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**EFFECT OF REED-COVERAGE ON THE MICROBIAL
COMMUNITY COMPOSITION OF LAKE FERTŐ**

-PhD thesis-

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I. Introduction

Lake Fertő/Neusiedler See, the westernmost steppe lake of Europe, is shallow with slightly alkaline water. The major ions dissolved in the moderately saline water are Na^+ , Mg^{2+} , HCO_3^- and SO_4^{2-} . Extensive reed-stand (*Phragmites australis*) covers 55% of the total lake area, encompassing several smaller open water bodies, i.e. inner ponds. The open water and the reed covered areas have similar ionic composition, pH and conductivity but there are great differences in their transparency and colour. The colour of the water in the reed-associated areas is brownish due to the high amount of coloured dissolved organic matter (humic substances) originated from plant residues. The increase of macrophyte dominance does not only mean increasing dissolved organic carbon (DOC) supply, but also causes changes in the composition and biological availability of the DOC pool depending on the type of macrophytes and environmental factors. The increase in macrophyte dominance can affect the bacterial abundance, activity and could modify the diversity of microbial communities, as well. However, little is known about the metabolic importance of bacterioplankton in macrophyte-dominated littoral systems, where a considerable amount of DOC originates from aquatic macrophytes instead of the phytoplankton. Elucidating the relationship between the bacterioplankton and the environmental factors regulating their activity is crucial to understand how aquatic ecosystems function.

II. Aims

Among the lakes in Hungary Lake Fertő proved to be one of the most suitable lakes for observing the effect of reed on the microbial community: more than the half of its area is covered by reed, but it has open water bodies as well. Thus, these two types of habitats could be compared. Most of the studies regarding the bacterial community composition of the water and sediment of Lake Fertő were based on cultivation techniques and there is a lack of knowledge of the archaeal community composition of this lake. Therefore, the followings were set as the aim of our study:

1. Revealing the effect of the reed-coverage on the bacterial and archaeal community composition and diversity of Lake Fertő in an open water area (B0) and two reed-associated habitats (Kis-Herlakni inner pond and reed-covered area) with (a) DNA-based analyses and (b) cultivation techniques.

2. Comparing the microbial community composition of the water and sediment samples.
3. Comparing the microbial community composition of the late autumn and summer samples of Lake Fertő.
4. Estimating the number of microbes participating in different metabolic activities in Lake Fertő.
5. Revealing the effect of the reed-coverage on the substrate utilisation profile of the microbial community of the water samples of Lake Fertő.
6. Estimating the role of microorganisms participating in the nutrient cycles (carbon, nitrogen and sulphur cycles) of Lake Fertő.
7. Since the cultivable bacterial community composition of Lake Fertő was previously studied only by applying media with high organic matter content, we assumed that some previously uncultured bacteria could also be isolated with the application of our media with low organic matter content. Consequently, the identification and characterisation of the previously uncultured bacterial taxa were set as one of the aims of this study.

III. Materials and Methods

The water and sediment samples were taken on 10 November 2015 and on 18 July 2016 from three different sites of Lake Fertő: the open water (B0 site; 47.73459 N 16.71941 E) situated on the Hungarian-Austrian border, the Kis-Herlakni inner pond (KH; 47.68460 N 16.70272 E) and from a reed-covered area accessed from the artificial External Belt Channel (R; 47.65432 N 16.72517 E) (Fig. 1). Water samples were collected using a tube sampler, providing representative samples of the entire water column. Sediment samples were collected with a sediment core sampler.



Figure 1. The open water site (point B0) (a), Kis-Herlakni inner pond (b) and reed-covered area (c) of Lake Fertő.

Water depth, temperature Secchi-transparency, pH and conductivity were measured at the sites. The concentration of total suspended solids (TSS) was determined according to Eaton *et al.*

(1995). CDOM content (coloured dissolved organic matter), expressed as platinum units [mg Pt L^{-1}], was measured according to Cuthbert and Del Giorgio (1992).

The bacterial and archaeal community composition of the water and sediment samples of Lake Fertő was revealed by 16S rRNA gene amplicon sequencing and cultivation-based techniques. Water samples (250-250 mL) were filtered through a 0.22 μm pore size mixed cellulose filter for next-generation sequencing. DNA was isolated from the filters and from the homogenised sediment samples using the Ultraclean[®] PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA). For the identification of bacterial and archaeal taxa the V3-V4 region of the 16S rRNA gene was amplified using primers with the following target-specific sequences: Bact 341F (Herlemann *et al.* 2011) and modified Bact 805R for Bacteria (Apprill *et al.* 2015) and Arch519F (Teske and Sorensen, 2007) and Arch855R (Yoshida *et al.* 2005) for Archaea. Methodological details of sequencing and the description of bioinformatic analysis were performed according to Benedek *et al.* (2019). Operational taxonomic units (OTUs) were assigned at 97% similarity threshold levels (Tindall *et al.* 2010). Shannon-Weaver and inverse Simpson (1/D) diversity indices (evaluating OTU evenness) and Chao-1 and ACE (Abundance-based Coverage Estimator) richness metrics (estimating the total number of species) were calculated from the subsampled reads using mothur v1.41.1. To determine significant differences between bacterial and archaeal communities of water and sediment samples, a one-way ANOSIM (ANalysis Of SIMilarities) test was carried out based on Bray-Curtis similarity using the Past 3 software (Hammer *et al.* 2001). To visually interpret the dissimilarities of the communities at OTU level non-metric multidimensional scaling (NMDS) ordinations were carried out. Bacterial strains were isolated from the six water samples using modified R2A (Reasoner and Geldreich, 1985) and a modified low carbon-containing medium (M4; Kéki *et al.* 2013). DNA from bacterial strains was isolated, then the 16S rRNA gene was amplified with primers 27F and 1492R as described by Kalwasiński *et al.* (2015). PCR products were grouped by amplified ribosomal DNA restriction analysis (ARDRA). Group representatives and ungrouped samples were chosen for 16S rRNA gene sequence analysis in order to identify the selected bacterial strains. To determine the most probable number [MPN mL^{-1}] of heterotrophic bacteria growing under aerobic conditions and fermentative acidogenic bacteria, 10-fold serial dilution was applied on microtiter plates (Rowe *et al.* 1977).

Biolog[®] EcoPlate was used for community level physiological profiling as previously described by Gryta *et al.* (2014) to test the utilisation of 31 different carbon sources in three replications.

Altogether, 202 ARDRA representative strains were used to test the utilisation of cellulose and reed extract. For reed extract utilisation, a solid medium based on Gordon and Mihm (1957) was applied with reed extract (2 w/v%) as the sole carbon source.

Two novel bacterial genera were described based on their genotypic (16S rRNA gene sequence and genomic DNA) and phenotypic data (classical and rapid identification methods, chemotaxonomic analysis).

IV. Results and Discussion

Among the physical and chemical parameters higher amounts of TSS (total suspended solids) values were detected at the open water site (where the fine sediment is permanently resuspended by the wind) than in the KH and R samples. At the open water site significantly lower ($p = 0.005$) Secchi-transparency was detected than in the reed-associated areas, which were transparent almost to their bottom. These sites were sheltered from wind-induced sediment resuspension. The concentration of CDOM was the lowest at point B0, while in the inner pond and the reed-covered area significantly higher values ($p = 0.004$) were present as a result of the humic substances that originated from the decomposition of organic plant material. The plant materials colour the water brownish (Schauer *et al.* 2015).

The MPN of aerobic heterotrophic bacteria was significantly higher at the reed-associated sites (KH, R) than at the B0 site in the water and sediment samples ($p = 0.014$ and $p < 0.001$, respectively). In case of the MPN of fermentative acidogenic bacteria a considerable difference was not detected between the reed-associated sites and the open water site.

Based on the results of amplicon sequencing, the difference was negligible between the ACE and Chao-1 indices of the bacterial community with respect to the reed coverage. However, significantly higher ($p < 0.001$) archaeal richness was detected in the samples of the reed-associated areas than at the B0 site in the water and sediment. Shannon diversity and inverse Simpson indices were similar in case of the bacterial communities of water samples. However, inverse Simpson indices showed significantly higher ($p < 0.001$) bacterial diversity in the sediment of the reed-covered area in November than in the other samples. A notably elevated ($p \leq 0.001$) archaeal diversity was detected in the reed-associated areas than at the open water site in the water and sediment samples. On the basis of our results we assume that plant material could result in increased archaeal diversity.

The indices of richness and diversity showed significantly higher ($p < 0.01$) bacterial diversity in the sediment samples than in the water samples, contrary to the archaeal richness and

diversity values, which were higher ($p < 0.05$) in the water samples. According to the presumption of Swan *et al.* (2010), the high concentration of carbon in the sediment of Lake Fertő could not provide a suitable environment for archaea to thrive. On the other hand, the abundance of archaea can be very low in these moderately saline soda lakes ($1\text{--}2 \text{ g L}^{-1}$ salinity), as it was observed in previous studies (Szabó *et al.* 2020).

Altogether, 55 bacterial and 8 archaeal phyla were identified by amplicon sequencing from the samples. Based on the ANOSIM test, the bacterial and archaeal communities of the water samples were significantly different ($p < 0.01$, $R = 0.98$ and $R = 0.83$, respectively) from the communities inhabiting the sediment of the same sites. The bacterioplankton was dominated by Proteobacteria, Bacteroidetes and Actinobacteria, while in the sediment samples Chloroflexi was also dominant in addition to Proteobacteria. In all of the water samples, phylum Nanoarchaeota was dominant, while in the sediment samples members of Euryarchaeota and Crenarchaeota were abundant.

According to the results of NMDS ordination, the bacterial and archaeal community composition in Kis-Herlakni inner pond and the reed-covered area was quite distinct from the prokaryotic community composition of the open water. In the open water, the ratios of Actinobacteria and Cyanobacteria were higher than in the water of the Kis-Herlakni inner pond and the reed-covered area. However, phyla Bacteroidetes appeared in higher relative abundance in the macrophyte-dominated regions than in the open water. Those taxa which prefer a lower concentration of dissolved organic matter (e.g. LD12, acTH1, acIV) and/or higher concentration of TSS (*Cyanobium*) were detected more often in the open water, while those taxa which could utilise CDOM as a carbon source (e.g. *Flavobacterium*, *Hydrogenophaga*) were abundant at the reed-associated sites during the two sampling times. In the case of the archaeal community, phylum Crenarchaeota was detected in the highest ratios in the water and sediment samples of point B0. Overall, reed coverage can significantly affect the microbial communities in the water and sediment samples, as the relative abundance of several taxa were different at the open water and the reed-associated sites.

Altogether, 559 bacterial strains were isolated and identified from the water samples of Lake Fertő. Most strains showed 98–100% similarity values to the reference sequences of type strains in the EzBioCloud database (Yoon *et al.* 2017) based on their 16S rRNA gene sequences. Members of 5 bacterial phyla were identified from the water samples by cultivation-based techniques. Mostly members of Proteobacteria were isolated from the samples, but representatives of Actinobacteria, Firmicutes and Bacteroidetes were also identified. Strains of

phylum Verrucomicrobia were only cultivated from Kis-Herlakni inner pond and the reed-covered area.

Significant differences between the bacterial community composition of the late autumn and summer samples of Lake Fertő were observed only at genus level. However, temporal changes had a greater effect on the archaeal community composition. Members of phylum Euryarchaeota were detected in higher relative abundance in November than in July in the water samples of point B0. Phylum Nanoarchaeota appeared as a major component only in the reed-covered area in November. Berdjeb *et al.* (2013) suggested that Bacteria and Archaea may be influenced by different factors and could occupy different ecological niches despite similar spatio-temporal dynamics.

Polymers were the most utilised substrates (Tween 40, Tween 80, glycogen) of planktonic microbial communities at all sampling sites. We hypothesise that the lake hosts active polymer decomposers due to the higher concentration of high-molecular-weight organic compounds released by the decaying reed. The utilisation of D-cellobiose was high in the reed-covered area in July. Most of our isolated bacterial strains (72%) were able to grow on a medium containing reed extract as the sole carbon source. Therefore, we hypothesise that they might have an actual role in the biodegradation of reed-derived organic matter.

On the basis of the results of amplicon sequencing, the carbon, sulphur and nitrogen cycles could be complete in Lake Fertő. However, the taxa that presumably play a role in these cycles were different in the open water and the reed-dominated areas.

40 strains isolated from the water of Lake Fertő showed similarities lower than 98% to the reference sequences of type strains in the EzBioCloud database (Yoon *et al.* 2017) based on their partial 16S rRNA gene sequences. From these strains two new bacterial genera were described. Members of the first new genus (strains DMA-K-7a^T, DMA-K-1, DMG-N-1) showed the highest level of sequence similarity to *Rhabdobacter roseus* R49^T (95.66 %) in the family *Cytophagaceae* based on their 16S rRNA gene sequences. The name *Arundinibacter roseus* gen. nov., sp. nov. was proposed (Szuróczki *et al.* 2019) for the strains of this new genus and species. Member of the second new bacterial genus (strain MG-N-17^T) showed the highest level of sequence similarity to *Verrucomicrobium spinosum* DSM 4136^T (94,38 %) in the family *Verrucomicrobiaceae*. The name *Phragmitibacter flavus* gen. nov., sp. nov. was proposed (Szuróczki *et al.* 2020) for the strain of the new genus and species.

V. Thesis

- The reed-coverage has an effect on the bacterial and archaeal community composition, as significant differences were detected between the prokaryotic composition of the open water and the two reed-associated habitats. This could be caused by the higher amount of decaying organic matter originated from the reed, since the concentration of CDOM (coloured dissolved organic matter) was significantly higher in the reed-associated areas than at the open water site.
- Significantly higher archaeal richness and diversity were detected at the reed-associated sampling sites than at the open water site.
- The reed-coverage has an effect on the most probable number of aerobic heterotrophic bacteria, as significantly higher amounts were estimated in the reed-associated areas.
- Microbial communities of water samples were considerably different from communities inhabiting the sediment of the same sites. The ACE and Chao-1 richness metrics and Shannon-Weaver and inverse Simpson (1/D) diversity indices showed significantly higher bacterial diversity in the sediment samples than in the water samples, contrary to the archaeal richness and diversity values, which were higher in the water samples.
- The bacterial and archaeal community composition of the late autumn and summer samples were different. While in case of the bacterial community composition significant differences were observed only at genus level, temporal changes had a greater effect on the archaeal community composition, as the relative abundance of archaeal phyla was also changed.
- On the basis of carbon source utilisation profile, polymers and carbohydrates were the most utilised substrates by the planktonic microbial community of Lake Fertő. The planktonic microbial communities of point B0 differ from the reed-associated areas according to the results of the carbon source utilisation. The utilisation of D-cellobiose was the highest in the water samples of the reed-covered area in July, which was probably due to the higher amount of organic plant matter. The open water and the reed-covered area had the strongest carbon source utilisation activity, which also depended on the seasons.
- The isolated bacterial strains from Lake Fertő play a role in the decomposition of organic matter originated from the reed, because almost 75% of the isolated strains could utilise reed extract as a sole carbon source.
- Microorganisms participating in the carbon, sulphur and nitrogen cycles could be detected in Lake Fertő. Therefore, we assume that these cycles could be complete at B0 site, in the Kis-Herlakni inner pond and in the reed-covered area.

- Some previously uncultured bacteria were also isolated from the water of Lake Fertő. Two new bacterial genera were described: the names of *Arundinibacter roseus* and *Phragmitibacter flavus* were proposed.

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