

## Short communication

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# Identification of heterotrophic phosphate and silica solubilizing bacterial strains isolated from Lake Baikal

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**ABSTRACT.** The taxonomic composition of phosphatase active bacteria in epilithic bio films is more diverse than that of phosphate solubilizing bacteria.  $\text{CaSiO}_3$  and phosphate solubilizing bacteria isolated from epilithic biofilms, water and sediments of Lake Baikal can release acid, alcohol and other compounds during the decomposition of sugars, thereby contributing to the destruction of mineral poorly soluble phosphates and  $\text{CaSiO}_3$  compounds. Microbial community of epilithic biofilms dominated by opportunistic microorganisms (*Buttiauxella*, *Serratia*, *Kosakonia*, *Yersinia*, *Aeromonas*, and *Acinetobacter*) that can survive in this biotope for a long time showed a high diversity of groups of phosphate and  $\text{CaSiO}_3$  solubilizing bacteria. In addition to opportunistic bacteria, we have isolated bacteria of the genera *Phytobacter*, *Serinibacter*, *Curtobacterium*, *Exiguobacterium*, *Paenibacillus*, and *Bacillus*, which are more often found in the soil, plant tissues or the rhizosphere. These bacteria can be the autochthonous microflora of the epilithic biofilms from Lake Baikal. Moreover, bacteria of the genera *Bacillus*, *Paenibacillus* and *Serratia* can transform both organic and inorganic phosphorus compounds. Such species as *Exiguobacterium* and *Serratia* can degrade both poorly soluble phosphorus compounds and calcium silicates.

**Keywords:** phosphorus, silica, heterotrophs, water, biofilms, sediments, 16S rRNA gene, strains, Lake Baikal.

## 1. Introduction

During the eutrophication of water bodies, a special role belongs to phosphorus as one of the most important nutrients necessary for the vital activity of all organisms. In water bodies, phosphorus is present in the form of inorganic and organic compounds. In the water of Lake Baikal, phosphorus in the form of phosphate ions is present in small amounts (5-15  $\mu\text{g/l}$ ) (Khodzher et al., 2017), because aquatic organisms rapidly use the soluble phosphorus compounds, or it has poorly soluble or insoluble forms that deposit. The bulk of its reserves has an organic form. Phyto- and zooplankton are the main source of organic phosphorus in the Baikal water. A wide range of aquatic microorganisms can transform phosphorus compounds: mineralize, solubilize and immobilize (Gen-Fu and Xue-Ping, 2005; Hupfer et al., 2007). At present, there is no consensus on the species specificity of bacteria involved in the phosphorus cycle in nature. Many organotrophic microorganisms are capable of mobilizing phosphates. According to V.V. Parfenova, in the water and bottom sediments of Lake Baikal, the genera *Pseudomonas*, *Micrococcus*, *Acinetobacter*, *Flavobacterium*, and *Bacillus* represent the microorganisms of the phosphorus cycle (Parfenova and Ilyaletdinov, 1985).

## 2. Materials and methods

In this study, we investigated two groups of cultured bacteria involved in microbial processes of the phosphorus cycle: 1) phosphatase active bacteria (PAB) having alkaline phosphatase activity and 2) phosphate solubilizing bacteria (PSB) that dissolve mineral poorly soluble phosphates. The presence of phosphatase activity in pure cultures was determined using the VITAL alkaline phosphatase kit (Saint Petersburg, Russia). PSB were grown on a medium containing insoluble mineral phosphate,  $\text{Ca}_3(\text{HPO}_4)_2$ ; glucose (10  $\text{g/l}$ ) and yeast extract were contained as carbon sources (Rodrigues and Fraga, 1999).

To determine the proportion of silica solubilizing bacteria capable of dissolving calcium silicates, in the microbial community of the investigated biotopes, 1 ml of a sample was inoculated in a special medium (Ehrlich, 1996). The medium consisted of four solutions that were mixed aseptically after sterilization. Mineral solution:  $\text{KH}_2\text{PO}_4$  0.54 g,  $\text{MgSO}_4 \times 7\text{H}_2\text{O}$  0.25 g,  $(\text{NH}_4)_2\text{SO}_4$  0.75 g,  $\text{FeCl}_3$  trace, yeast extract 2 g per 300 ml of water. Sugar medium: glucose 40 g per 100 ml of water. Silica salt solution:  $\text{CaSiO}_3$  10 g per 200 ml of water and 400 ml of 3% agar. The inoculation time was 10-13 days, after which the results were recorded.

The strains were identified by the 16S rRNA

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gene fragment according to the previously described methods (Sukhanova et al., 2019).

### 3. Results

Our studies of epilithic biofilms from Lake Baikal resulted in obtaining the 2012 collection consisting of 170 items, in which 137 strains had alkaline phosphatase activity. These were the members of the genera *Bacillus*, *Pseudomonas*, *Aeromonas*, *Serratia*, *Massilia*, *Rhodococcus*, *Glacihabitans*, *Stenotrophomonas*, *Yersinia*, *Achromobacter*, *Micrococcus*, *Streptomyces*, *Microcella*, *Staphylococcus*, *Brevundimonas*, *Virgibacillus*, *Devosia*, and *Paenibacillus*.

On the medium for PSB, we isolated the strains of heterotrophic bacteria from the epilithic biofilms, water and sediments of Lake Baikal, and obtained the collection consisting of 70 cultures. Molecular genetic identification of 23 PSB strains determined their taxonomic diversity: three phyla in the epilithic biofilms, *Proteobacteria* (*Kosakonia*, *Acinetobacter*, *Buttiauxella*, *Serratia*), *Actinobacteria* (*Curtobacterium*) and *Firmicutes* (*Exiguobacterium*), two phyla in the water, *Proteobacteria* (*Phytobacter*) and *Actinobacteria* (*Curtobacterium*); one phylum in the sediments, *Firmicutes* (*Bacillus*, *Paenibacillus*).

Strong acids destroy calcium silicate. Molecular genetic identification of nine strains isolated from the epilithic biofilms of Lake Baikal, which can dissolve the  $\text{CaSiO}_3$  solutions, indicated the following diversity: *Firmicutes* (*Exiguobacterium*), *Proteobacteria* (*Serratia*, *Yersinia* and *Aeromonas*), and *Actinobacteria* (*Serinibacter*).

### 4. Discussion

The term “fermenting” more precisely defines physiological properties of bacteria of the family Enterobacteriaceae (*Buttiauxella*, *Serratia*, *Kosakonia*, *Yersinia*), because they produce acids during glucose fermentation (ferment glucose to form acid and gas). Some of these genera belong to opportunistic microorganisms that are normally devoid of pathogenic properties and do not cause infectious diseases. Enterobacteriaceae colonize mucous membranes and are relatively resistant. On various objects of the external environment, they can survive from 10 days to six months; they are resistant to high concentrations of sodium chloride and drying, survive at below-zero temperatures, are viable in raw well and tap water, and quickly die at a temperature of 68°C or higher. Chemoheterotrophs of the genus *Aeromonas* use various sugars and organic acids as a carbon source.

*Acinetobacter* have versatile metabolic activity, which ensures their amazing phenotypic plasticity. The variety of substances that *Acinetobacter* use as a food source is striking in its breadth: from simple carbons (glucose and others) and oil to tissues of the human body. Most strains of *Acinetobacter* are indole negative; they decompose sugars to release alcohol only through oxygen-depending metabolism. The

optimum temperature for clinical isolates is 37-38°C, and they can grow and multiply under psychrophilic conditions, which is especially typical of saprophytic strains (Chebotar' et al., 2014).

Members of the phylum *Firmicutes* of the genus *Exiguobacterium* reduce nitrates to nitrites. The acids are produced from trehalose, sorbitol, sucrose, d-fructose, and d-mannitol (Raichand et al., 2012). The *Exiguobacterium* members are globally diverse organisms that are found in various environments, including microbialites, ocean, freshwater lakes, Himalayan ice and soil, hydrothermal vents, shrimps, microbial biofilms, ancient Siberian permafrost, plant rhizosphere, and food processing environments. Strains of these poorly known bacteria, which were obtained from such different (and often extreme) environments, deserve attention, because they are probably specially adapted to these environments and cause changes in the genome, which may correspond to psychrophilic and thermophilic adaptations (Vishnivetskaya, 2009).

In the water and epilithic biofilms, we detected PSB of the genus *Curtobacterium*. The traditional characterization of this taxon mainly associates it as a causative agent in dry bean crops. *Curtobacterium* PSB are widespread with a predominant presence in soil ecosystems. Moreover, this genus has a great diversity of the genome potential for the degradation of carbons, especially in regard to structural polysaccharides, i.e. exists as cellulose bacteria (Chase et al., 2016). Some species of the genus *Curtobacterium* can produce acids from adonitol (Behrendt et al., 2002).

Another PSB representatives from the water column of Lake Baikal are Gammaproteobacteria of the genus *Phytobacter* that belongs to the endophytic origin. Some strains produced acids from various sugars at 35°C for two days (Pillonetto et al., 2018).

Gram-positive Actinobacteria of the species *Serinibacter* were isolated from the rhizosphere of mangrove forests of Pramuka Island, Indonesia (Hamada et al., 2015), and another species was isolated from the intestinal tract of fish (Hamada et al., 2009). These bacterial species can produce acids from starch, sucrose, etc.

PSB isolated from the bottom sediments of Lake Baikal belong to the members of the phylum *Firmicutes* of the genus *Paenibacillus*, which can produce acetone and acetic acid as the end products of glucose fermentation (Nelson et al., 2009). Some *Bacillus* strains showed the synthesis of acids from various carbon sources, which uses some sugars, amino acids and other carbon compounds as the only carbon sources (Shivaji et al., 2009).

### 5. Conclusion

The taxonomic composition of PAB in epilithic biofilms is more diverse than that of PSB.  $\text{CaSiO}_3$  and phosphate solubilizing bacteria isolated from epilithic biofilms, water and sediments of Lake Baikal can release acid, alcohol and other compounds during the decomposition of sugars, thereby contributing to the

destruction of mineral poorly soluble phosphates and  $\text{CaSiO}_3$  compounds. Microbial community of epilithic biofilms dominated by opportunistic microorganisms (*Buttiauxella*, *Serratia*, *Kosakonia*, *Yersinia*, *Aeromonas*, and *Acinetobacter*) that can survive in this biotope for a long time showed a high diversity of groups of phosphate and  $\text{CaSiO}_3$  solubilizing bacteria. In addition to opportunistic bacteria, we have isolated bacteria of the genera *Phytobacter*, *Serinibacter*, *Curtobacterium*, *Exiguobacterium*, *Paenibacillus*, and *Bacillus*, which are more often found in the soil, plant tissues or the rhizosphere. These bacteria can be the autochthonous microflora of the epilithic biofilms from Lake Baikal. Moreover, bacteria of the genera *Bacillus*, *Paenibacillus* and *Serratia* can transform both organic and inorganic phosphorus compounds. Such species as *Exiguobacterium* and *Serratia* can degrade both poorly soluble phosphorus compounds and calcium silicates.

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