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RAPID ASSESSMENT OF CARBON SUBSTRATE UTILIZATION IN THE EPILIMNION OF MEROMICTIC URSU LAKE (SOVATA, ROMANIA) BY THE BIOLOG ECO PLATE™ APPROACH

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SUMMARY. Ursu Lake is a large saline, meromictic and heliothermal lake located in the eastern part of the Transylvanian Basin (Sovata, Mureș County, Romania). The investigated lake is characterized by a strong vertical stratification of physical and chemical parameters that indicate a corresponding stratification of biodiversity. Using BIOLOG Ecoplate™ method we were able to describe the community-level physiological profile of the microbial population inhabiting the moderately saline epilimnion (5-6% salinity) of Ursu Lake. The metabolization of 31 different carbon sources was monitored in the water samples collected at 0.5 m depth from two different seasons: October 2013 and March 2014. Physico-chemical parameters (temperature, salinity, pH, oxidation-reduction potential, and dissolved oxygen) were measured along with the estimation of the total chlorophylls, carotenoids, and prokaryotic cell count. The results revealed a higher rate of C substrate consumption in the water sample collected in spring compared to that found in the autumn sample. This finding is paralleled by the differences observed in some of the chemical parameters (salinity, dissolved oxygen) between the seasons suggesting a time-based modification of the microbial activity. Alpha-cyclodextrin, glycogen, D-cellobiose, D-mannitol, and N-acetyl-D-glucosamine were the fastest metabolized C sources in both seasons. This is the first report of using BIOLOG Ecoplate™ approach in profiling the microbial activity in a Romanian deep, meromictic and heliothermal salt lake and one of the very few attempts reported to use the BIOLOG system for the characterization of microbial communities in hypersaline ecosystems.

Keywords: BIOLOG Ecoplate™, biopolymer degradation, community-level physiological profile, meromictic lake, microbial activity.

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Introduction

Saline and hypersaline lakes are natural (karstosaline) or man-made (anthroposaline) environments that provide living place for halotolerant and halophilic organisms. These ecosystems are characterized by salt concentration exceeding that of the sea water (i.e., salinity > 30 $g \cdot L^{-1}$ or 3 %) and sometimes reaching the saturation point (i.e., salinity > 300 $g \cdot L^{-1}$) (Oren, 2002). The majority of organisms flourishing in these conditions are prokaryotes belonging to *Bacteria* (Ventosa *et al*., 1998) and *Archaea* (Andrei *et al*., 2012), and only a few are representatives of fungi, protozoa, and algae (Oren, 2002; 2008).

The Romanian territory comprises a significant number of anthroposaline and karstosaline salt lakes dispersed between two distinct geographic areas: the Transylvanian Basin (Central and north-western) and the Dacic Basin (Southern, SE and eastern Romania) (Alexe, 2010; Bulgăreanu, 1996). More than 40 saline and hypersaline lakes are found in the Transylvanian Basin, with locations following a circular line of the inner Carpathians belt. Among these bodies of water, several lakes (e.g. Ocnei and Rotund Lakes in Turda, Fără Fund and Brâncoveanu Lakes in Ocna Sibiului, Ursu Lake in Sovata etc.) are characterized by a strong and relatively stable stratification of physical and chemical parameters, a property termed meromixis (Boehrer and Schultze, 2008). In a similar manner as other meromictic lakes worldwide, the Transylvanian stratified saline lakes display an upper water layer (epilimnion or the uppermost part of the mixolimnion), down to about 2 m depth, an intermediate stratum showing a steep change of physico-chemical parameters (chemocline or the lowermost part of the mixolimnion) at around 2.5-3.5 m depth, and a deep, stable and hypersaline layer (monimolimnion) that starts right below the chemocline (Alexe, 2010; Baricz *et al*., 2014; Máthé *et al*., 2014). Although some of these atypical lakes have long been known and exploited for their touristic value, there is a scarcity of data concerning their biodiversity, especially with respect to their microbial diversity and activity. Systematic investigation of the microbial communities hosted by a number of Transylvanian meromictic salt lakes is just at its beginning and it dealt with the diversity of phototrophic algae and cyanobacteria (Keresztes *et al*., 2012), heterotrophic bacteria (Borsodi *et al*., 2010; 2013; Crognale *et al*., 2013; Máthé *et al*., 2014) and halophilic archaea (Baricz *et al*., 2014). In an earlier study, Muntean *et al*. (1996) were the first to explore the biological activity in the sediment (mud) samples of therapeutic value collected from the bottom of many Romanian salt lakes, including a few of Transylvanian ones.

A fast and convenient approach for profiling the metabolic requirements, sole carbon source utilization and community level physiological profiles (CLPP) was developed by BIOLOG Inc., an R&D company based in Hayward, CA, USA. BIOLOG Ecoplate™ microplates are tools designed for the analysis of whole microbial community from soil and water. One plate has 96 wells containing 31

different carbon sources and a negative control (no carbon substrate) in triplicate. Each well also contains a redox dye indicator (tetrazolium violet) which indicates the positivity of metabolization with color development toward blue-violet (Garland and Mills, 1994; Weber *et al*., 2008). The benefit of the triplicate nature of the microplates is the capacity to use different samples on the same plates or to have an indicator of experimental variation (Weber *et al*., 2007). The Ecoplates offer the opportunity to calculate several substrate-based diversity indices including substrate richness, substrate diversity, and substrate evenness (Zak *et al*., 1994). Several CLPPs of aquatic ecosystems were performed using BIOLOG Ecoplate™ predominantly for freshwater systems such as lakes (Dickerson and Williams, 2014), ponds (Lear *et al*., 2013) or wetland mesocosms (Weber *et al*., 2008). Only a few data exist on CLPP applied in saline environments (Litchfield and Gillevet, 2002; Phillips *et al*., 2011) for the main reason that this approach seems to work unreliably at high salt concentration (Litchfield *et al*., 2001; Pierce *et al*., 2014). CLPP using BIOLOG Ecoplate approach was performed by Crognale *et al*. (2013) in the top and bottom water samples from shallow saline Mierlei Lake, nearby Ursu Lake in Sovata, but the tests were apparently applied to samples with salinities exceeding 10%. However, to date, no approach for a direct estimation of the microbial activity within the water mass has been employed in the Romanian deep, meromictic salt lakes.

The present work intended to provide a first glimpse at the metabolic diversity of microbial community populating the moderately saline epilimnion of the meromictic and heliothermal Ursu Lake. Our aims were: 1) to assess the capacity of carbon substrate metabolization *via* BIOLOG Ecoplate™ approach in the water samples collected during two different seasons (autumn and spring), and 2) to scrutinize the influence of some physico-chemical parameters on the metabolic requirement for carbon sources of the epilimnetic, heterotrophic microbial community of Ursu Lake.

Materials and methods

Description of sampling site

Ursu Lake is located in Sovata (Mureş County), in the eastern part of the Transylvanian Basin, and is the largest heliothermal lake in Romania with an area of about 41000 sqm and maximum depth of 18 m (Muntean *et al*., 1996; Alexe, 2010; Máthé *et al.*, 2014). Ursu Lake is a karstosaline lake formed following a natural event in the late nineteenth century. Continuous water input is provided by brackish Topliţa River and the freshwater Auriu River (Alexe, 2010).

Measurement of physico-chemical parameters and sampling of water

Measurements of physico-chemical parameters of lake water as well as sampling were performed as described previously in Baricz *et al.* (2014), during October 2013 and March 2014. The choice for these sampling times was justified by: 1) ease of accessibility and sampling, 2) the favorable thermal conditions that might support the microbial activity, and 3) the absence of bathing activity. The temperature, salinity, concentration of dissolved oxygen (DO), oxidation-reduction potential (ORP), and pH of the water were measured *in situ*, at a depth of 0.5 m, using a portable water multiparameter device (HI 9828/20, Hanna Instruments, USA). Water samples (1 L) aimed for testing of total microbial activity by BIOLOG Ecoplate approach were collected from 0.5 m depth in sterile plastic containers using an electrical layer sampler. The samples were kept on ice during transportation to laboratory.

Total cell counts

Ten milliliters of water samples were fixed with glutaraldehyde at 2% final concentration and stored for 24 hours at 4^{o}C, in the dark. Fixed samples were filtered through 0.45 µm-pore-size, black, gridded, MCE membrane filters (Fioroni, France). The cells retained on the membrane filters were directly stained with DAPI (4', 6'-diamidino-2-phenylindole) at 5 µg ml^{-1} final concentration and examined by epifluorescence microscopy (BX60, Olympus Optical, Tokyo, Japan). The images were recorded using the microscope's digital camera (Olympus XC50) and analyzed with the CellC software (Porter and Feig, 1980).

Measurement of single-carbon substrate degradation

To test for the CLPP in the collected environmental samples, all the 96 wells of the plates were filled with 150 µl of untreated water samples in sterile conditions, by using an 8-channel automatic pippetor. After inoculation, the plates were incubated and daily monitored during 5 days (120 hrs) in aerobic condition at 30^{o}C. As we aimed for a quick approach to estimate the bulk metabolic activity in the water samples, the consumption of various carbon sources was followed by direct observation of color change at every 24 hours. A reaction was considered roughly positive when the clear blue-violet hue appeared. An extension of incubation (up to 10 days) was employed to estimate whether there are carbon sources that need a prolonged time for degradation. In this situation, the plates were wrapped in plastic foil to avoid evaporation.

Results

Physico-chemical and biological properties of the sampled water layer

The *in situ* measurement of the physical and chemical parameters at 0.5 m depth indicated the moderately saline nature (estimated salinity 57-68 $g \cdot L^{-1}$) of the upper water layer (epilimnion) of Ursu Lake (Table 1). Two environmental factors, temperature and pH, were similar, while other parameters were slightly (salinity and ORP) or significantly (DO) different. The lower salinity found in the epilimnion during March 2014 compared to that estimated in October could be a consequence of the higher fresh water input during late autumn and winter seasons (i.e. rainfalls and ice/snow melting). Other physical and chemical features of the surface lake water are very susceptible to changes that reflect the fluctuations of the surrounding environment. While the temperature value measured during October (around 23^{o}C), followed warm days recorded in summer, the similar value recorded in the epilimnion during March was probably due to the heliothermal effect of the lake water. The measured pH values were also comparable (8.44 in October and 8.53 in March, respectively) but the explanation lays in the buffering effect of the HCO_3^- ions that are present in millimolar concentration (data not shown).

Dissolved oxygen (DO) measurements showed a higher value in October 2013 (6.71 $mg \cdot L^{-1}$) than in March 2014 (0.17 $mg \cdot L^{-1}$). This finding could be explained by an intense activity of oxygenic phototrophic community in deeper layer during warm season (summer 2013) that allowed accumulation of oxygen in the epilimnion. On the turn, the drop of oxygen level during spring season might be triggered either by high metabolic activity of aerobic heterotrophs and/or by a reduced production of photosynthetic oxygen following winter season. The ORP measurements indicated a slightly higher reduction capacity of the water layer during October (-20.7 mV) than that of March sample (+ 6.6 mV). The difference is however too small to assume a clear external cause, ORP probably fluctuating as a consequence of daily or weekly biological activity.

Some of the biological properties were estimated in the epilimnion (0.5 m) (Table 1). In October 2013 the water sample collected at 0.5 m depth presented higher values of total chlorophyll and carotenoid concentrations as well as of cell density compared to that estimated in the March sample. Based on these results one might expect a significantly denser microbial community and possibly higher biological activity in the epilimnion of Ursu Lake during October than March. As stated above, the imbalance of biological parameters recorded in the epilimnion of Ursu Lake could be a direct consequence of varying external environmental factors such as air temperature and amount of water inflow. Higher chlorophyll and carotenoid concentrations during autumn (14.91 and 668.13 $\mu g \cdot L^{-1}$, respectively) than that found during spring (2.30 and 319.60 $\mu g \cdot L^{-1}$, respectively) might be due to the warm, sunny summer season that favored the blooming of primary producers.

Table 1.

Physical, chemical, and biological properties of sampled water layer (0.5 m depth) from Ursu Lake

Parameter (Measurement Units)	Season	
	October 2013	March 2014
Salinity ($g \cdot L^{-1}$ or approx. psu)	68.3	57.5
Temperature (^oC)	23.7	23.6
Dissolved oxygen ($mg \cdot L^{-1}$)	6.71	0.17
ORP (mV)	-20.7	6.6
pH	8.44	8.53
Total chlorophylls ($\mu g \cdot L^{-1}$)	14.9	2.3
Total carotenoids ($\mu g \cdot L^{-1}$)	668.1	319.6
Total cell count ($*10^6$ cells $\cdot$ mL^{-1})	5.36	2.96

Single-carbon substrate degradation

The two BIOLOG Ecoplates were monitored for color changes every 24 hrs during 5 days of incubation. The triplicate format of the plates warrants a reliable repetition of the experiment and provides exact results of different substrate metabolization. The reaction was accounted as positive when color turns to blue-violet in at least two duplicate wells (Fig. 1.).

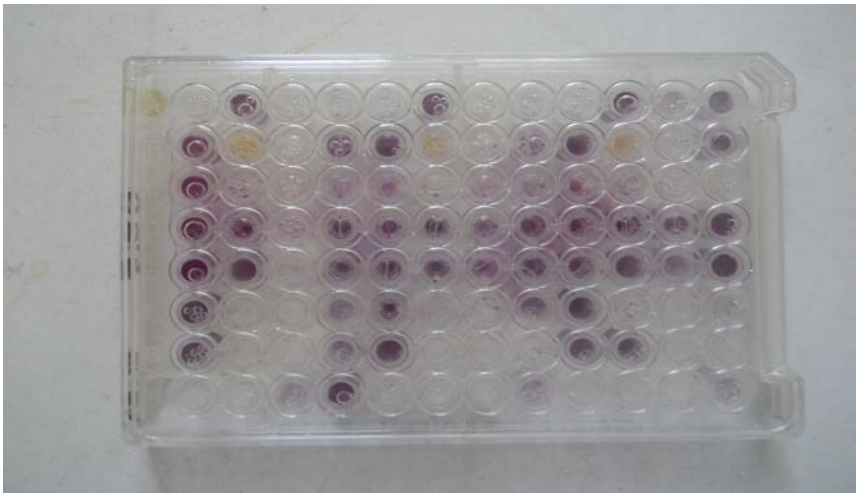

Figure 1. Photographic image of BIOLOG™ EcoPlate inoculated with water sample collected at 0.5 m depth from Ursu Lake, during March 2014, after 48 hrs. of incubation.

The full list of the 31 carbon substrates is presented in Table 2 along with the results (positive or negative) of their degradation.

Table 2.

Microbial utilization of single-carbon sources in the water sample collected at 0.5 m depth from the Ursu Lake during two seasons (October 2013 and March 2014). For the positive samples, the incubation time at which the color change was observed is given

ID	Carbon source	Incubation time (hrs)	
		Oct-13	Mar-14
C1	Pyruvic acid methyl ester	72	24
C2	Tween 40	72	48
C3	Tween 80	72	24
C4	Alpha-cyclodextrin	24	24
C5	Glycogen	24	24
C6	D-cellobiose	24	24
C7	Alpha-D-lactose	-	-
C8	Beta-methyl-D-glucoside	48	24
C9	D-xylose	-	-
C10	i-erythritol	-	-
C11	D-mannitol	24	24
C12	N-acetyl-D-glucosamine	24	24
C13	D-glucosaminic acid	120	120
C14	Glucose-1-phosphate	120	120
C15	D,L-alpha-glycerol phosphate	-	120
C16	D-galactonic acid-gamma-lactone	-	-
C17	D-galacturonic acid	-	-
C18	2-Hydroxy benzoic acid	-	-
C19	4-Hydroxy benzoic acid	-	72
C20	Gamma-hydroxybutyric acid	72	72
C21	Itaconic acid	-	72
C22	Alpha-ketobutyric acid	-	-
C23	D-malic acid	120	120
C24	L-arginine	72	120
C25	L-asparagine	48	48
C26	L-phenylalanine	72	96
C27	L-serine	48	48
C28	L-threonine	48	24
C29	Glycyl-L-glutamic acid	72	72
C30	Phenylethylamine	-	96
C31	Putrescine	72	48

Note: *For a better visualization, we emphasized the name of substrates with colored background: light gray for substrates metabolized in both water samples; blue for substrates metabolized by only one water sample, and white for carbon sources that were not degraded after 10 days of incubation. To highlight the metabolization rates of C sources the backgrounds of positive scores were colored in black (24 hrs), red (48 hrs), orange (72 hrs), dark yellow (96 hrs), and light yellow (120 hrs).*

Out of 31 carbon sources, five substrates were quickly metabolized (i.e., after 24 hours of incubation) by both samples: alpha-cyclodextrin, glycogen, D-cellobiose, D-mannitol, and N-acetyl-D-glucosamine. The first two are polymers, while the latter are a disaccharide, a sugar alcohol, and a monosaccharide derivative, respectively. Other substrates that are degraded in a fairly short time (within 48 hrs.) by both samples are the monosaccharide derivative beta-methyl-D-glucoside and the amino acids L-asparagine, L-serine, and L-threonine. Substrates such as methyl pyruvate, Tween 40, Tween 80, gamma-hydroxybutyric acid, L-arginine, L-phenylalanine, glycyl-L-glutamic acid, and putrescine are equally used in both samples during first 72 hours, apparently slightly quicker in the sample collected during spring season. Three substrates were metabolized in 120 hrs. in both samples: D-glucosaminic acid, glucose-1-phosphate, and D-malic acid. The first two carbon sources are monosaccharide derivatives and the last one is a carboxylic acid.

There are some carbon sources metabolized in only one sample, namely in the March sample: 4-hydroxy benzoic acid and itaconic acid (24 hrs.); phenylethylamine (96 hrs.); D, L-alpha-glycerol phosphate (120 hrs.).

Seven different carbon sources were not metabolized in any of the two samples after 5 days of incubation and up to 10 days of monitoring: alpha-D-lactose, D-xylose, i-erythritol, D-galactonic acid-gamma-lactone, D-galacturonic acid, 2-Hydroxy benzoic acid, and alpha-ketobutyric acid.

In the sample collected and inoculated in October 2013, the BIOLOG Ecoplate™ results indicated that 20 different carbon sources (64.5 % of total carbon sources) were biologically degraded within 120 hours. In the March sample, positive results were observed for 24 different carbon sources (77.4 % of total C sources) within same time interval (Table 3). A number of seven C sources were not metabolized in any of the samples up to 10 days of monitoring: the monosaccharides D-xylose, D-galactonic acid-gamma-lactone, the disaccharide alpha-D-lactose, the sugar alcohol i-erythritol, the phenolic derivative 2-hydroxy benzoic acid (also known as salicylic acid), and the carboxylic acid alpha-ketobutyric acid and D-galacturonic acid.

Table 3.

Summary table of carbon substrate utilization in water samples tested by BIOLOG Ecoplates™ approach

Parameters	**Sampled season**	
	October 2013	**March 2014**
Total metabolized substrates	20 (64.5%)	24 (77.4%)
Number of substrates metabolized within 24 hrs	5	9
Number of substrates metabolized within 72 hrs	17	17
Number of substrates with slow metabolization (after 96 hrs)	3	7
Substrates metabolized in only one sampled season	0	4
Total substrates metabolized in both sampled seasons	20	

Discussion

The data attained from the present study is a first reported indicative for the range of metabolic requirements of heterotrophic microbial community residing the saline epilimnion (0.5 m depth, 5-6 % salinity) of meromictic Ursu Lake (Sovata, Romania). Since the microorganisms are considered the primary decomposers in any environment, revealing the metabolic availability of whole microbial community brings valuable information on their functioning status. As already stressed in the introductory part of the present work, no similar studies were performed for the water collected from meromictic salt lakes located in Romania. Comparable studies were, however, performed in saline environments worldwide: solar salterns from Newark, California and Eilat, Israel (Litchfield *et al*., 2001; Litchfield and Gillvete, 2002), La Sal del Rey salt lake from Texas (Phillips *et al*., 2012), and alkaline Mono Lake, California (Litchfield and Gillevet, 2002).

Our results showed that in October 2013 (autumn), the salinity of water layer (0.5 m) was around 68.3 $g \cdot L^{-1}$ (or 6.8 %) with a total cell number of 5.36 $x10^6$ $cell \cdot mL^{-1}$. This sample accommodated a heterotrophic microbial community capable of using at least 20 different compounds as sole carbon sources. In the sample collected in March 2014 (spring), the salinity was estimated as 57.5 $g \cdot L^{-1}$ (5.75 %) and cell density as 2.96 $x10^6$ $cell \cdot mL^{-1}$. Twenty-four different carbon sources were metabolized by the microbial community existing here at a two-fold lesser cell density than in October. In the case of La Sal del Rey salt lake (Texas, USA), the samples were collected during June 2010 and July 2010 (Phillips *et al*., 2012). The results indicated that in the surface sample with low salinity (4 ppt or 0.4%) and a cell density of around 5.2 x 10^3 cfu mL^{-1}, a total of 29 carbon substrates were metabolized. In a moderately saline water sample (86 ppt, 8.6%), at a higher cell density, the entire range of 31 carbon sources from the plate were metabolized (Phillips *et al*., 2012). In Eilat solar saltern (Israel) and Mono Lake (California, USA), the capacity of the microbial community to use different C sources was tested with BIOLOG GN (Gram-negative) plates. This type of plate contains 95 different carbon sources of which 25 are identical with those in BIOLOG Ecoplates (Table 4). In a previous comparative work it was statistically demonstrated that the two types of plates could reliably be used to discern between the aerobic heterotrophic bacterial communities from various aquatic environments (Choi and Dobbs, 1999). The approach of BIOLOG GN Plates indicated that the Eilat-4 sample (3.9% salinity) from the inlet of the saltern, had the highest carbon metabolization capacity a number of 57 substrates (15 also found in Ecoplates) being used at an estimated cell count of 6 x 10^2 – 3.1 x 10^3 $cfu \cdot mL^{-1}$. The Eilat-3 sample (same 3.9% salinity), also collected from the inlet of solar saltern, had a total capacity of degrading 39 C sources at a higher cell density (2 x 10^4 – 3.5 x 10^5 $cfu \cdot mL^{-1}$). In Mono Lake, the BIOLOG GN plates results revealed that a total of 23 carbon sources were used (5 matches with Ecoplates) at a microbial density of 5.4 x 10^4 $cfu \cdot mL^{-1}$ and a salinity of about 8% (Litchfield and Gillevet, 2002). These findings may suggest that at similar salinity

(3.9 to 8%), the epilimnetic microbial community of Ursu Lake would be more active or at least more receptive to a larger range of C sources than those tested in Eilat solar saltern and the surface shore water of the alkaline Mono Lake (Table 4). The high metabolic potential of Ursu Lake is supported by a recent study of Máthé *et al*. (2014) that dealt with the cultivable and molecular diversity of microbial population along the salinity gradient in the water column. The molecular analysis based on the 16S rRNA gene amplification and analysis showed that all three domains of the life are present at the depth of 0.5 – 1 m. The heterotrophic bacterial isolates likely to degrade some of the C sources present in the Ecoplates under aerobic conditions were assigned to *Pseudoalteromonas* sp., *Idiomarina* sp., *Vibrio* sp., *Marinobacter* sp., *Halomonas* sp., *Thalassospira* sp., *Roseovarius* sp., *Bacillus* sp. and *Staphylococcus* sp. (Máthé *et al*., 2014).

Table 4.

Comparison of carbon utilization pattern assessed by BIOLOG GN plates and BIOLOG Ecoplates in three saline systems. The positive reactions were emphasized with black background

Carbon substrates shared between the two types of BIOLOG plates	**Saltern pond (E-4, Eilat, Israel)[a]**	**Shore water (Mono Lake, USA)[a]**	**Epilimnion (Ursu Lake, Romania)[b]**
Pyruvic acid methyl ester	+	+	+
Tween 40	+	+	+
Tween 80		+	+
Alpha- Cyclodextrin	+	-	+
Glycogen	+	-	+
Cellobiose	+	-	+
Alpha-D-Lactose	+	-	-
Beta-methyl-D-glucoside	-	+	+
i-erythritol	-	-	-
D-mannitol	+	-	+
D,L-alpha-glycerol phosphate	-	-	+
Glucose – 1- phosphate	+	-	+
N-acetyl-D-glucosamine	+	+	+
D- Galacturonic acid lactone	-	+	-
Gamma-hydroxybutyric acid	-	-	+
Itaconic acid	-	-	+
Alpha-ketobutyric acid	+	-	-
L-arginine	+	-	+
L-asparagine	+	-	+
L-phenylalanine	-	-	+
L- serine	+	-	+
L-threonine	+	-	+
Glycyl-L-glutamic acid	+	-	+
Phenylethylamine	-	-	+
Putrescine	-	-	+
Total substrates used	**15**	**6**	**21**

[a] *- data from Litchfield and Gillevet (2002).*
[b] *- Data from March 2013 sample, present study.*

The capacity to use different carbon sources by the microbial community plays a key role in maintaining the equilibrium of the organic substrate composition and implicitly in the carbon cycling within aquatic ecosystems (Christian and Lind, 2006; Weber *et al*., 2008). For example, the hypothetical occurrence of N-acetyl-D-glucosamine in the epilimnion of Ursu Lake and subsequent appetite for its metabolization can be explained through the presence of Gram-positive bacterial population. This monosaccharide is a major component of the bacterial cell wall that could be released in the surroundings by bacterial decomposition and further used as carbon substrate by other microbes. Plant materials originating from the lush temperate vegetation neighboring Ursu Lake could provide other organic compounds like D-cellobiose, D-mannitol, 4-Hydroxy benzoic acid (also present in algae). Some of the amino acids used in the investigated Ecoplates (L-arginine, L-serine, L-asparagine, L-threonine, L-phenylalanine) as well other compounds such as itaconic and malic acids (derived from the Krebs cycle) or phenylethylamine, might be released by microbial decomposition of the organic matter.

The epilimnion is the upper layer of water (Baricz *et al*., 2014), a place with a strong interplay between the freshwater inflow, organic matter input, and fluctuating air conditions that ensures the presence of a large spectrum of microbial diversity with a potent metabolic availability. The ability of the epilimnetic microbial community in Ursu Lake (and elsewhere) to metabolize various carbon sources is crucial in preserving the balance between the formation of organic compounds or toxic substances (e.g. putrescine) and the normal biochemical parameters of water layer. Interestingly, biopolymers such as alpha-cyclodextrin, glycogen and non-ionic detergents Tween 40 and Tween 80 are quickly metabolized in both samples, suggesting that the epilimnion of Ursu Lake may host very active polymer decomposers that could be isolated and used as organisms with potential in food industry and bioremediation.

Conclusions

The results of assessing the carbon utilization pattern by the BIOLOG Ecoplate™ approach indicated a promising metabolic potential of the microbial community from the saline epilimnion of Ursu Lake. By a comparative analysis of water samples collected during two seasons (autumn and spring) it has been observed that the epilimnetic aerobic microbial community is readily degrading a broad spectrum of carbon sources at moderate salinity values (5–6%).

Apparently, our results suggested a higher activity of carbon substrate degradation during spring season, a broader range of C substrates being oxidized by a less denser cell population, at slightly lower salinity and higher ORP. The peculiar nature of meromictic, heliothermal Ursu Lake as well as its importance for the local economy encourages further in-depth investigations on the composition and diversity

of the microbial community, its metabolic activity and role in biochemical cycling of major elements with respect to varying enviornmental conditions and human impact on the lake.

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