EXPLORING THE UNKNOWN DIVERSITY OF PHOTOTROPHIC BACTERIA IN SODA PANS

Grant No. PD 105407

- F I N A L R E P O R T -

As stated in the proposal, the project focused on the identification, diversity and distribution of PPB (phototrophic purple bacteria) in soda pans and aimed to answer the following questions:

1. Which genera and species of PPB are present in the soda pans of the Carpathian Basin?
2. What kind of environmental conditions favor the rise of PPB taxa in soda pans?
3. What is the seasonal distribution of the PPB community in soda pans? Which environmental factors are the most important drivers of community changes?
4. Where are these bacteria preferably found in soda pans, in the sediment surface layer or in the water phase?

Since we had the opportunity to collect samples from other saline habitats and it seemed that some PPB are associated with green algae, the project was amended with two additional research questions:

5. Are the Hungarian soda pans unique regarding their bacterial communities compared with other saline lakes?
6. Which chlorophyte genera are present in these soda pans and what is their distribution?

And as we did not have the opportunity to study as much soda pans in the first years as we planned (a lot of pans were completely desiccated), a soda pan having a very diverse bacterial community was selected for a detailed functional diversity analysis. Therefore an additional research question was formed:

7. How bacteria adapt to the multiple environmental stress conditions present in the soda pans of the Carpathian Basin?

Acknowledgement

First of all, I would like to thank the hard work of my PhD students, Attila Szabó and Kristóf Korponai (Department of Microbiology, Eötvös Loránd University) regarding molecular microbiological analyses. I am very grateful for Boglárka Somogyi, Balázs Németh, Emil Boros and Lajos Vörös (Balaton Limnological Institute, HAS, Tihany) for providing continuous support in sample collection, limnological analyses and for the fruitful scientific discussions. I also express my sincere gratitude to all my students and co-authors who contributed to the results of this project.

I.
BACTERIAL COMMUNITIES IN THE SODA PANS OF KISKUNSÁG

For a high-throughput pyrosequencing study, samples were collected monthly from April 2013 to July 2014 (>30 samples) from two soda pans, Sós-ér representing the coloured type and Zab-szék representing the turbid type according to Boros *et al.* (2014). In general, their bacterial composition was dominated by *Actinobacteria* (30%), *Proteobacteria* (25%), *Bacteroidetes* (23%), *Cyanobacteria* (6%) and *Verrucomicrobia* (5%). Genus-level composition with the co-occurring patterns of taxa is shown on **Figure 1**.

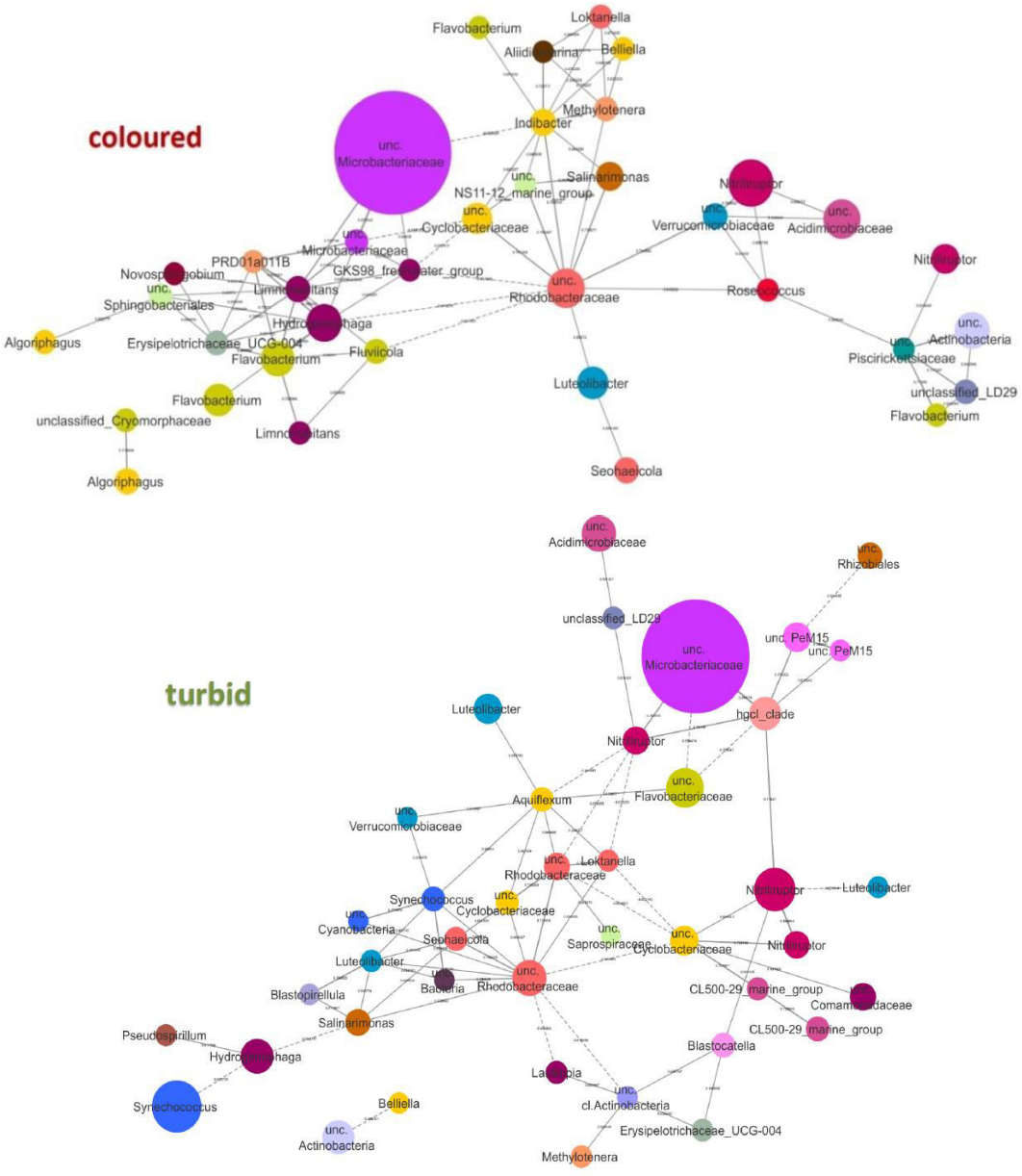

Figure 1 Network of co-occurring OTUs based on the correlation analysis of the 16S rRNA gene pyrosequencing dataset of soda pan samples

A connection stands for a strong (> 0.6) and significant ($p < 0.005$) correlation. Node size corresponds to the relative abundance of the OTU in the dataset. OTUs were assigned to the closest genus, node-colouring is based on the phylogenetic class assignment. OTUs were created using 97% 16S rRNA gene sequence similarity cut-off. Analysis was based on 156,106 high-quality sequences.

The most abundant phylum in the studied soda pans was *Actinobacteria* with a relative contribution ranging between 1.6-56.8% (average 19.7%) and 2.5-88.6 (average 33.6%) in the coloured and turbid soda lake, respectively (**Figure 2**). In the latter habitat, lineages (acTH1, acSTL and acIV) having no cultivated members (Newton *et al.*, 2011) were present as a major fraction within this group. Furthermore, recently it was discovered that several aquatic actinobacteria are capable to use the energy of sunlight via an actinorodopsin-based light-driven proton-pump (Sharma *et al.*, 2009).

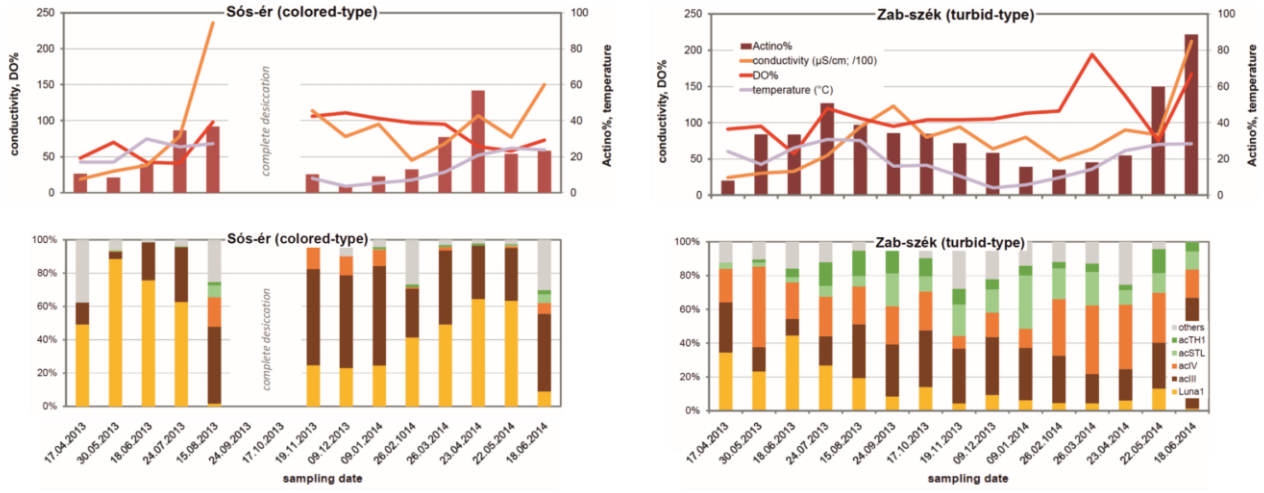

Figure 2 Seasonal distribution of phylum *Actinobacteria* in the bacterioplankton of the studied coloured and turbid soda pan of the Carpathian basin

Upper graphs, relative abundance the members of phylum *Actinobacteria* based on pyrosequencing reads (Actino%) and fluctuation of selected environmental parameters; *lower graphs*, relative abundance of main actinobacterial taxa according to Newton *et al.* (2011).

Within the second largest phylum, *Proteobacteria*, the dominance of class *Alphaproteobacteria* was observed (representing 41.2% and 54.7% of *Proteobacteria* in the coloured- and turbid-type pan, respectively). Several genera (*Roseococcus*, *Rhodobaca* and *Salinarimonas*) with strains capable (or putatively capable) to photoheterotrophic growth (Brenner *et al.*, 2005; Cai *et al.*, 2011) were identified in this class, mainly affiliated with the order *Rhodobacterales*. In general, other identified genera contained mainly aerobic heterotrophic bacteria with many halophilic or halotolerant members [*Altererythrobacter*, *Loktanella*, *Seohaeicola*, *Pseudospirillum*, *Salinarimonas*, *Aliidiomarina*, *Idiomarina*, *Flavobacterium* and *Indibacter* (Cai *et al.*, 2011; Krieg *et al.*, 2010; Jung *et al.*, 2014; Chiu *et al.*, 2012; Van Trappen *et al.*, 2004; Anil Kumar *et al.*, 2014; Yoon *et al.*, 2009; Satomi *et al.*, 2002)], some of them were even alkaliphilic [*Mongoliicoccus* and *Nitriliruptor* (Liu *et al.*, 2005; Goodfellow *et al.*, 2012)] corresponding with the relatively high pH (~9.5) and salinity (~7 g/L) of these pans.

We identified tight connections among photoautotropic and heterotrophic (including also photoheterotrophic) microorganisms. Two main metabolic types of this latter group were (i) bacteria growing on algal-derived small organic compounds, e.g. amino acids and sugars (represented by the previously mentioned clade *Rhodobacterales*), and (ii) bacteria metabolizing high molecular weight organic compounds from decomposing algal cells (represented by the clade *Flavobacteria*). Similar phenomenon was reported recently in the oceans by Buchan *et al.* (2014) (see additional details in the section describing the results of shotgun metagenomic analysis). Relationships among the relative abundance of bacterial taxa and measured environmental variables were also retrieved by statistical analyses (**Figure 3**).

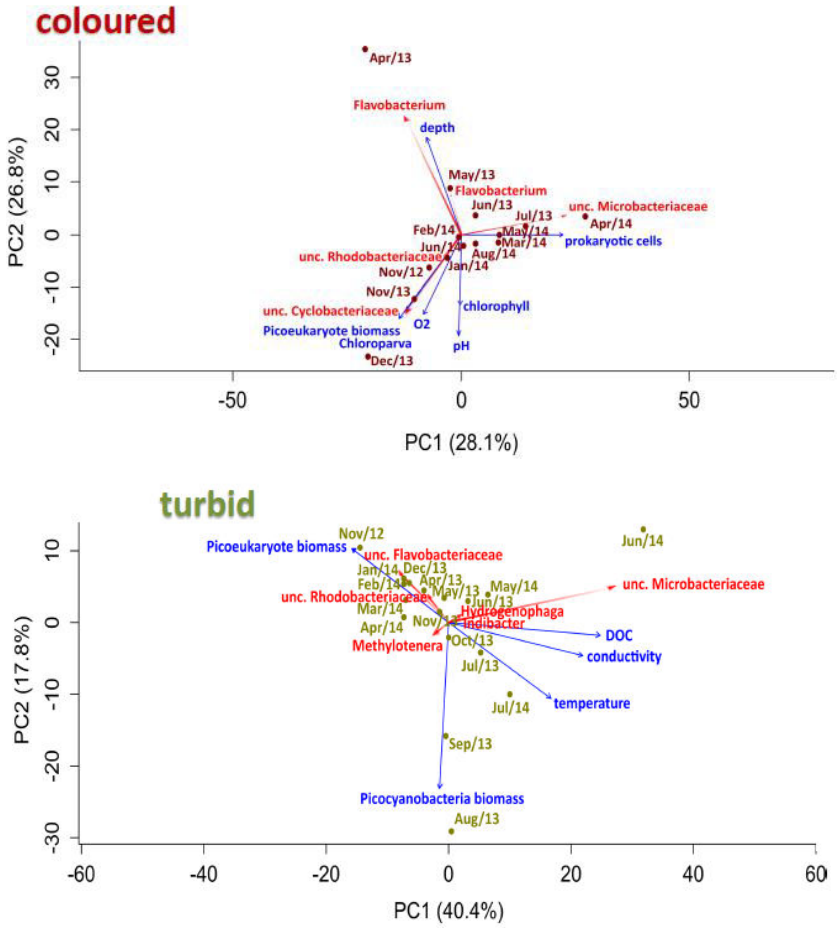

Figure 3 Principal component analysis of samples to retrieve the relation between the OTU composition of the studied soda pans and measured environmental parameters

Codes of the samples refer to the month/year of sampling. Genus-level taxonomic affiliation of OTUs responsible for the variation among samples based on *simper test* appears in red as biplot on the ordination. Significantly-fitted ($p < 0.01$) environmental parameters appear in blue. OTUs were created using 97% 16S rRNA gene sequence similarity cut-off.

Regarding species richness, the turbid-type Zab-szék harbored more bacterial species (171-1090, average 479) compared with the coloured-type Sós-ér (84-436, average 268; based on the ACE richness estimator using 97% OTU similarity cut-off values for the 16S rRNA gene).

Taken together, it seems that desiccation and algal blooms have fundamental effect on the bacterial communities of the studied soda pans, and the turbid environment of Zab-szék [i.e. continuous mixing, a 'fluid sediment' *sensu* Eiler *et al.* (2003)] provides a specific milieu and supports a much diverse microbial community with higher contribution of uncultivated taxa compared to the coloured-type Sós-ér.

In the shotgun approach, quality-filtering resulted 497,312 high-quality reads with a mean length of 170 ± 48 nt (overall 84.6 Mbp sequence data) with the following taxonomic assignment: 96.0% *Bacteria*, 0.1% *Archaea*, 2.0% *Eukaryota* and 1.9% viruses. Viral sequences were dominated by hits assigned to bacteriophages (mainly *Caudovirales*), which may control bacterial community composition and through host cell lysis affects the availability of organic carbon compounds and nutrients (Wilhelm and Shuttle, 1999; Mühling *et al.*, 2005; Atanasova *et al.*, 2015). The surprisingly low abundance of *Archaea* in the Hungarian soda pans was confirmed recently by real-time PCR (Attila Szabó and Tamás Felföldi, unpublished results).

Table 1 Process-related assignment of shotgun reads retrieved from the Büdös-szék soda pan sample (29th November 2012)

Metabolic pathways/Adaptation mechanisms	*Count*	*%*	*Examples*	*Count*
Algae-derived organic matter uptake (TBDT system and related enzymes)	981	2.23		
TonB-dependent transporter system	793	1.80	TonB-dependent receptors	758
Glycoside hydrolases	151	0.34	COG2152 predicted glycoside hydrolase	45
			COG1649 predicted glycoside hydrolase	34
Aminopeptidases	37	0.08	Proline iminopeptidase (EC 3.4.11.5)	14
			Asp-X dipeptidase	11
			Deblocking aminopeptidase (EC 3.4.11.-)	7
One-carbon metabolism	1253	2.84		
One-carbon metabolism by tetrahydropterines	93	0.21	Formate-tetrahydrofolate ligase (EC 6.3.4.3)	44
			Methanol dehydrogenase large subunit protein (EC 1.1.99.8)	40
Serine-glyoxylate cycle	1135	2.57	Serine hydroxymethyltransferase (EC 2.1.2.1)	61
			Phosphoglyceromutase	60
Ribulose-monophosphate pathway	25	0.06	Formaldehyde activating enzyme	16
Autotropic CO_2-fixation	928	2.11	Carbonic anhydrase (EC 4.2.1.1)	32
			Carboxysome NADH dehydrogenase (EC 1.6.99.3)	21
DNA repair mechanisms - UV stress	1959	4.44	Excinuclease ABC subunits A, B and C	421
			DNA polymerase I (EC 2.7.7.7)	158
			ATP-dependent DNA helicase RecQ	96
			DNA mismatch repair protein MutS	94
			ATP-dependent DNA helicase UvrD/PcrA	89
Oxidative stress – reactive oxigen species	757	1.72	Thioredoxin	87
			Gamma-glutamyltranspeptidase (EC 2.3.2.2)	64
			Hydrogen peroxide-inducible genes activator	30
			Manganese superoxide dismutase (EC 1.15.1.1)	25
			Superoxide dismutase [Fe] (EC 1.15.1.1)	22
Adaptation to alkalinity	544	1.23		
Na^+/H^+ antiporters	196	0.44		
Smf-driven mechanisms	133	0.30	Acetate permease ActP (cation/acetate symporter)	27
			Na^+/pantothenate symporter (TC 2.A.21.1.1)	17
			Na^+/glycine symporter GlyP	16
Biogenesis of cbb_3-type cytochrome-c oxidases	98	0.22	Type cbb3 cytochrome oxidase biogenesis protein CcoI	51
			Type cbb3 cytochrome oxidase biogenesis protein CcoG	37
Rnf complex	117	0.27	Na^+-translocating NADH-quinone reductase subunit B (EC 1.6.5.-)	22
			Na^+-translocating NADH-quinone reductase subunit F (EC 1.6.5.-)	22
			Electron transport complex protein RnfC	10
Adaptation to salinity - Osmotic stress	426	0.97		
'Salt-out' osmoadaptive strategy (e.g. ectoine, betaine)	83	0.19	Choline-sulfatase (EC 3.1.6.6)	24
			Sarcosine oxidase (EC 1.5.3.1)	21
			Choline dehydrogenase (EC 1.1.99.1)	10
'Salt-in' osmoadaptive strategy (e.g. KCl accumulation)	343	0.78	Trk system K^+ uptake protein TrkA	26
			K^+ uptake protein TrkH	26
			Glutathione-regulated K^+-efflux system ATP-binding protein	25
			K^+ voltage-gated channel subfamily KQT	18
			Osmosensitive K^+-channel histidine kinase KdpD (EC 2.7.3.-)	15

Abbreviations: *Smf* – Na^+-motive force, *Rnf* – Ferredoxin-NAD-oxidoreductase

A total of 165,823 functional hits of shotgun reads were identified using the SEED classification in MEGAN and 44,083 were assigned to subsystems. Results showed a functionally complex community with several genes related to the harsh environmental conditions present in the studied soda pans (**Table 1**).

According to the obtained data, the following processes and mechanisms related to planktonic bacteria could be presumed. Residence of aquatic birds and algal blooms provide high nutrient supply (Boros *et al.*, 2008; Somogyi *et al.*, 2009), which results in the high abundance of heterotrophic organisms (Vörös *et al.*, 2008), such as members of phylum *Bacteroidetes*. These bacteria (especially from the order *Flavobacteriales*) favor to attach to organic particles and have high abundances in nutrient-rich habitats (Williams *et al.*, 2013), since they participate in the degradation of biopolymers, such as algae-derived particulate organic matter (Buchan *et al.*, 2014; Xing *et al.*, 2015). Genes encoding receptors of the TonB-dependent transporter (TBDT) systems, responsible for biopolymer uptake (Williams *et al.*, 2013), were among the most abundant genes in the shotgun metagenomic dataset. TBDT-related degradative enzymes (e.g. glycoside hydrolases) were also identified. Furthermore, similarly to *Flavobacteriales*, members of *Rhodobacterales* are also abundant during phytoplankton blooms in marine environments using algal exudates as substrate (Williams *et al.*, 2013; Buchan *et al.*, 2014; Teeling *et al.*, 2012). Based on the results of shotgun metagenomics and the community structure profile, it could be hypothesized that these bacterial groups could have similar functions in the studied soda lakes as in the oceans.

Genes involved in the serine-glyoxalate cycle and other pathways related to one-carbon metabolism were also abundant, most probably due to methane and C_1-compounds originating from the sediment, which are subsequently utilized by methylotrophic bacteria (Sorokin *et al.*, 2015). Genus *Methylotenera* was a characteristic methylotrophic bacterium in the 16S rRNA gene amplicon sequencing data (**Figure 1**). Although most of the inhabiting microorganisms have chemoheterotrophic lifestyle, several gene components related to CO_2-fixation were also found.

Since Büdös-szék was close to desiccation in the time of sampling with low water depth, the whole water body could have been exposed to UV radiation, probably this resulted the high abundance of genes related to DNA repair mechanisms. Genes (e.g. thioredoxin, superoxide dismutase) involved in the response to oxidative stress caused by reactive oxygen species generated in the aerobic water by solar irradiation (Williams *et al.*, 2013) were also abundant. Furthermore, organisms have to adapt to the high pH, and the high ionic content could be another source of stress. To maintain the optimal intracellular pH, bacteria can use Na^+/H^+ exchange channels, while for maintaining osmotic balance in the studied habitat, it seems that the 'salt-in' strategy (Oren, 1999; Banciu and Muntyan, 2015) is preferred rather than the synthesis and uptake of compatible solutes ('salt-out') (Oren, 1999).

Additionally, a purple bacterium bloom was studied in detail (with microscopic, cultivation-based and molecular microbiological methods), which was observed in a small unnamed soda lake in April 2013 (**Figure 4**). In this case, two dominant genera, *Ectothiorhodospira* and *Rhodobaca* caused this unusual bloom event. The characteristic vibrio-shaped, motile cells with sulphur granules of the former genus were recorded during the microscopic investigations. These cells showed also the phenomenon phototaxis.

For the isolation of bacreria, two new culture media were developed and applied parallel with a conventionally used medium (R2A). We also applied an unconventional solidifying agent, gellan gum, in our new culture media, which proved to be a very effective component. Both aerobic and anaerobic plate counts were recorded, which were above 10^7 CFU/mL.

A potential new bacterial genus (related to the genus *Aquiflexum*) and several new species candidates were isolated (potential new species of the genera *Belliella*, *Pseudomonas*, *Nitrincola*, *Rhodobaca* and *Roseinatronobacter*). The taxonomic characterization one of the new species candidates, *Nitrincola* sp. R4-8

has been initiated. The strain was also deposited in two culture collections (DSMZ in Germany and NCAIM in Hungary). The proposed name for the new species is *'Nitrincola schmidtii'* (in the honour of Antal Schmidt, who has extensively studied the soda pans in Hungary). Other isolated species included members of the genera *Porphyrobacter*, *Loktanella*, *Roseicitreum*, *Halomonas*, etc.

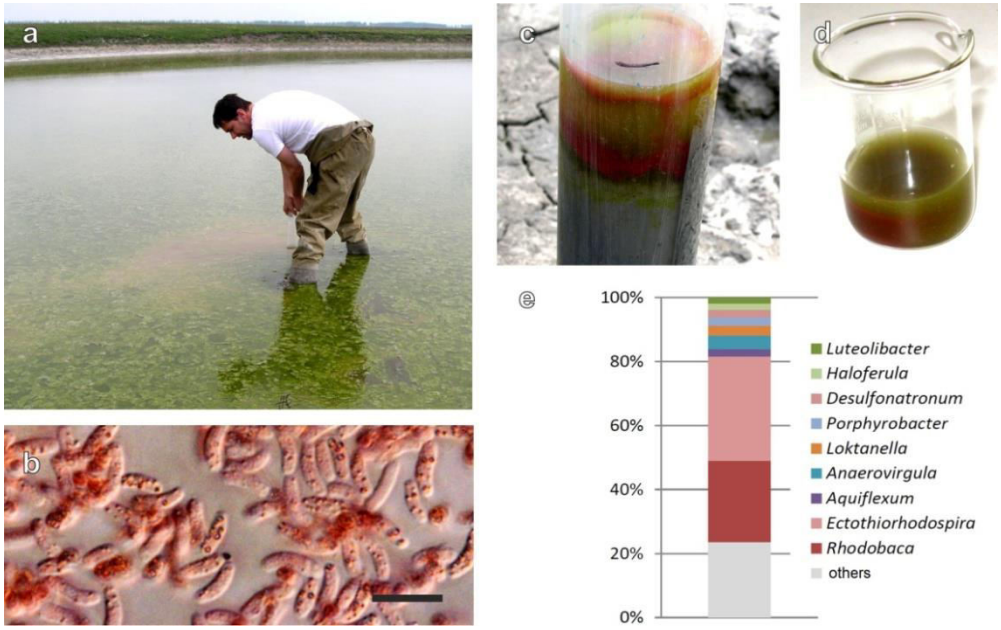

Figure 4 Dual bloom of green algae and purple bacteria observed in a small soda pond near Soltszentimre on 23rd April 2014

a Surface water covered by cells of *Oocystis* (Chlorophya) with Balázs Németh conducting sample collection; **b** micrograph of purple bacteria in the lower sample layer; *scale bar*, 10 µm; **c, d** separation of the green- and purple-coloured layer after sample collection; **e** composition of the bacterial community revealed by pyrosequencing (contribution of genera expressed as relative abundance); *(Photos Fig. 4ac are the courtesy of Boglárka Somogyi).*

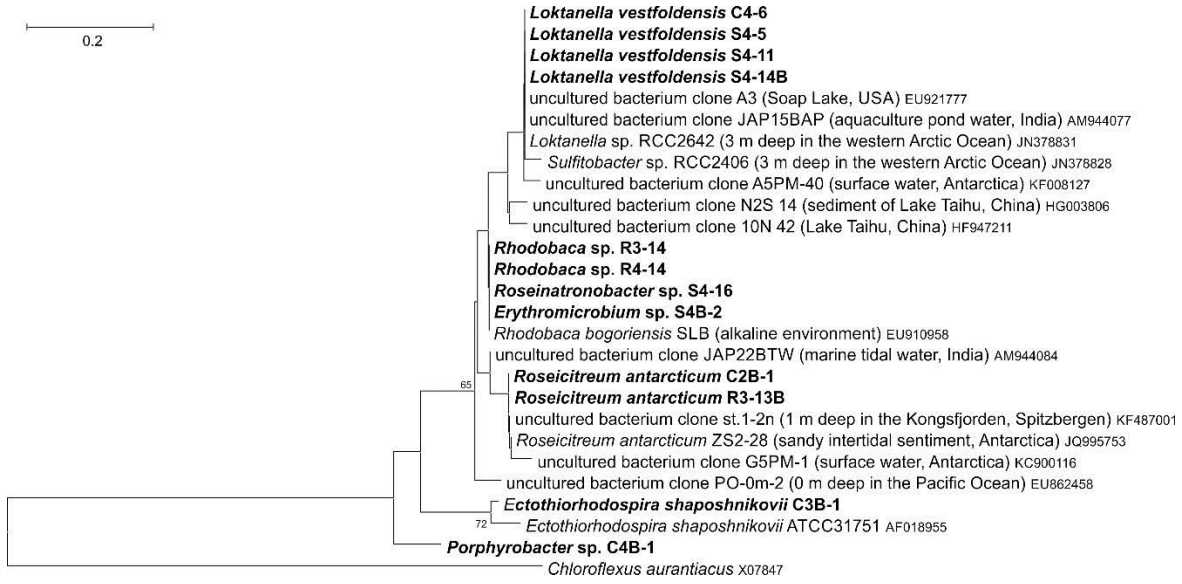

Figure 5 *PufM*-based phylogenetic postion of bacterial isolates retrieved from the dual bloom observed in a small soda pond near Soltszentimre on 23rd April 2014

Tree was reconstructed using the Neighbor-Joining method, evolutionary distances were computed using the JTT matrix-based method based on 35 amino acid positions. Sequences determined in this project appear in bold.

Several purple- or red-colored soda lake isolates contained bacteriochlorophyll or catorenoids, which were confirmed by *in vivo* absorption spectrum analysis and infrared microscopy. The gene that encodes a part of the light reaction center complex, *pufM*, was amplified and sequenced from 12 isolates (**Figure 5**). The deviation of *pufM* phylogeny from 16S phylogeny in case of some isolates indicated horizontal gene transfer event, as it observed by others (Koblížek *et al.*, 2015) among members of the order *Rhodobacterales*.

Providing bacteriochlorophyll-containg and non-phototrophic strains to our colleagues (Boglárka Somogyi, Nóri Tugyi and Lajos Vörös) at the Balaton Limnological Institute of the HAS, we helped the installation of their infrared microscopic camera, which enabled the enumeration of purple bacteria in soda lakes. The results showed that the biomass of bacteriochlorophyll-containing bacteria usually exceeds 10% of the total bacterial biomass in Hungarian soda pans, but this value could even reach 30-40%.

Project publications

Korponai, K., Somogyi, B., Szabó, A., Boros, E., Vörös, L., Felföldi, T. 2015. Bíborbaktérium-közösség összetételének megismerése újgenerációs DNS-szekvenálási és tenyésztéses technikák kombinálásával [Determination of purple bacterium community composition combining next-generation DNA sequencing and cultivation techniques]. Hidrológiai Közlöny 95: 29-31. (in Hungarian with English abstract)

Korponai, K., Szabó, A., Somogyi, B., Vörös, L., Vajna, B., Boros, E., Felföldi, T. 2016. A planktonikus bakteriális közösségek szezonális alakulása különböző karakterű szikes tavakban [Seasonal dynamics of the planktonic bacterial community in two distinct types of soda pans]. Hidrológiai Közlöny (különszám): 44-52. (in Hungarian with English abstract)

[Szabó, A., Korponai, K., Somogyi, B., Kerepesi, Cs., Vörös, L., Bartha, D., Márialigeti, K., Felföldi, T. Soda pans of the Pannonian steppe harbor unique bacterial communities adapted to multiple extreme conditions. Submitted to Microbial Ecology]

Szabó, A., Korponai, K., Somogyi, B., Vörös, L., Vajna, B., Kerepesi, Cs., Márialigeti, K., Felföldi, T. Algal blooms and desiccation drives microbial community composition in alkaline soda pans. 16th International Symposium on Microbial Ecology (ISME-16), Montreal, Canada, 2016. augusztus 21-26. (Conference abstract)

Szabó, A., Korponai, K., Somogyi, B., Vörös, L., Jurecska, L., Márialigeti, K., Felföldi, T. 2015. Egy asztatikus szikes tó planktonikus mikrobaközösségének taxonómiai és funkcionális genomikai analízise [Taxonomic and functional genomic analysis of the planktonic microbial community inhabiting an astatic soda pond]. Hidrológiai Közlöny 95: 73-76. (in Hungarian with English abstract)

Tugyi, N., Vörös, L., Boros, E., Felföldi, T., Márialigeti, K., Somogyi, B. 2015. Ismeretlen fototróf mikroorganizmusok hazai vizekben – az infravörös mikroszkópi technika jelentősége a limnológiában [Phototrophs in near-infrared – first data on the occurrence of aerobic anoxygenic bacteria in Hungarian shallow lakes]. Hidrológiai Közlöny 95: 91-95. (in Hungarian with English abstract)

II.
EUKARYOTIC ALGAE IN HUNGARIAN SODA PANS

Hungarian soda pans have special limnological characteristics as their water is generally turbid because of the high amount of inorganic suspended solid particles and/or have high humic substance content which gives a brownish color to the water (Felföldi *et al.*, 2009; Somogyi *et al.*, 2009; Boros *et al.*, 2013; Pálffy *et al.*, 2014). Under the resulted light-limited conditions, the dominance of small-sized phytoplankton (i.e. picophytoplankton, <3 µm cell size) is favored due to their increased surface to volume ratio (Raven, 1998), while excrements of aquatic birds (Boros *et al.*, 2008, 2013) provides the nutritional basis of their growth.

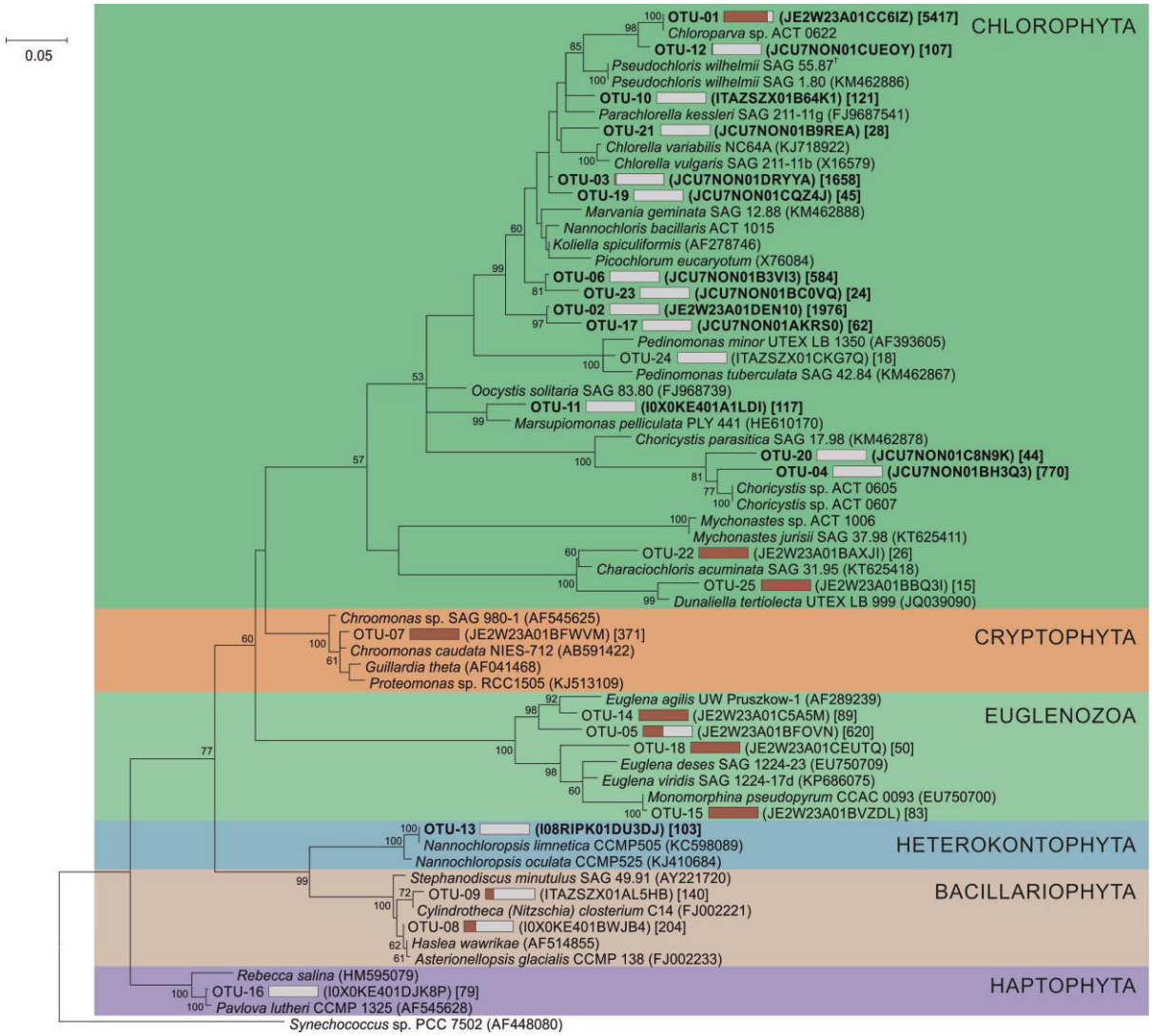

Figure 6 Distribution of eukariotic algal sequences in the whole soda lake pyrosequencing dataset based on plastid 16S rRNA gene sequences

OTUs representing potentially pico-sized algae are highlighted with bold. Sequence read numbers are given in brackets. Bars are showing the distribution of each OTU between the two sites (grey, turbid site, Zab-szék; brown, coloured site, Sós-ér). 16S rRNA gene sequence of 5 reference chlorphyte strains (ACT, Algal Collection Tihany) was also determined within this project. Tree was reconstructed using the Maximum Likelihood method with the General Time Reversible nucleotide substitution model based on 441 nucleotide positions.

In the case of small green algae, a similar phenomenon was observed as in the case of bacteria, since the turbid environment of Zab-szék harbored much abundant and diverse picophytoplankton community with many uncultivated taxa compared to the coloured-type Sós-ér (**Figure 6 and 7**). Namely, Sós-ér contained almost exclusively members of the genus *Chloroparva* (recently described form a soda pan by our research group, Somogyi *et al.*, 2011), while the Zab-szék community was dominated with uncultivated members of class *Trebouxiophyceae* (*Chlorophyta*).

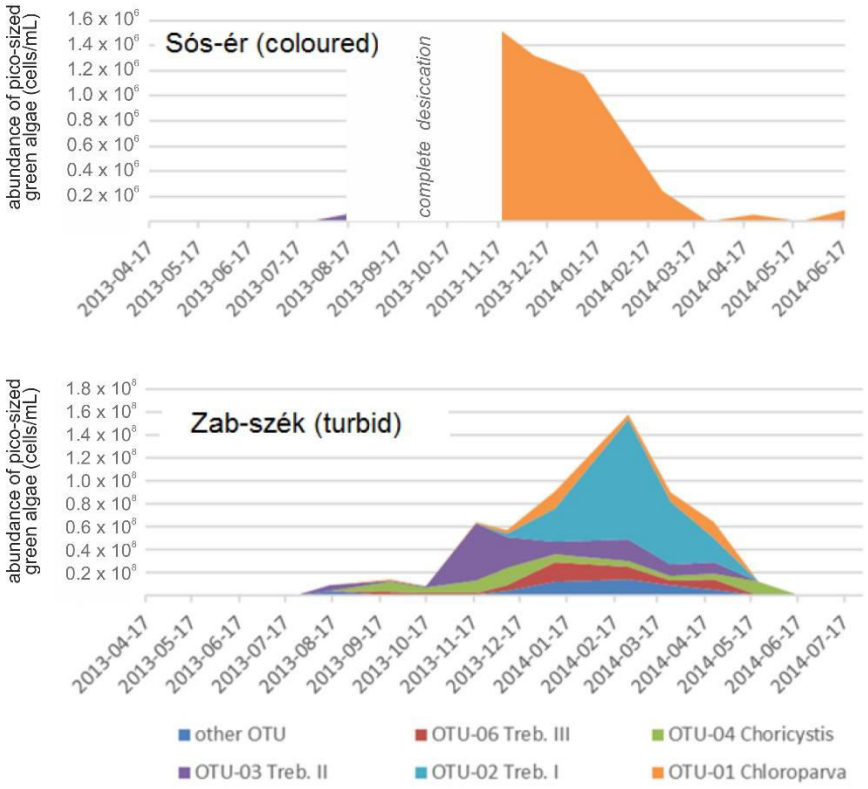

Figure 7 Seasonal dynamics of pico-sized green algae in the soda pans of the Carpathian Basin
Absolute cell numbers were calculated based on the combination of epifluorescence microscopic cell counts and the relative abundance of plastid pyrosequencing reads. For the phylogenetic position of each OTU, see **Figure 6**. Note that on Y axis, two magnitudes higher data are shown on the lower part of the figure.

Project publications

Selvarajan, R., Felföldi, T., Tauber, T., Sanniyasi, E., Sibanda, T., Tekere, M. 2015. Screening and evaluation of some green algal strains (Chlorophyceae) isolated from freshwater and soda lakes for biofuel production. Energies 8: 7502-7521. (IF: 2.077)

Selvarajan, R., Felföldi, T., Sanniyasi, E., Tekere, M. 2016. Assessing the potential of some freshwater and saline microalgae as biodiesel feedstock. J. Biobased Mat. Bioenergy 10: 50-62. (IF*: 0.635)

Somogyi, B., Felföldi, T., V.-Balogh, K., Boros, E., Pálffy, K., Vörös, L. 2016. The role and composition of winter picoeukaryotic assemblages in shallow Central European great lakes. J. Great Lakes Res. (in press) (IF*: 1.910)

III.
COMPARISON OF BACTERIAL COMMUNITIES IN SALINE LAKES: UNIQUENESS OF HUNGARIAN SODA PANS

Hungarian soda pans (or probably better to say Pannonian soda pans, referring to the geographic region, since some similar pans could be found in Austria and Serbia) have some characteristic limnological features compared with other soda lakes worldwide. Compared to the deep soda lakes in North America and Africa (Antony *et al.*, 2013; Dimitriu *et al.*, 2008; Grant, 2004; Lanzén *et al.*, 2013), soda pans in our region are shallow that frequently dry out completely by the end of the summer; while shallow, hypersaline soda lakes of the Kulunda Steppe have much higher salinity (Foti *et al.*, 2007), as the salinity of Pannonian soda pans varies generally within the hyposaline range (Boros *et al.*, 2014). And a very unique feature is that their water is generally turbid because of the high amount of inorganic suspended solid particles and/or have high humic substance content which gives a brownish color to the water (Felföldi *et al.*, 2009; Somogyi *et al.*, 2009; Boros *et al.*, 2013, Pálffy *et al.*, 2014). It seems that these unique features are reflected in the planktonic microbial communities (both pro- and eukaryotes, photo- and chemotrophs) inhabiting the pans.

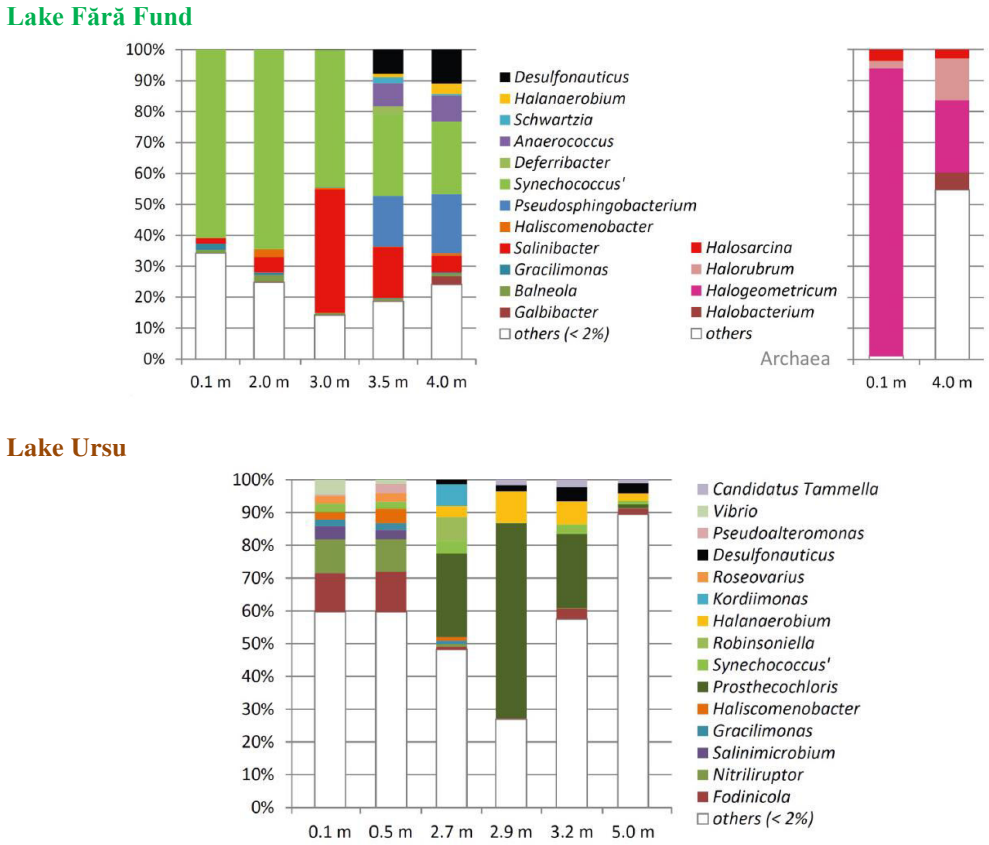

Figure 8 Distribution of prokaryotic genera in two heliothermal meromictic saline lakes in Romania based on 16S rRNA gene pyrosequencing

Contrary to other sites where *Gammaproteobacteria* was the most abundant class within *Proteobacteria* (Dimitriu *et al.*, 2008; Vavourakis *et al.*, 2016), in our case the dominance of *Alphaproteobacteria* was observed. Based on the phenotypic properties deduced from species descriptions, functional groups of bacteria were also represented by markedly different genera from those observed in other soda lakes

worldwide [reviewed by Sorokin et al., (2015)], e.g. *Methylotenera* was the dominant methylotropic bacterium (Kalyuzhnaya *et al.*, 2006) not *Methylomicrobium* and *Methylophaga* as in other soda lakes. Similarly, as our previous studies have shown that planktonic cyanobacteria are dominated by *Synechococcus* (Somogyi *et al.*, 2010, 2011; Felföldi *et al.*, 2011) contrary to *Spirulina*, *Arthrospira* as in other soda pans/lakes (Schagerl *et al.*, 2015), i.e. by different genera; in this project it was revealed that eukaryotic algae are dominated by *Chloroparva*, *Choricystis* and uncultivated green algae rather than *Picocystis* and *Dunaliella* (Krientiz and Kotut, 2010; Sorokin *et al.*, 2015).

Bacterial communities of sodium-hydrogen carbonate-dominated soda ponds were also compared to those of other alkaline samples (soda lime from Poland) and sodium-chloride-dominated salt lakes (samples collected in Romania and Kazakhstan, **Figure 8 and 9**). Interestingly, in the latter, members of the globally distributed cyanobacterium *Synechococcus* are belong to the obligate marine clade, while in the soda ponds distinct clades related to freshwater isolates are more typical. Members of phototrophic archaea (such as halobacteria) are common in some salt lakes, contrary to the soda lakes (although similarly high electrical conductivity values could be present), where these bacteria could be found in very low abundance (as mentioned above), which is a unique and previously unknown feature of the soda ponds. A genus-level comparison is presented in **Table 2**.

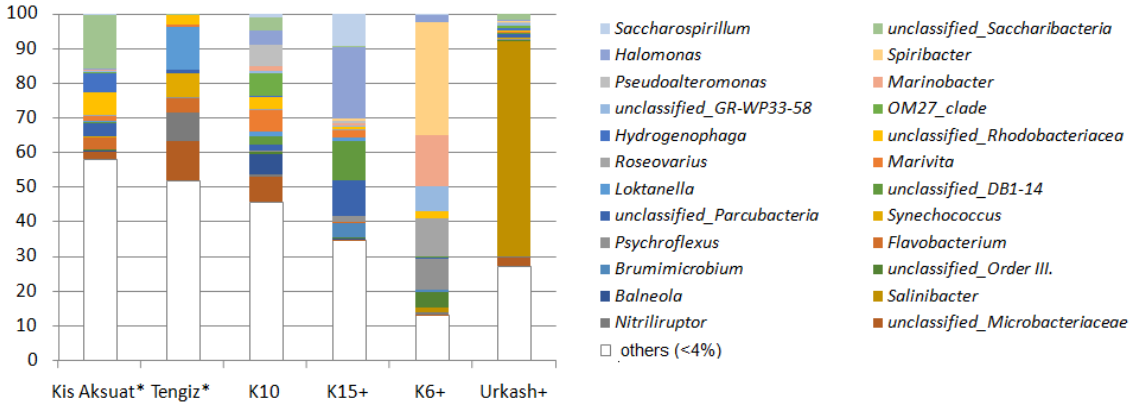

Figure 9 Distribution of bacterial genera in the studied saline lakes of Kazakhstan based on 16S rRNA gene pyrosequencing
Hypersaline lakes are marked with +, hypertrophic lakes are marked with *.

Table 2 Comparison of characteristic prokaryotic genera in saline lakes
Genera abundant in several sites appear with the same colour.

Component	*Hungarian soda lakes*	*Transylvanian saline lakes*	*Kazakh saline lakes*	*saline lakes of the Kulunda steppe*	*Oceans and seas*
Bacteria	*Fluviicola* *Flavibacterium* *Hydrogenophaga* *Rhodobaca* *Aquiflexum* *Belliella* *Nitrilruptor*	*Halomonas* *Gracilimonas* *Owenweeksia* unc. Micrococcinae *Psychroflexus* *Marinobacter* unc. Frankineae	*Halomonas* *Spiribacter* *Saccharospirillum* *Marivirga* *Psychroflexus* *Marinobacter* *Brumimicrobium*	*Halomonas* *Gracilimonas* *Balneola* *Rhodobaca* *Psychroflexus* *Halorhodospira* unc. Rhizobiales	*Halomonas* *Prochlorococcus* *Marinicella* *Beggiatoa* *Glaciecola* *Marinobacter* *Brumimicrobium*
Archaea	*Halogeometricum* *Haloarcula* *Archaeoglobus* *Natronomonas*	*Halorubrum* *Halosarcina* *Methanothermus* *Natronomonas*	*Halorubrum* *Haloarcula* *Halovenus* *Natronomonas*	*Halorubrum* *Haloarcula* *Methanolobus* *Natronomonas*	*Haloplanus* *Haloarcula* *Staphylothermus* *Natronomonas*
Dominant ions	sodium, hydrogen carbonate	sodium, chloride	sodium, chloride, sulfate	sodium, carbonate, hydrogen carbonate	sodium, chloride

Project publications

[Bell, T.A.S., Sen-Kilic, E., Felföldi, T., Vasas, G., Fields, M.W., Peyton, B.M. Bacteria and Eukarya community dynamics during eutrophication and toxic cyanobacterial blooms in alkaline Lake Velence, Hungary. Submitted to Aquatic Microbial Ecology]

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SUMMARY – SHORT ANSWERS TO RESEARCH QUESTIONS

1. Which genera and species of PPB are present in the soda pans of the Carpathian Basin?

The studied soda pans harbor bacterioplankton abundant in uncultivated members of the family *Rhodobacteraceae*, while genera *Roseococcus* and *Rhodobaca* and occasionally (under a green algal layer) *Ectothiorhodospira* are also important members of the community.

2. What kind of environmental conditions favor the rise of PPB taxa in soda pans?
3. What is the seasonal distribution of the PPB community in soda pans? Which environmental factors are the most important drivers of community changes?

Answers for these questions could be combined. Many members of the PPB community are associated with small green algae, which are abundant in winter/spring. Lower water temperature favors the rise of picoalgae, and algal exudates favor the rise of PPB.

4. Where are these bacteria preferably found in soda pans, in the sediment surface layer or in the water phase?

Different communities are associated with aerobic and anaerobic environments. Under special meteorological and hydrological conditions, PPB could form a visible purple layer just above the sediment, however, usually the water of the soda pans is continuously mixed, which hinders the development of such bloom above the sediment.

5. Are the Hungarian soda pans unique regarding their bacterial communities compared with other saline lakes?

Contrary to other saline habitats, members of the domain *Archaea* are practically absent in these habitats, however, on lower taxonomic level, differences from other soda lakes/pans were also identified (e.g. dominance of the freshwater *Synechococcus* clade in the cyanobacterium community, *Methylotenera* as the dominant methylotropic bacterium).

6. Which chlorophyte genera are present in these soda pans and what is their distribution?

Two type of soda pans harbored different green algal communities, the coloured Sós-ér contained almost exclusively members of the genus *Chloroparva*, while in the turbid Zab-szék, planktonic algal community was dominated with uncultivated members of class *Trebouxiophyceae* (*Chlorophyta*).

7. How bacteria adapt to the multiple environmental stress conditions present in the soda pans of the Carpathian Basin?

For maintaining osmotic balance of cells, the synthesis and uptake of compatible solutes ('salt-out' strategy) is preferred. Additionally, genes related to response to high UV radiation (e.g. DNA repair), oxidative stress (e.g. thioredoxin, superoxide dismutase) and high pH (e.g. Na^+/H^+ exchange channels) were also detected.

In the beginning of the project, we introduced two next-generation DNA sequencing methods (pyrosequencing and semiconductor sequencing) in our lab, therefore the developed pipeline of sample processing is given as Appendix.

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A P P E N D I X

N e x t - g e n e r a t i o n D N A s e q u e n c i n g m e t h o d s

1. Amplicon sequencing

Genomic DNA was directly extracted from the water samples/filters using the UltraClean Soil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) according to the manufacturer's instructions with the exception that cell disruption step was carried out by shaking the sample at 30 Hz for 2 min in a Mixer Mill MM301 (Retsch, Haan, Germany). Extracted DNA was stored at -20 °C until further processing. DNA concentrations were determined with a Qubit dsDNA HS assay (Invitrogen, Carlsbad, CA, USA) in a Qubit 2.0 Fluorometer (Invitrogen).

For the determination of the community composition, V3-V4 region of the 16S rRNA gene was amplified using universal bacterial primers: S-D-Bact-0341-b-S-17 forward (5'-CCTACGGGNGGCWGCAG-3') and S-D-Bact-0785-a-A-21 reverse (5'-GACTACHVGGGTATCTAATCC-3') [A1], fused with proper sequencing barcodes and adapters. To minimize the stochastic effects of the reaction, the PCR amplification was performed in triplicates in 20 µL final volume containing 5× Phusion HF Buffer (Thermo Fisher Scientific Inc., Waltham, MA, USA), 0.2 mM dNTPs (Fermentas Vilnius, Lithuania), 0.4 µg μL^{-1} Bovine Serum Albumin (Fermentas), 0.5 µM of each primer, 0.02 U μL^{-1} Phusion High-Fidelity DNA Polymerase (Thermo Fisher). The following thermal conditions were used: initial denaturation at 98 °C for 5 minutes, followed by 25 cycles of denaturation (95 °C for 40 s), annealing (55 °C for 2 minutes) and extension (72 °C for 1 minute) and a final extension step at 72 °C for 10 minutes. Amplicons were pooled before the purification step, resulted libraries were purified with the High Pure PCR Cleanup Micro Kit (Roche/454 Life Sciences, Branford, CT, USA). Quality control of the amplicon libraries was carried out using a model 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Emulsion PCR, amplicon library processing and pyrosequencing were performed on a GS Junior sequencing platform according to the Lib-L protocol of the manufacturer (Roche/454 Life Sciences). Initial data processing was performed using a gsRunProcessor 3.0.

Resulting sequence reads were processed using the mothur v1.35 software [A2] based on the 454 standard operating procedure (http://www.mothur.org/wiki/454_SOP - downloaded at 04/07/2015 [A3]). To minimize the amplification and pyrosequenceing bias, sequences were quality filtered and denoised, furthermore the removal of chimeric sequence reads using the uChime program [A4] and singleton sequences according to Kunin et al. [A5] were carried out. Sequence alignment was performed with the SINA v1.2.11 aligner tool [A6] using the ARB-SILVA SSU NR 99 reference database – SILVA Release 123 [A7] for alignment and classification. Sequences classified as Archaea, Chloroplast, Mitochondria and unknown were excluded from further analysis. Operational taxonomic units (OTUs) were assigned at 97 % similarity threshold levels, representing bacterial species [A8]. The ratio and distribution of reads are shown at different taxonomic levels corresponds to their relative abundance in the dataset in decreasing order; taxonomic assignments were made when the bootstrap values were higher than 80 based on the ARB-SILVA SSU NR reference database. For subsequent statistical analysis read numbers were normalized to the size of the smallest data set. Richness estimators and diversity indices were calculated in mothur.

2. Shotgun metagenomic sequencing

For the shotgun metagenomics analysis, three libraries were prepared from three DNA isolates. Libraries were sheared and prepared for sequencing with the Ion Xpress Plus Fragment Library Kit and the Ion PGM Template OT2 200 Kit (Life Technologies). Sequencing was performed with the Ion PGM Sequencing 200 Kit v2 on 314 chips using Ion Torrent PGM (Life Technologies). Raw sequence signals were analyzed with the Ion Torrent Suite software 3.6.2 (Life Technologies). Resulted fastq files were merged together for further processing.

Shotgun reads were filtered based on their average quality score ($Q \geq 24$) with PRINSEQ v0.20.4 [A9], also sequence duplicates were removed and bases less than phred=10 were trimmed from the end of the sequences. Filtered reads containing gene sequences were identified with the blastx command of DIAMOND [A10] against the NCBI NR database (downloaded at 22/02/2015) in sensitive mode with 0.001 e-value cutoff (default) and set the max target sequences option to 250 (default is 25). Taxonomic and functional assignments were made with MEGAN 5.11 [A11] against the NCBI and SEED classification (downloaded at 12/05/2015 NCBI and 01/11/2014 SEED) using the default parameters.

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