2.7 m deep	1,17 m deep	2 m deep
VL (Heterotrophic anaerobic bacteria)	$0.4 \times 10^3$	$1.5 \times 10^3$	$11 \times 10^4$	$3 \times 10^3$	$11 \times 10^6$
Postgate (Sulphate reducing bacteria)	$4.5 \times 10^3$	$1.1 \times 10^5$	$9.5 \times 10^3$	$2 \times 10^5$	$11 \times 10^5$
Nutrient broth (Heterotrophic aerobic and facultative anaerobic bacteria)	$25 \times 10^3$	$25 \times 10^3$	$9.5 \times 10^3$	$9.5 \times 10^3$	$15 \times 10^7$
Medium with starch (Amylolytic bacteria)	$9.5 \times 10^3$	$9.5 \times 10^3$	$9.5 \times 10^3$	$9.5 \times 10^3$	$1.5 \times 10^6$
Vinogradsky (Iron bacteria)	$3 \times 10^3$	$0.4 \times 10^2$	$9.5 \times 10^4$	$11 \times 10^6$	$15 \times 10^8$
Medium for nitrite bacteria (Nitrite bacteria)	$11 \times 10^7$	$11 \times 10^7$	-	-	-
Giltay medium (Denitrifying bacteria)	-	-	$45 \times 10^3$	$11 \times 10^4$	$45 \times 10^6$

In the water samples taken from Busurca Channel, at a depth of approx. 2 m, the highest values of bacterial density were recorded in all analyzed physiological groups.

In the water samples taken from the Mândra and Roşu lakes, from the channels connecting the Rosu and Roşuleţ lake, and from the Impuţiţa channel, which connects the Red lake with the Puiu lake, the density values of the 7 present physiological groups of bacteria are relatively constant, but inferior to the concentration values recorded in the Busurca channel.

At the same time, in order to select the bacterial strains with biotechnological potential for metabolizing oil and oil-derived products, isolations on solid LB medium were performed.

**Table 2. Quantitative estimation of some physiological groups of microorganisms present in sediment samples collected in the proximity of Sulina harbor**

Selective culture media / Physiological groups of bacteria	No. of cells/ml of sample			
	Roşu lake	Împuţiţa channel	Musura bay	Sulina harbour
	Sediment, 2.7 m	Sediment 1.1 m	Sediment 1.6 m	Sediment 1 m
VL / Heterotrophic anaerobic bacteria	$3 \times 10^7$	$4.5 \times 10^7$	$15 \times 10^7$	$14 \times 10^8$
Nutrient broth / Heterotrophic aerobic and facultative anaerobic bacteria	$11 \times 10^9$	$1.6 \times 10^6$	$45 \times 10^4$	$3 \times 10^4$
Medium with starch / Amyolytic bacteria	$4.5 \times 10^2$	$2.5 \times 10^2$	$25 \times 10^4$	$2.5 \times 10^2$
Medium for nitrite bacteria / Nitrite bacteria	$2.5 \times 10^2$	-	$2.5 \times 10^2$	$2.5 \times 10^2$
Medium for nitrate bacteria / Nitrate bacteria	-	-	$4.5 \times 10^3$	$11 \times 10^5$
Giltay medium / Denitrifying bacteria	$15 \times 10^6$	$3.5 \times 10^6$	$11 \times 10^9$	$25 \times 10^6$
9K medium / Iron- oxidizing chemolithotrophic bacteria	$4.5 \times 10$	$1.5 \times 10$	$9.5 \times 10$	$2.5 \times 10$
Postgate medium / Sulphate reducing bacteria	$3 \times 10^8$	$14 \times 10^6$	$45 \times 10^6$	$2 \times 10^7$

Concerning the distribution and bacterial density in the samples of sediment located at depths between 1 and 2.7 m, the preponderance of reducing sulphate bacteria, optional anaerobic aerobes and anaerobic heterotrophs in the mud at the bottom of the Red lake was noticed, as well as denitrifying bacteria, with an increased concentration in all analyzed sediment samples.

As expected, sediment samples collected in the Sulina harbor area confirm the presence of bacteria belonging to the physiological groups that grew on the used selective media.

#### 4. CONCLUSIONS

1. The presence of bacterial populations belonging to seven and eight, respectively, physiological groups of microorganisms in the Danube water and sediment samples was confirmed.
2. Higher levels of cellular concentration were found in denitrifying bacteria and optionally aerobic heterotrophic anaerobic bacteria, while nitrite and nitrate bacteria were not identified in all samples.
3. The biodiversity of the Danubian microbiota fulfills the complexity of the general ecological map of the Danube Delta Biosphere.

## 5. ACKNOWLEDGEMENTS

The studies and researches undertaken by the bioremediation team of the Center for Microbiology at the Bucharest Biology Institute were carried out within the framework of the Scientific Research Project Nr. RO1567-IBB 05/2017, entitled and endorsed by the Romanian Academy.

## 6. REFERENCES

- Kolarević, S., Knežević-Vukčević, J., Paunović, M., Tomović, J., Gačić, Z., Vuković-Gačić, B. (2011). The anthropogenic impact on water quality of the river Danube in Serbia: Microbiological analysis and genotoxicity monitoring, *Archives of Biological*, 63, 4, 1209-1217.
- Lazăr, I., Voicu, A., Dobrotă, S., Ștefănescu, M., Petrișor, I.G., Cișnașiu, C. (2005). New contributions to the microbiota naturally occurring in the Movile cave, *Travaux de l' Institut de Speologie "Emile Racovitza"*, Tomes XLIII-XLIV 2004 - 2005, Ed. Academiei Române.
- Păceșilă, I., Ionică, D., Coman A. (2008). Evaluation of metabolic intensity of benthic microbial communities from Musura Bay (Black Sea) using dehydrogenase activity method, *Proceedings of the 37th IAD Conference*, 29.10-1.11.2008, Chisinau, Moldova, 194-198.
- Postgate, J.R. (1966), *Media for sulfur bacteria*, Laboratorii Practice, Univ. Press, Cambridge, 15, 1240-1245.
- Postgate, J.R., (1984). *Sulfate-reducing bacteria*, 2<sup>nd</sup> ed Cambridge, Univ. Press, 57-58.
- Rodina, A.G., 1972, *Methods in Aquatic Microbiology*, University Park Press, Baltimore, Butterworths, London, 461 p.  
[http:// www.caraiman.home.ro/delta/danubedelta2.htm](http://www.caraiman.home.ro/delta/danubedelta2.htm)