ROMANIAN ACADEMY

INSTITUTE OF BIOLOGY BUCHAREST

Ph.D.THESIS

SUMMARY

GENERAL STUDIES ON THE MICROBIOLOGICAL COMPLEXITY OF LETEA SALT LAKE

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CONTENT

KEYWORDS

LETEA LAKE

BIOGEOCHEMICAL CIRCUIT

ALKALINE SALTY LAKES

HALOHILE BACTERIA

BIOTECHNOLOGY

EXTRACELLULAR ENZYMES

INTRODUCTION

Widespread geographically, salt lakes are ecosystems of economic, cultural, recreational and scientific importance. Being considered lifeless environments with low economic and scientific theft, they have not been of interest to the community for a long time (Florn, 2013).

From an economic point of view, salt lakes are important sources of minerals (lithium, uranium, borax, etc.), biochemicals (eg proteins; carotenoids, for example isolated from the cultivation of Dunaliella salina) or can be habitat for flamingo birds. These types of ecosystems have an important ecological value due to the diversity of species that can develop under the conditions offered by such an ecosystem. At the same time, they can be points of attraction for recreational activity and of tourist, social or economic interest (Dead Sea or Mono Lake, California).

At the same time, salt lakes are exposed to climate change, but also to factors that call into question their scientific and socio-economic existence (evaporation process, dramatic drops in water levels, increased salinity, UV radiation, human intervention - mining , pollution, capture activities, etc.). Unlike freshwater, these ecosystems have long been considered unimportant.

It is important to be aware of the socio-economic, balneological value of salt lakes, but also the negative impact of anthropogenic intervention and to develop plans for their conservation and protection.

A common feature of all saltwater bodies is salinity, which is the sum of the concentrations of dissolved ions (magnesium, calcium, potassium, sodium) and anions (carbonate, bicarbonate, chloride, sulfate). In general, the chloride anion and the sodium cation predominate, classifying the salt lakes into carbonates, chlorocarbons, carbochlorides, chlorosulfates and chlorides. At the same time, salinity being a parameter influenced by different biotic and abiotic factors, varies seasonally and spatially, being lower at the lake surface compared to deeper water, which defines the heliothermal phenomenon of salt lakes (Grant and Jones, 2016).

Depending on the salinity, the salt lakes are divided into hyposalines, with values of this parameter ranging between 3-20 g L^{-1} and hypersaline, with salinity values over 50 g L^{-1}. The pH values of water bodies group them into acidic, alkaline and neutral lakes, the latter

being the most common. The pH value directly influences the diversity at the lake level so that the best represented from this point of view are the alkaline lakes (Hammer, 1986).

Halophilic bacteria are a complex group of microorganisms adapted to survive in hypersaline environments. They were isolated from hypersaline lakes, saline soils or sea salt crystallization ponds (salterne). Although many of the halophilic bacteria were isolated from saline environments, they could still be isolated from freshwater (Larsen, 1962; Sarkar et al., 1985; Ramos-Cormenzana, 1990). In addition to the ecological significance of this group, moderately halophilic microorganisms have important biotechnological potential because they accumulate high concentrations of compatible solutions in the cytoplasm that can function as osmoprotectors and enzymatic and cellular stabilizers, and produce extracellular enzymes with high salt tolerance, applicable in biotechnological processes (Ramos-Cormenzana, 1990).

PURPOSE AND OBJECTIVES OF THE THESIS

This paper aims to highlight in general the microbiological complexity (including the microbial loop) of Lake Letea and is the first study of its kind, being therefore of particular importance to the scientific and local community, with an impact on the development of studies and research. future for this unique ecosystem, as well as for the Danube Delta.

Since 1991, the Danube Delta has become a UNESCO World Heritage Site, when it was classified both as a national biosphere reserve and as a national park according to the IUCN. The site is protected as a wetland of international importance by the Ramsar Convention (Marinescu, 2010).

Lake Letea is located near the village and the forest of the same name, in the region between the arms of Chilia and Sulina (Popescu et al., 1987). It is a permanent lake, not covered by aquatic vegetation (Moldoveanu et al., 2020). The lake is also called ghiol, a term derived from the Turkish language (Șăineanu, 1932; Lucaci et al., 2019). It is assumed that it was born after the collapse of the earth, in 1970, and the depth varies between 4 and 5 meters (Torică, 2006).

The main objectives that formed the basis of the elaboration of the doctoral thesis are:

1. assessment of microbial diversity in Salt Lake Letea, with seasonal conditioned salinity,

2. characterization of microbial food webs in Salt Lake Letea,
3. the role of microbial communities in the functioning of the saline system,
4. isolation and identification of microorganisms with biotechnological potential from Salt Lake Letea.

In order to achieve the objectives, techniques were used:

- ✓ *classical microbiology*,
- ✓ *optic microscopy*,
- ✓ *spectroscopy* (XRF),
- ✓ *biochemistry* - screening of halophilic and halotolerant bacteria in the production of extracellular enzymes,
- ✓ *molecular biology* - genomic DNA extraction, PCR amplification with specific primers, agarose gel electrophoresis, 16S rRNA sequence analysis, construction of phylogenetic trees.

THESIS STRUCTURE

The paper consists of two parts and contains 36 figures, of which 24 in the personal contributions part and 25 tables, of which 13 in the second part.

The first part presents general information about the Danube Delta (location, climatic factors and diversity in this region).

The second part presents personal contributions on the role of microorganisms involved in biogeochemical cycles. A number of moderately halophilic and halotolerant bacteria were isolated, characterized and tested. At the same time, their ability to synthesize extracellular enzymes was followed. Each chapter is structured in subchapters that include: introduction, materials and methods, results and discussions, conclusions.

ORIGINAL CONTRIBUTIONS

1. CHARACTERIZATION OF THE PHYSICAL-CHEMICAL PARAMETERS OF LETEA LAKE

1.1. Materials and methods

1.1.1. Establishment of sampling points

At the level of Letea Lake, three sampling points were established and with the help of GPS the coordinates were determined (Rasooli et al., 2016). Sampling took place over two years, October 2016 - May 2019, seasonal: autumn 2016, spring, summer, autumn 2017 and spring 2018. Water samples were taken from the three points in sterile containers and kept at 4^0C in during transport to the laboratory for further investigations (Azhar et al., 2014).

1.1.2. Determining the physico-chemical parameters of Letea Lake

1.1.2.1. Determination of physico-chemical parameters

Using the Hanna multiparameter probe, the following were measured *in situ*: pH, water temperature, salinity, redox potential (ORP), total dissolved solids (TDS), conductivity and oxygen saturation (OD%).

1.1.2.2. XRF analysis of samples

XRF analysis of water samples taken from Letea Lake was performed by X-ray fluorescence spectrometry using the Supermini X-Ray Fluorescence Spectrometer (Rigaku

Corporation, Japan). A semi-quantitative method of determining the percentage of light elements in a helium atmosphere was used. A volume of 10 ml of sample was used to perform the tests (Neagu et al., 2014).

1.1.3. Determination of the chloride anion

The determination of the chloride content dissolved in water was performed by the Mohr method (Nielsen, 2017). The principle of this method is the reaction of chloride ions with silver ions, in the presence of 10% potassium dichromate as an indicator. Under vigorous stirring, a silver chloride precipitate forms. In the titration process, when a red-brick color appears, specific for silver chromate, it is considered that the reaction between chloride and silver ions (Nielsen, 2017) marks the end of the titration. The total volume used for the test was 50 ml.

1.2. Results and discussions

The sampling points, denoted L1, L2 and L3 (Table 1.2.1) were chosen so as to cover approximately the entire surface of the lake taking into account that, depending on the season and conditioned by the evaporation rate, it undergoes changes, in meaning that in the spring and autumn season its area is larger compared to the summer season. The geographical coordinates of the sampling points are detailed in Table 1.2.1.

Table 1.2.1.GPS coordinates of sampling points (L1- Letea1, L2-Letea2, L3- Letea3).

Punct de prelevare	N	E
L1	45º16'51.978"	29º33'10.4184"
L2	45º16'53.0688"	29º33'11.6244"
L3	45º16'51.8628"	29º33'12.0204"

The Hanna multi-parameter probe provided data on certain physico-chemical parameters (Table 1.2.2).

Table 1.2.2. Determination of physico-chemical parameters using the Hanna multiparameter probe.

Seasons	Spring						Summer			Autumn					
Year	2017			2018			2017			2016			2017		
Sampling point	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3	L1	L2	L3
pH	10,0	9,5	9,4	9,3	9,6	9,5	9,2	8,7	9,3	9,6	9,4	9,5	10,2	9,4	10,4
Water temperature (ºC)	5,2	6,6	5,4	19,4	20,2	20,3	27,5	27,4	27,4	19,2	19,2	19,2	8,7	8,7	8,6
Conductivity (mS/cm)	27,7	27,4	27,9	13,9	14,2	14,0	42,2	42,1	41,2	59,3	56,2	59,6	58,3	58,4	58,4
DO (mg O_2/L)	4,1	4,9	4,4	3,2	1,2	1,2	2,7	2,6	2,6	6,7	6,9	7,2	17,0	11,7	15,6
Salinity (ppm)	16,9	16,8	16,6	7,8	8,2	8,1	27,1	27,0	26,6	39,8	38,4	39,9	38,6	38,7	38,7

Legendă: DO =total dissolved oxygen; Sampling points: Letea1 (L1), Letea2 (L2) și Letea3 (L3)

As for the pH value, in the case of waters with a high content of chlorides and sulphates, it is in the area of neutral values. The presence of Ca and Mg carbonates determines that the pH value of the water should be in the range of 7.5 - 8.5 pH units. A pH value between 8.5-12 defines alkaline and slightly alkaline waters and is characterized by a high content of silicon salts. On the other hand, acid saline waters characterized by a pH value in the range of 2-3 units possess this characteristic mainly due to the absence of carbonates and the low capacity as silicate buffering agents (Benison et al., 2007; Bowen and Benison, 2009; Hines et al., 1992).

In Letea Lake there is a variation of the pH value starting from 8.7 units, a value registered in the summer of 2017, up to a maximum value of 10.4 units, in the fall of 2017 (table 1.2.2). These values, of this physico-chemical parameter constantly recorded above values of 7 units, lead to the conclusion that Letea Lake can be included in the category of lakes with slightly / slightly alkaline pH values. In this regard, comparing the average pH values for the fall of 2017 recorded for Lake Letea, with data from the literature, it is found that the values found by 10 pH units are similar to the values obtained in Lake Altai (Russia), respectively 10 - 10.3 units (Foti et al., 2008).

In relation to the data from the specialized literature (Ramos - Cormenzana, 1993) and in the case of Letea Lake, it was observed that the relationship between water temperature and

dissolved oxygen is inversely proportional. From the analysis of the data obtained during the five sampling periods, it was found that the values recorded for dissolved oxygen decrease seasonally, compared to the increase in water temperature.

In this context, in the autumn of 2016, as well as in the summers of 2017 and 2018, the same decrease of dissolved oxygen values was registered in relation to the increased values of the lake water temperature. This is confirmed by the high value of dissolved oxygen in the fall of 2017 and the relatively low value of the lake temperature.

The research carried out for this paper showed that after analyzing the water samples it was found that a number of compounds were: frequently encountered (HgO, SiO_2, Al_2O_3, K_2O, CaO, Fe_2O_3, Cr_2O_3), encountered in certain periods (MgO, Br, SO_3, Co_2CO_3, SrO, CuO, GeO_2 , Y_2O_3, HoO_3, Ir_2O_3) found in traces (TiO_2, ZnO, Ga_2O_3, MnO, NiO, HfO_2, Nb_2O_5, Ag_2O, Ta_2O_5, ZrO_2, As_2O_3, Cs_2O).

From the analysis of the data obtained by spectroscopy (Figure 1.2.1), it was found that the highest values of the most common compounds were obtained in the spring of 2018. Thus, in the sampling point Letea1 (L1), the mass percentages for Al_2O_3 and HgO were 3,814 and 5,303, respectively. For Fe_2O_3, the maximum value was recorded at sampling point Letea2 (L2). In the last point, the mass percentages for SiO2, K2O and CaO were 6,737; 20,014 and 9,688.

Figure 1.2.1. Supermini X-Ray Fluorescence Spectrometer

Analyzing the results obtained in this study, depending on the season, at the sampling point Letea1 (L1), in autumn 2016 and summer 2017 it was found that CaO and K_2O predominated, as later, in spring 2017, these compounds no longer be identified in the water samples to be analyzed. On the other hand, the compound Cr_2O_3 was identified only in the spring season of the mentioned years. Of all the elements determined by this technique, three

of them, respectively SiO_2, Al_2O_3 and Fe_2O_3 were identified in each sampling season, respectively spring, summer and autumn (Figure 1.2.2).

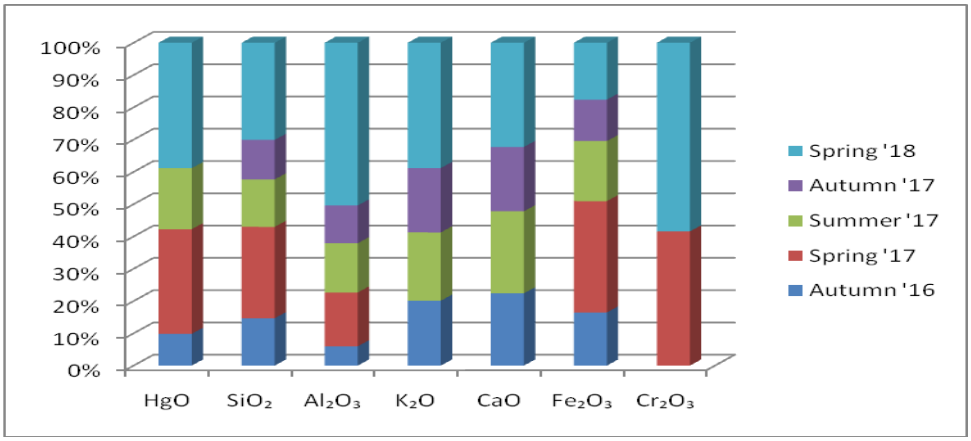

Figure 1.2.2. Presence of chemicals at sampling points Letea1 (L1) ('16- 2016; '17- 2017; '18- 2018).

During the five sampling campaigns of the water samples from Letea Lake, it was found that in the sampling point marked with L2 (Letea2) three elements predominate, respectively SiO_2, K_2O, Fe_2O_3. On the other hand, at this sampling point, the element Cr_2O_3 was identified by XRF analysis only in the summer of 2017.

Another element with an impact on the environment, namely Al_2O_3 was identified in autumn 2017 and at higher values (3.81% mass) in the spring of 2018. Another element with a high degree of pollution for the investigated system is HgO which was identified in the summer and autumn of 2017. On the other hand, the specific presence of chemical elements with negative impact on the quality of the investigated ecosystem, could be attributed to their character of airborne heavy metals (Ștefănuț et al., 2018 ; Buekers et al., 2011) that reach and deposit in the water of Lake Letea by air depending on the speed and direction of the winds.

Figure 1.2.3 graphically illustrates the data presented above showing the impact of each element on the state of the ecosystem and the evolution (growth and development) of microorganisms in lake water.

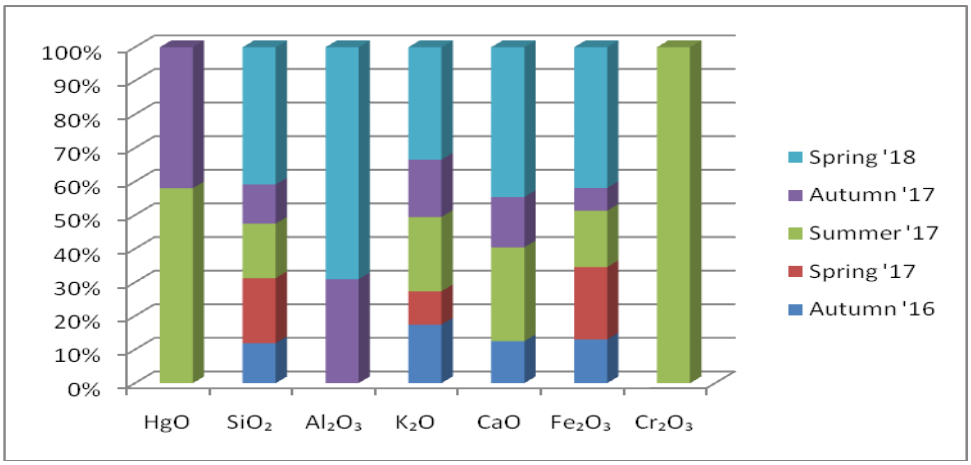

Figure 1.2.3. Presence of chemicals at sampling points Letea2 (L2) ('16- 2016; '17- 2017; '18- 2018).

In contrast to the sampling points identified as L1 and L2, in the sampling point noted L3 (Letea3) the share of items identified by XRF analysis is different in all seasons investigated in this paper. It can be noted, however, that the compound SiO_2 is present, similar to sampling points L1 and L2 and that the compound Cr_2O_3, identified in the summer of 2017 in point L2, is absent.

Regarding the compound Fe_2O_3, which was identified in points L1 and L2 in the period 2016 - 2018, in the sampling point identified as L3, it was detected in water samples taken in spring and summer of 2017. Also from this sampling point, in the spring of 2018, the presence of heavy metals was not detected, such as: HgO, Fe_2O_3 and Cr_2O_3.

The data in Figure 1.2.4 show that in the spring of 2018, the most abundant compound was Al_2O_3 (as an effect of anthropogenic impact and airborne household waste under the action of winds), and the weakest concentration was identified in the case - SiO_2, in the fall of 2016.

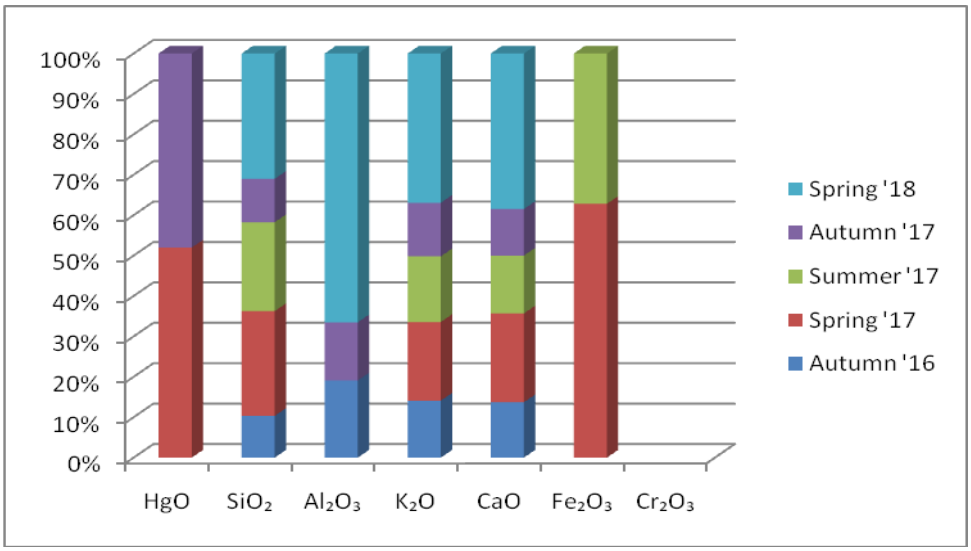

Figure 1.2.4. Presence of chemicals at sampling points Letea3 (L3) ('16- 2016; '17- 2017; '18- 2018).

Salinity depends on the supply and export of water from the ecosystem, so any change in the hydrological regime determines the change in the nature of the lake. The water supply must be closely related to the evaporation process.

From the data presented in table 1.2.3, it is observed that the salinity varies from 7.81 g L^{-1}, value recorded in spring 2018, to 46.86 g L^{-1}, value recorded in autumn 2017.

Table 1.2.3. Seasonal variation in chloride content.

Sampling points	Chloride (g L^{-1})				
	Spring		Summer	Autumn	
	2017	2018	2017	2016	2017
L1	11,36	7,81	18,82	25,56	29,11
L2	12,43	9,59	18,46	25,56	39,05
L3	11,72	9,59	19,88	25,56	46,86

L1 - Letea1, L2 - Letea2, L3 - Letea3

From the data analysis, it is observed that there is a similarity between the values recorded in spring 2017 and spring 2018 (Figure 1.2.5). Thus, in the spring of 2017 there was a value of 11.8 g L^{-1} which is close to the value obtained in the spring of 2018 (8.9 g L^{-1}). Following the autumn sampling, after analyzing the water samples, different values were

obtained for the two years. In the fall of 2016, the average chloride content is 25.5 g L^{-1} compared to the average content in 2017 (38.3 g L^{-1}).

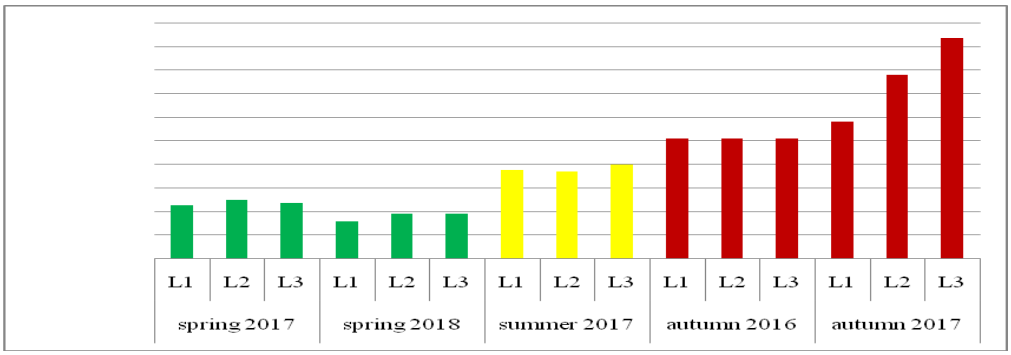

Figure 1.2.5. Variation of the chloride content (g L^{-1}) from water samples taken between 2016 and 2018.

The particular feature of this lake is the variation of salinity. An explanation for the decrease in salinity from autumn to spring would be the supply of fresh water through rain and snow. The summer months are characterized by an increase in temperatures, induction of evaporation and a decrease to the absence of rain (Trotsenko and Khmelenina, 2002). This is also clear from the analysis of weather data from the Sulina weather station. Thus, the highest temperatures were recorded in July and the lowest in January. The precipitation amounts are high in autumn compared to other seasons. In the autumn of 2016 and 2018, there was more significant precipitation compared to 2018, an aspect also observed in chloride values (Table 1.2.3). As the temperatures from spring to summer increased and the evaporation phenomenon intensified and the precipitations decreased, an increase of salinity was observed (Table 1.2.3).

2. THE ROLE OF MICROORGANISMS IN BIOGEOCHEMICAL CIRCUITS IN LETEA LAKE

2.1. Materials and methods

2.1.1. Microorganisms involved in the carbon biogeochemical cycle

For the quantitative determination of the number of heterotrophic bacteria a solid medium with the following composition (g L^{-1}) was used: yeast extract, 3; peptone, 10; NaCl, 5; agar 20.

Sterilization was performed by autoclaving at 120°C for 30 minutes.

For analysis, from the water samples, serial dilutions were performed, two repetitions per dilution. A volume of 1 ml of each dilution was transferred dropwise over the entire surface of a Petri dish, after which liquefied culture medium was added and brought to 55-60°C (Cojoc et al., 2013).

To determine the number of heterotrophic bacteria, Petri dishes were incubated at 37° C for 48 hours, after which they were quantified (Halder et al., 2016).

2.1.2. Microorganisms involved in the biogeochemical cycle of nitrogen

2.1.2.1. Ammonifying bacteria

To determine the number of ammonifying bacteria, a culture medium with the following composition (g L^{-1}) was used: asparagine, 0.2; solution of trace elements (H_3BO_3, 2.8; $MnSO_4x4H_2O$, 0.2; $NaMoO_4x2H_2O$, 0.75 $ZnSO_4x7H_2O$, 0.24; $Cu(NO_3)_2x3H_2O$, 0.04); Winogradski standard solution (K_2HPO_4, 5; $MgSO_4x7H_2O$, 2.5; NaCl, 2.5; $Fe_2(SO4)_3$, 0.05 g; $MnSO_4$, 0.05; distilled water, 1000 ml) 50 ml.

The medium (5 ml) was distributed in test tubes and sterilized by autoclaving at 20°C for 30 minutes (Lazăr et al., 2004).

From the samples under analysis, they were performed in triplicate decimal serial dilutions. From each dilution, 0.2 ml was transferred to the three test tubes with culture medium. Their incubation was performed at 28°C for a period of 15 days (Lazăr et al., 2004).

After the incubation period, 1-2 drops of Nessler reagent were added, and in the test tubes in which a yellow-orange color was observed, the reaction was considered positive (Lazăr et al., 2004).

2.1.2.2. Denitrifying bacteria

To determine the number of denitrifying bacteria, Pochon medium with the following composition (g L^{-1}) was used: KNO_3, 2; Winogradski standard saline (K_2HPO_4, 5; $MgSO_4x7H_2O$, 2.5; NaCl, 2.5; $Fe_2(SO_4)_3$, 0.05; $MnSO_4$, 0.05; distilled water, 1000 ml); calcium carbonate, 5; glucose, 10; distilled water, 950 ml.

The medium was sterilized at 110°C for 30 minutes (Lazăr et al., 2004).

Decimal dilutions in triplicate were performed from the investigated samples. A volume of 0.2 ml of each dilution was transferred to the tubes containing 2.5 ml of culture medium. The test tubes were incubated for a period of 7-15 days, at a temperature of 28°C (Lazăr et al., 2004).

After incubation, Griess I and Griess II reagents were added, and the appearance of a red color led to the conclusion of a positive result (Lazăr et al., 2004).

2.1.3. Microorganisms involved in the biogeochemical cycle of sulfur

For the quantitative determination of the number of sulfate-reducing bacteria, the Postgate culture medium with the following composition (g L^{-1}) was used: potassium phosphate, 0.5; ammonium chloride, 1; sodium sulfate, 0.5; magnesium sulfate, 2; calcium lactate, 3.5.

Sterilization of the medium was performed at 120°C for 30 minutes. After sterilization, 5% 10 ml yeast extract, 5% $FeSO_4$, 5 ml, 2% Na_2S 2 ml, 10% $NaHCO_3$ 1-5 ml were added to the medium until the pH was 7.2-7. 4 (Lazăr şi colab., 2004).

Decimal dilutions in triplicate were made from the water samples, and 2 ml of them were transferred to sterile tubes. Subsequently, medium in the high column was added to each tube, and the tubes were incubated for 7 days at 28°C (Lazăr et al., 2004).

The appearance of a black FeS precipitate is a positive response, the lack of the precipitate being a negative response (Lazăr şi colab., 2004).

2.1.4. Microorganisms specific to salt lakes - the case of Letea Lake (halophilic / halotolerant microorganisms)

For the quantitative determination of the number of halophilic / halotolerant microorganisms, a culture medium with the following composition (g L^{-1}) was used: yeast extract, 10; proteose-peptone, 5; glucose 1; NaCl, 100; $MgCl_2x6H_2O$, 7; $MgSO_4x7H_2O$, 9.6; $CaCl_2x2H_2O$, 0.36; KCl, 2; $NaHCO_3$ 0.06; NaBr 0.026; agar 20 (Ventosa et al., 1989).

From the water and sediment samples subjected to analysis, serial dilutions (10^{-1}-10^{-4}) were made in sterile physiological water (1 ml of sample is suspended in 9 ml of sterile physiological water). In Petri dishes, 1 ml of the sample and their dilutions were distributed, over which the culture medium was added. The tests were performed in duplicate, and the incubation was performed at 30°C for 7 days.

2.2. Results and discussions

The role of microorganisms in the process of degradation of substrates is extremely important. Data from the literature show that heterotrophic bacteria represent the significant component of the microbial loop with a role in ensuring the productivity of the entire ecosystem (Besemer et al., 2005).

The determination of the number of heterotrophic bacteria (colony forming units) was performed by seeding the samples on an agarized medium. From the data centralized in Table 2.2.1, the lowest number of heterotrophic bacteria was determined in the water samples taken in the summer of 2017 ($3.3x10^1$ C.F.U./mL), and the highest number was recorded in the spring of 2017 ($3,1x10^3$ C.F.U./mL). Starting with the spring of 2017, the number of heterotrophic bacteria decreases, and this trend is maintained throughout the year.

Table 2.2.1. Seasonal dynamics (spring, summer and autumn) of heterotrophic bacteria.

Sampling points	C.F.U./ mL				
	Spring		Summer	Autumn	
	2017	2018	2017	2016	2017
L1	**$3{,}1x10^3$**	$18x10^2$	$3{,}4x10^1$	$1{,}71x10^2$	$7{,}2x10^1$
L2	$7x10^2$	$6x10^2$	$8{,}5x10^1$	$2{,}06x10^2$	$1{,}5x10^2$
L3	$8{,}5x10^2$	$12x10^2$	**$3{,}3x10^1$**	$1{,}49x10^2$	$1{,}3x10^2$

L1 - Letea1, L2 - Letea2, L3 - Letea3

The data recorded in Figure 2.2.1 show the presence of heterotrophic bacteria in water samples taken during 2017 and 2018. If in the spring of 2017, the colonies were larger in area and more numerous, as the seasons progressed a decrease is observed both of their number as well as of their morphology.

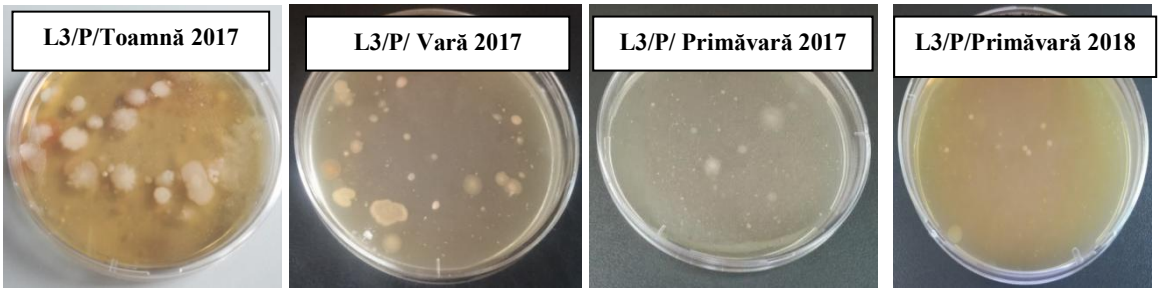

Figure 2.2.1. The presence of heterotrophic bacteria isolated from water samples taken at point L3 (Letea3), in the spring, summer, autumn seasons (2017 and 2018).

The nitrogen circuit in nature involves four stages: molecular nitrogen fixation, ammonification, nitrification (nitritation and nitration) and denitrification (Zarnea, 1994).

The analysis of the data obtained and presented in table 2.2.2 shows that most ammonifying bacteria were identified in spring 2018 ($140x10^2$ cel./mL) and the fewest in autumn 2017 (2.5 cel./mL) . Similar values were obtained in the spring season in both 2017 and 2018. There is an increase in the number of ammonifying bacteria in the autumn and spring period followed by a decrease in the summer season.

Table 2.2.2. Seasonal dynamics (spring, summer, autumn) of ammonifying bacteria.

Sampling points	MPN				
	Spring		Summer	Autumn	
	2017	2018	2017	2016	2017
L1	$11x10^3$	**$140x10^2$**	2,5x10	$1,5x10^2$	**2,5**
L2	$11x10^3$	**$140x10^2$**	2,5x10	$9,5x10^2$	**2,5**
L3	$2,5x10^3$	$45x10^2$	2,5x10	$9,5x10^2$	**2,5**

L1 - Letea1, L2 - Letea2, L3 - Letea3

From the data obtained from the analyzes performed and centralized in table 2.2.3 it is found that in the fall of 2017, the denitrifying bacteria were in the largest number ($11x10^2$ cel./mL). In the spring of 2017, they could not be highlighted and it is observed that denitrifying bacteria are abundant during the year with lower temperatures (autumn), their number remaining small and relatively constant in the rest of the year.

Table 2.2.3. Seasonal dynamics (spring, summer, autumn) of denitrifying bacteria.

Sampling poitns	MPN				
	Spring		Summer	Autumn	
	2017	2018	2017	2016	2017
L1	**0**	45	4,5	4x10	**$11x10^2$**
L2	**0**	110	4,5	20	$15x10^1$
L3	**0**	140	9,5	20	**$11x10^2$**

L1 - Letea1, L2 - Letea2, L3 - Letea3

From the data presented in table 2.2.4 it is observed that the largest number was registered in the spring of 2017 ($4.5x10^2$ cel./mL). Their number determined from the water samples taken from Letea Lake is relatively low and constant, except for the Letea1 (L1) sample from autumn 2016.

Table 2.2.4. Seasonal dynamics (spring, summer, autumn) of sulfate-reducing bacteria.

Sampling points	MPN				
	Spring		Summer	Autumn	
	2017	2018	2017	2016	2017
L1	**$4,5x10^2$**	**2,5**	4,5	9,5x10	7,5
L2	**$4,5x10^2$**	**2,5**	**2,5**	**2,5**	7,5
L3	**$4,5x10^2$**	**2,5**	9,5	**2,5**	**2,5**

L1 - Letea1, L2 - Letea2, L3 - Letea3

The results presented in table 2.2.5 demonstrate the evolution of the number of halophilic bacteria, between October 2016 and April 2018. The largest number of colony forming units was in the spring of 2017, ranging between $2.1x10^3$ C.F.U./ mL at the sampling point Letea3 (L3) and $3.7x10^3$ at the Letea1 (L1) sampling point. The water temperature during this period was 5.7^0C and the pH was 9.61 units. In the following spring (2018), the lowest number of halophilic bacteria, 280 C.F.U./mL, was registered in the Letea1 (L1) and Letea2 (L2) sampling points. The water temperature during this period was 19.9^0C, and the pH value of 9.46 units.

Table 2.2.5. Seasonal dynamics (spring, summer, autumn) of halophilic bacteria.

Sampling poitns	C.F.U. / mL				
	Spring		Summer	Autumn	
	2017	2018	2017	2016	2017
L1	**$3,7x10^3$**	**280**	$4,2x10^2$	770	$1,7x10^2$
L2	$3,6x10^3$	**280**	$4,1x10^2$	410	$1,9x10^2$
L3	$2,1x10^3$	350	$1,4x10^3$	560	$2,3x10^2$

L1-Letea1, L2- Letea2, L3-Letea3

The results obtained centrally and presented in Figure 2.2.2 show that, after the spring of 2017, the number of halophilic bacteria decreases. If in the spring of 2017, the size of the colonies was small, in the following period, there is an increase in the area of the colonies and a decrease in the diversity of bacteria, their number remaining constantly quite low.

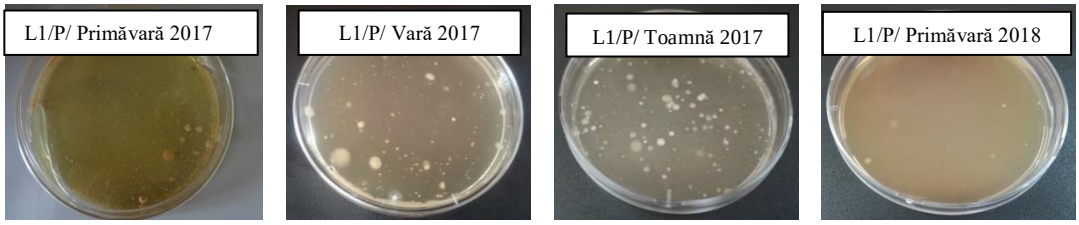

Figure 2.2.2. Images with colonies of halophilic bacteria from the Letea1 (L1) sampling point (P-sample).

3. CHARACTERIZATION OF HALOPHILIC BACTERIA

3.1. Materials and methods

3.1.1. Morphological characterization of halophilic bacteria

Gram staining is a method of taxonomically differentiating bacteria based on tinctorial affinities (Roohi et al., 2012). After fixing the bacterial culture, the smear is stained with gentian violet; the dye reacts with the acidic components of the cytoplasm forming a stable intracellular complex after treatment with Lugol's solution. In the case of Gram-positive bacteria, this complex insoluble on contact with alcohol and acetone does not discolor. Gram-negative bacteria, not forming this stable complex, discolor. Subsequently, Gram-negative bacteria must be recolored with another contrast dye (fuchsia) (Lazăr et al., 2004; Rhode, 2011).

The Gram staining method (described in 1884), however, is time consuming, expensive, and reagents are frequently replaced. It has been observed that the same results are obtained with the help of a treatment with a 3% KOH solution (Buck, 1982).

Place a 3% KOH drop on a glass slide. With the help of a sterile loop, a small amount of culture is taken from the growing medium for 24 hours, which is transferred to the KOH solution. After mixing, check the consistency by raising the loop 1 cm away from the blade. String formation certifies the presence of Gram-negative bacteria, the absence of strings being characteristic of Gram-positive bacteria (Buck, 1982).

The test is based on the chemical difference of the bacterial cell wall between Gram-positive and Gram-negative bacteria. Thus, in the presence of alkaline solutions, the cell wall of gram-negative bacteria is affected (Davis et al., 1968).

3.1.2. Biochemical characterization of halophilic bacteria

The ability of a bacterial strain to synthesize **oxidase** is highlighted using the Kovacs test. This test involves the oxidation of phenyl diamine to indophenol oxidase, in the presence of atmospheric oxygen, with the formation of a dark purple color (indophenol). The microbial culture must be fresh (18-24 hours). It is considered a positive reaction to change color immediately or in a maximum of 30 seconds. If no change occurs or the color remains light pink / purple, then the answer is considered negative.

Catalase is an enzyme synthesized by many aerobic and optionally anaerobic bacteria and whose role is to catalyze the breakdown of hydrogen peroxide (H_2O_2) into water and oxygen. Following the contact of a strain with hydrogen peroxide, the presence of gas bubbles is considered a positive reaction (Azhar et al., 2014).

3.1.3. Taxonomic classification of isolates in a Domain

For taxonomic classification in a field of the living world, the selected bacteria were seeded by the stria technique on MH medium 10% NaCl supplemented, in two variants, with chloramphenicol (0.002%) and sodium deoxycholate (0.004%). The plates were incubated at 30°C for 48 hours (Oren, 1991).

3.1.4. The influence of salinity on the growth of bacterial strains

To highlight the growth of bacterial strains depending on salinity, a culture medium with the following composition (g L^{-1}) was used: NaCl, 100; $MgCl_2x6H_2$, O 7; MgSO4x7H_2O, 9.6; $CaCl_2x2H_2O$, 0.36; KCl, 2; $NaHCO_3$ 0.06; NaBr 0.026; yeast extract, 10; proteose peptone, 5; glucose 1; agar 20 (Halder et al., 2006).

Inoculation was performed by the loop depletion technique, and the plates were incubated at 37^0C for 48 hours.

3.2. Results and discussions

Decimal serial dilutions were made from the water samples taken. Samples and dilutions were seeded on a specific medium by incorporation into the medium. After incubation and determination of viable cell numbers (Rasooli et al., 2016), 105 isolates were randomly selected.

The selected bacterial isolates were purified by successive passages and characterized morphologically. To highlight the diversity of microorganisms isolated on solid MH medium, colonies of: white, cream, pink, yellow, orange, brick were selected; with full edge, radiating, wavy or irregular; circular or irregular shape; flat profile, convex, umbonate, raised flat; glossy or rough surface.

It was found that out of the 105 isolates described, a large part of the selected microorganisms (23) could no longer be cultured.

Based on the tinctorial affinities of the cell wall (Gram staining method), the 72 pure cultures isolated from water samples and 10 pure cultures isolated from sediment were classified as Gram-negative and Gram-positive. It was observed that in the fall of 2016, only Gram-negative bacteria were isolated. In the fall of 2017, both Gram-positive and Gram-negative bacteria were isolated, the latter number being dominant.

Regarding the ability to produce oxidase and catalase, a similarity was observed between seasons. Thus, in the fall of 2016 and the fall of 2017, isolates were identified that responded positively, predominantly, to the oxidase test. In the water samples taken in the spring of 2017 and 2018, isolates producing both oxidase and catalase predominated. In the summer season, of the selected isolates, three responded positively to both tests and two negatively.

Of the 82 isolates from the water and sediment samples, 70 were oxidase-producing, 43 were catalase and 35 were positive in both tests.

Of the total number of isolates, 83% were oxidazo-positive and 52% were catalazo-positive (Figure 3.2.1).

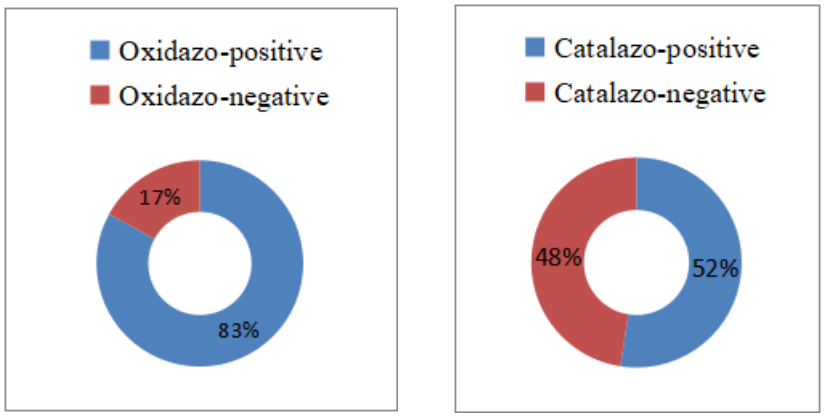

Figure 3.2.1. Framing of selected isolates according to the response to the oxidase-catalase test.

To classify the isolates in a domain (Bacteria or Archaea), the MH medium was supplemented with sodium deoxycholate (0.004%) and chloramphenicol (0.002%). After performing the tests, an increase of the isolates was observed on medium supplemented with sodium deoxycholate, which places them in the field of Bacteria. The action of chloramphenicol on halophilic bacteria is to inhibit their growth and development (Oren, 1991; Ghosh et al., 2010).

To place them in a salinity range, the tested cultures were seeded on MH medium with different salinities (1M, 2M, 3M, 4M), including in the absence of NaCl.

From the data analysis and using the classification of Kushner and Kamekura (1988), the isolates from the water and sediment samples taken from Lake Letea are divided into halotolerant and moderately halophilic. In the spring of 2017 and 2018, bacteria with a strong growth on the environment without NaCl were isolated.

In general, an inability of selected isolates to develop in the presence of 4M NaCl was observed, with the exception of 3 isolates identified in spring 2017, 1 isolate identified in summer 2017, 1 isolate identified in autumn 2017 and 5 isolates identified in spring year 2018. The vast majority of isolates developed in the salinity range 0 - 3 M (a number of 49) (Figure 3.2.2).

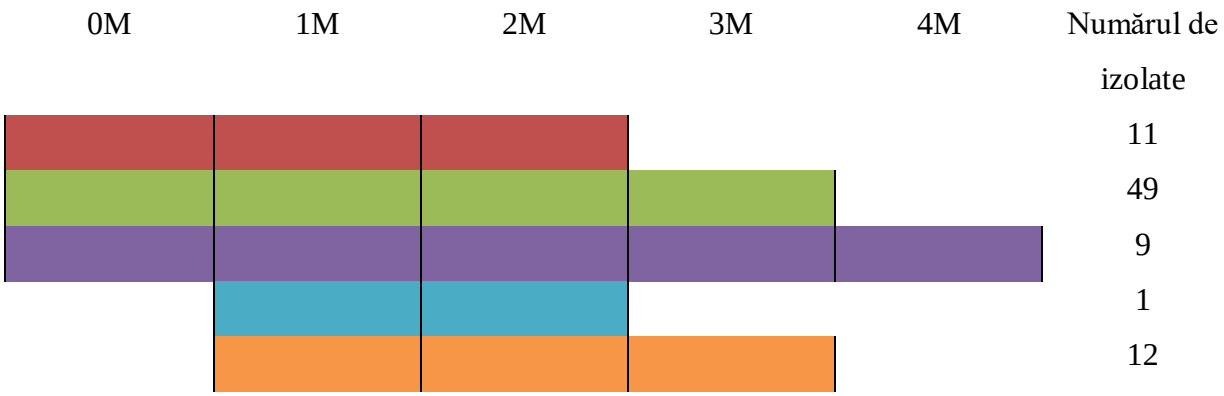

Figure 3.2.2. Increase of selected isolates in a certain salinity range.

4. SCREENING OF ISOLATES PRODUCING EXTRACELLULAR ENZYMES IN THE CLASS OF HYDROLASES

4.1. Materials and methods

An MH medium with the following composition (g L^{-1}) was used: yeast extract, 10; NaCl, 100; $MgCl_2x6H_2O$, 7; $MgSO_4x7H_2O$, 6; $CaCl_2x2H_2O$, 0.36; KCl, 2; $NaHCO_3$, 0.06; NaBr, 0.026; agar, 20; to which was added a substrate characteristic of each enzymatic activity, at different concentrations of NaCl (0M-4M).

4.1.1. Starch hydrolysis

The ability of some strains to degrade the starch-supplemented culture medium was highlighted by spot seeding of an MH medium to which 2 g of soluble starch was added.
The plates thus seeded were incubated for 48 hours at 28°C by flooding the plates with Lugol's solution (I_2/KI), the amylolytic activity was observed by the appearance of a halo around the stem (Rohban et al., 2009).

4.1.2. Casein hydrolysis

In order to highlight the protease activity, the previously described MH medium was seeded in spots to which 1% casein was added.

The appearance of a precipitation ring, around the suction, after incubation for 48 hours at 28ºC, indicates the presence of a proteolytic activity (Enache and Kamekura, 2010).

4.1.3. Hydrolysis of esterases

4.1.3.1. Tween 80 hydrolysis

By adding sorbitol monooleate (Tween 80) to the agarized MH medium the esterase activity of the selected strains can be observed. They will be seeded in the spot and then incubated at 28°C for 48 hours. The presence of calcium oleate crystals around bacteria certifies the existence of Tween-esterase (Rohban, 2008).

4.1.3.2. Tributyrin hydrolysis

MH medium supplemented with 1% tributyrin was used to highlight lipase activity. Inoculation was performed as a spot, and incubation was performed at 280 ° C for 48 hours. The appearance of a clear area around the colony indicates the presence of lipase activity (Kumar et al., 2012).

4.1.4. Carboxymethyl-cellulose(CMC) hydrolysis

MH medium (described above) supplemented with carboxymethylcellulose was used to highlight cellulase activity (Rohban et al., 2009).

4.1.5. Inulin hydrolysis

Inulinases can be detected by using an MH medium having the inulin as the only carbon source. The medium was seeded in the spot, and the incubation was performed at 28ºC for 48 hours. The presence of halo around bacterial culture indicates the existence of inulinase activity (Babavalian et al., 2014). After 48 h at 28ºC and flooding of the plates with Lugol's solution (I_2 / KI), the inulinase activity was observed by the appearance of a halo around the stem.

4.1.6. Hydrolysis of gelatin

To highlight gelatines, the MH medium described above was supplemented with 150g L^{-1} gelatin (Zarnea et al., 1992).

After inoculating the medium by puncturing the medium with the loop, it was incubated at 30^0C for 24 hours. Subsequently, after incubation, the samples were kept in the refrigerator for 10 minutes. The appearance of the phenomenon of liquefaction of the environment highlighted the production of gelatinases.

4.2. Results and discussions

After being isolated, described, characterized and purified, the isolates were tested to identify the ability to synthesize extracellular enzymes with possible biotechnological potential. Thus, of the isolated and purified bacterial cultures, only 49 showed at least one enzymatic activity.

In the autumn of 2016, eight isolates with the ability to synthesize inulases (7), esterases (7) and cellulases (1) were identified. It was also observed that no isolate degraded the starch-supplemented medium.

In the spring of 2017, the isolates showed amylolytic (2), inulinase (2), cellulase (4), esterase (5) and proteolytic (12) activity. If in the fall of 2016, inulinase activity predominated, in the spring of 2017, the isolates had predominantly proteolytic activity. Of the 14 isolates with the ability to synthesize extracellular enzymes, six isolates degraded a single substrate type, five isolates degraded the supplemented medium with two substrate

types, and three isolates degraded three substrate types. Although four isolates had the ability to synthesize casein, the hydrolysis radius was still small (0.1 cm). No isolate degraded the medium supplemented with tributyrin.

In the summer of 2017, 11 bacterial cultures with extracellular enzymatic activity were isolated. All 11 isolates had the ability to degrade the inulin supplemented medium. Eight isolates also showed proteolytic activity and three isolates were also highlighted by amylolytic activity. Of the 11 isolates, three synthesized a single type of enzyme (inulinase), five isolates degraded the medium supplemented with two types of substrate (inulin and casein), and three isolates degraded three types of substrate (inulin, casein, and starch). . As in the spring of 2017, in the summer of the same year, no isolates with the ability to synthesize esterases and cellulases were identified. At the same time, no isolate degraded the environment supplemented with tributyrin.

In the autumn of 2017, 7 isolates producing extracellular enzymes were identified, the predominant activity being the proteolytic one (5 isolates). The presence of three isolates capable of degrading the medium supplemented with Tween 80 is noted. Strain C3L2-1 has an extremely complex enzymatic activity, being able to degrade the medium supplemented with casein, starch, Tween 80 and inulin. The second isolate with combined extracellular enzymatic activity is C3L1-3, which has the ability to synthesize proteases and esterases. No microbial culture capable of degrading the medium supplemented with tributyrin was isolated.

From the samples taken in the spring of 2018, nine isolates showed proteolytic, amylolytic and cellulase activity. It was observed that isolates capable of synthesizing proteases predominated. Strain C4L1-4 also synthesized amylolytic enzymes. No isolates with esterase and inulinase activity were identified. No microbial culture capable of degrading the medium supplemented with tributyrin was isolated.

It should be noted that during the five seasons, no bacterial cultures capable of degrading the environment supplemented with gelatin were isolated.

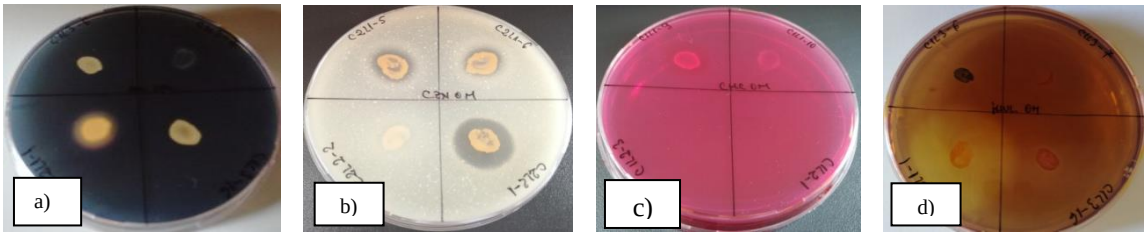

Figure 4.2.1. The ability of some isolates to degrade the supplemented environment with: starch (a), casein (b), CMC (c), inulin (d).

5. TAXONOMIC CLASSIFICATION OF SOME HALOPHILIC BACTERIA INVESTIGATED

5.1. Materials and methods

5.1.1. DNA extraction

The DNeasy Blood & Tissue kit was used for DNA extraction; following the manufacturer's working protocol. After extraction and purification, 16S rRNA amplification was performed by the chain polymerization reaction. For this stage, 8 strains were randomly selected, detailed in table 5.2.1.

For this, universal primers for bacteria were used: 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GCTTACCTTGTTACGACTT). The PCR mixture contained: 1 µl DNA, 12.5 µl GoTaq G2 Hot Start Green Master Mix2x, 1 µl for each primer (10 µl), 9.5 µl non-nucleated water, in a final volume of 25 µl. The amplification was performed in Eppendorf Mastercycler pro S following the following steps: incubation (95˚C for 2 minutes), denaturation (35 cycles of 30 seconds, at 95˚C), alignment (54˚C for 30 seconds), elongation at 72˚C for 90 seconds and a single final elongation at 72˚C for 5 minutes (Ruginescu et al., 2018). PCR products were purified using QIAquick PCR purification kit.

5.1.2. 16S rRNA sequence analysis

The purified products were sent to a commercial company for sequencing - CeMIA SA (Greece). The obtained sequences were compared with the sequences in the database using the NCBI BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

5.2. Results and discussions

Depending on the intensity of the hydrolytic activity, eight bacterial strains were selected for further investigations (one bacterial strain - autumn 2016; five bacterial strains - spring 2017; two bacterial strains - summer 2017). The bacterial strains chosen for identification were halotolerant. Four strains (C1L1-2, C1L1-4, C2L1-1, C2L2-5) show the ability to synthesize two different types of extracellular enzymes. Strain C1L1-9 has the ability to hydrolyze the medium supplemented with casein, carboxymethyl-ellulose and Tween 80.

Halophilic bacteria with multiple hydrolytic capacity are involved in numerous biotechnological processes (Rasooli et al., 2016).

Using the gene sequencing technique encoding 16S rRNA synthesis (Amoozegar et al., 2008), according to the protocol described above, the eight bacterial strains were identified (Table 5.2.1).

Table 5.2.1 contains information on the nearest phylogenetic taxon according to the GenBank identification number. The isolates were identified as belonging to the genera: *Marinobacter, Halobacillus, Virgibacillus, Bacillus, Halomonas* and *Salinivibrio*.

Table 5.2.1. Taxonomic affiliation of the eight isolated bacterial strains from Lake Letea.

Izolate	**Cel mai apropiat taxon de izolat (GenBank accession number)**	**Similaritate (%)**
CNL1-9	*Marinobacter* sp. ME108 (AJ302707.1)	99
C1L1-2	*Halobacillus* sp. 4TMC2 (MK251570.1)	99
C1L1-4	*Virgibacillus* sp. Bac332 (CP033046.1)	98
C1L1-6	*Marinobacter* sp. M71 (FM992844.1)	99
C1L1-9	*Bacillus zhangzhouensis* c9 (MK696234.1)	99
C1L3-9	*Halomonas* sp. ALS9(KU714727.1)	98
C2L1-1	*Salinivibrio* sp. JSM 114060 (JX220752.1)	98
C2L2-5	*Salinivibrio proteolyticus* AB116 (KY646049.1)	98

The tree was built by the maximum probability method. Similarity ≥ 70% is shown. The rate of 0.02 shows the substitution rate for 1000 reconstruction variants.

The results presented in Figure 5.2.2 show that the 8 strains investigated, having the property to hydrolyze at least two or more types of organic substrates, are phylogenetically grouped into six genera of moderately halophilic or halotolerant microorganisms, respectively: *Marinobacter, Halobacillus, Virgibacillus, Bacillus, Halomonas* and *Salinivibrio*.

Strain C1L3-9 has a degree of similarity of 99% with species of the genus *Halomonas*, but is grouped separately within the phylogenetic tree. Similarly, strains C1L1-6 and C1L1-9 are grouped with members of the genus *Marinobacter*, but separated from each other and from each other. The same aspect can be observed for the C2L2-5 and C2L2-1 strains that are grouped with members of the genus *Salinivibrio*. Unlike the first two cases, these two strains form an individual group between them with a maximum probability value of 83%.

Strain C1L1-9 is grouped with members of the genus *Bacillus* with a maximum probability of 100%.

Strain C1L1-4 is positioned together with members belonging to the genus *Virgibacillus* with a maximum probability of 98%, and strain C1L1-2 with the same maximum probability is found together with members of the genus Halobacilus.

Analyzing the data presented in figure 5.2.2, two phylogenetically distinct groups can be observed. One, with a maximum probability of 84% is made up of members of the genera *Halomonas, Marinobacter* and *Salinivibrio*. Within this group are found five of the strains investigated.

The other group, with a maximum probability of 100% includes the genera *Bacillus*, *Virgibacillus* and *Halobacillus*, and three of the analyzed strains are found in this group.

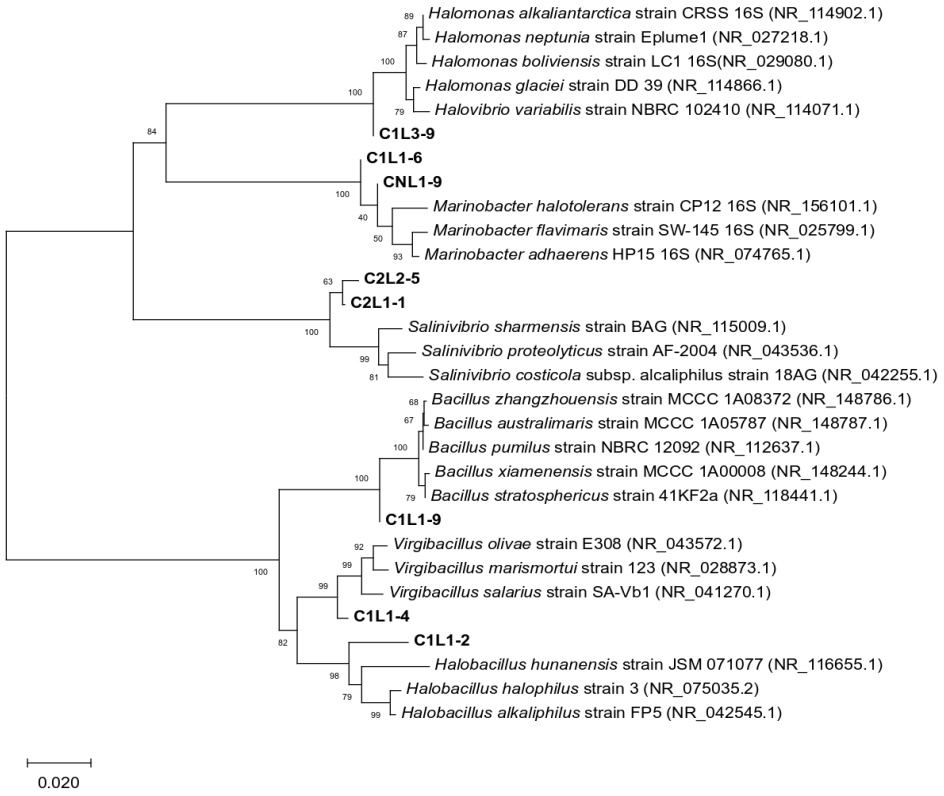

Figure 5.2.2. The phylogenetic tree built on the basis of 16S rRNA partial sequences demonstrating the position of the investigated strains between species of the genera Marinobacter, Halobacillus, Virgibacillus, Bacillus, Halomonas and Salinivibrio. The tree was built by the maximum probability method. Similarity ≥ 70% is shown. The rate of 0.02 shows the substitution rate per 1000 reconstruction variants.

The previous results are also supported by data on the salinity range in which the investigated strains can develop as well as the need for NaCl for optimal development. Thus, the strains that fall into group I of the phylogenetic tree structure (Figure 5.2.2) grow between 0-3M NaCl with an optimum growth at 1M. In the second group are found the strains that have an optimal development in the similar salinity range but the optimal salt concentration for growth tends to 0M NaCl, given that no other sodium salts have been eliminated from the composition of the medium.

GENERAL CONCLUSIONS

- Letea Lake has a salinity that varies seasonally. From autumn to spring, salinity decreases due to the intake of fresh water from snow and rain. In spring there is a concentration of salinity due to the phenomenon of evaporation and small amounts of precipitation.

- The chloride content varies from 8.9 g L-1 (spring 2018) to 38.3 g L-1 (autumn 2017).

- The pH value varies between 8.7 units (summer 2017) and 10.4 units (autumn 2018).

- The highest number of halophilic bacteria was recorded in the spring of 2017, ranging between 2.1x103 C.F.U./mL at the Letea 3 sampling point and 3.7x103 C.F.U./mL at the Letea1 sampling point. In the other seasons, the number of halophilic bacteria remained relatively low.

- The lowest number of heterotrophic bacteria was determined in the water samples taken in the summer of 2017 (3.3x101 C.F.U./mL), and the highest number of heterotrophic microorganisms was quantified in the samples from spring 2017 (3.1x103 C.F.U./mL). Since the spring of 2017, the number of heterotrophic bacteria has been declining, remaining relatively constant throughout the year.

- The highest number of ammonifying bacteria was identified in the spring of 2018 (140x102 cel./mL) and the lowest in the fall of 2017 (2.5 cel./mL), similar values being obtained in the spring season both in year 2017 as well as in 2018. There was an increase in the number of ammonifying bacteria during autumn and spring followed by a decrease in the summer season.

- In the fall of 2017, denitrifying bacteria recorded the highest number (11x102 cel./mL), and in the spring of 2017, they could not be identified. It is observed that denitrifying bacteria are abundant during the year with lower temperatures (autumn - winter), their number remaining small, relatively constant over the rest of the time.

- The highest number of sulfate-reducing bacteria was registered in the spring of 2017 (4.5x102 cel./mL). It was found that the number of reducing sulfate bacteria determined from the water samples taken from Lake Letea is relatively low and constant, with the exception of the Letea1 (L1) sample from autumn 2016.

- Of the 105 halophilic bacteria described, after the successive passages in order to obtain pure cultures, 82 isolates could be cultured and subsequently tested.

- Of the 82 isolates from the water and sediment samples, 70 responded positively to the oxidase test, 43 to catalase and 35 isolates responded positively to both tests.

- All isolates belong to the Bacteria domain.

- Depending on the interval at which it develops in the presence of NaCl, the isolates from the water samples taken from Letea Lake are divided into halotolerant (69) and moderately halophilic (13).

- The 82 isolates were screened to identify the ability to produce extracellular enzymes. Thus, a number of 49 isolates showed enzymatic activity (16 isolates - esterase activity; 22 isolates - inulinase activity; 36 isolates - proteolytic activity; seven isolates - amylolytic activity; seven isolates - cellulose activity).

- Depending on the intensity of the hydrolytic activity, eight bacterial strains were selected for further investigations (one bacterial strain - October 2016; five bacterial strains - April 2017; two bacterial strains - July 2017). The bacterial strains chosen for identification are halotolerant. Four strains (C1L1-2, C1L1-4, C2L1-1, C2L2-5) show the ability to synthesize two different types of extracellular enzymes. Strain C1L1-9 has the ability to hydrolyze the medium supplemented with casein, carboxymethyl-cellulose and Tween 80.

- 16S rRNA analysis showed that strains CNL1-9 and C1L1-6 belong to the genus *Marinobacte*r, strain C1L1-2 belongs to the genus *Halobacillus*, C1L1-4 belongs to the

genus *Virgibacillus*, strain C1L1-9 belongs to the genus *Bacillus*, strain C1L3-9 belongs to the genus *Halomonas*,C2L1-1 and C2L2-5 belong to the genus *Salinivibrio*.

Selective bibliografy

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1. **Lucaci A.I.**, Moldoveanu M., Florescu L., Cojoc R., Neagu S., Ruginescu R., Enache M., 2019. The seasonal dynamics of the cultivable microbial communities in Letea saline lake. *AgroLife Scientific Journal*, 8, 1, 160-166.

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