

Genetic diversity of Actinobacteria inhabiting water and sponges of Lake Baikal

Lipko I.A.^{1*}, Krasnopeev A.Yu.¹, Tikhonova I.V.¹, Timoshkin O.A.¹, Kabilov M.R.², Belykh O.I.¹

¹ Limnological Institute, Siberian Branch of the Russian Academy of Sciences, Ulan-Batorskaya Str., 3, Irkutsk, 664033, Russia

² Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Lavrentiev Ave., 8, Novosibirsk, 630090, Russia

ABSTRACT. Actinobacteria is a ubiquitous major group in freshwater bacterioplankton and sponge microbial communities. We were first to analyse the diversity of Actinobacteria inhabiting Lake Baikal water and endemic sponges *Lubomirskia baikalensis* (healthy and diseased) using high-throughput 16S rRNA sequencing. In total, we have assigned of three classes, fourteen orders and twenty-six families of Actinobacteria. The diversity and composition of actinobacteria were common in bacterioplankton and healthy sponges. The environmental crisis in the coastal zone of Lake Baikal led to a colonization of the *L. baikalensis* by benthic and soil-derived representatives of Actinobacteria. Some amplicon sequences of opportunistic genera were detected in the coastal water. The example of Actinobacteria has dramatically demonstrated the response of bacterial populations caused by negative environmental impact.

Keywords: freshwater sponge; actinobacteria; high-throughput 16S rRNA sequencing; Lake Baikal

1. Introduction

Phylum Actinobacteria is one of the largest taxonomic units in the domain Bacteria (Ventura et al., 2007). They represent a significant part of the microbial community in freshwater reservoirs (Garcia et al., 2014) and freshwater sponges (Gernet et al., 2005; Costa et al., 2013). In Lake Baikal, the phylum Actinobacteria is the prevalent component of the microbial communities in the water column and healthy sponges (Parfenova et al., 2013; Gladkikh et al., 2014; Seo et al., 2016; Kulakova et al., 2018). Negative ecological changes in the coastal zone of the lake, which began in 2010–2011, caused sickness and mass mortality of endemic sponges. The bleached and diseased *L. baikalensis* revealed a change in the composition and structure of the sponge microbial community, including Actinobacteria (Denikina et al., 2016; Kulakova et al., 2018;).

Through all studies, there are still no papers on the detailed composition of actinobacterial communities in baikalian sponges and bacterioplankton. We were aimed to reveal actinobacterial diversity in the lake water as well as healthy and diseased *L. baikalensis* and to analyse the actinobacteria composition before and during environmental crisis in the coastal zone of Lake Baikal.

2. Materials and Methods

We investigated 6 samples of diseased *L. baikalensis* (bleached, green with brown patches and maroon-violet biofouling) and 6 water samples collected in the local bays Bolshiye Koty and Listvennichny of Lake Baikal in 2011–2015. Total DNA was extracted using the DNA-Sorb B kit according Gladkikh et al. (2014). DNA amplification was performed using 343F and 806R bacterial primers targeting V3 and V4 regions of the 16S rRNA gene. High-throughput sequencing was carried out on the Illumina platform in SB RAS Genomics Core Facility (Russia). Bioinformatics analysis also included already published data on amplicon sequencing analysis of healthy *L. baikalensis* sponges and water samples from Lake Baikal (Parfenova et al., 2013; Gladkikh et al., 2014). Quality filters, chimera removal and operational taxonomic units clustering (threshold 97%) were performed using Mothur v.1.39.5 (<http://www.mothur.org>). The sequences were aligned using SILVA database (v.132 release, <https://www.arb-silva.de>). All calculations were performed with HPC-cluster «Akademik V.M. Matrosov» (Irkutsk, Russia; <http://hpc.icc.ru>).

*Corresponding author.

E-mail address: irinalipko@yandex.ru (I.A. Lipko)

3. Results and Discussion

The phylum Actinobacteria dominated the microbial community of the water column, particularly in the coastal zone, and contained from 32% to 69% of all sequences. They constitute a significant part of the microbial community in healthy and diseased *L. baikalensis*. In healthy sponges, actinobacteria ranged from 3.5% to 14% (Gladkikh et al., 2014; Seo et al., 2016), but in diseased ones their fraction remained at the same level of up to 8.6%, except for bleached specimens.

Totally, we have assigned of three classes (Actinobacteria, Acidimicrobiia, and Thermoleophilia), fourteen orders and twenty-six families. Among them, classes Actinobacteria and Acidimicrobiia were ubiquitous and dominant in all *L. baikalensis* and water samples, whereas Ilumatobacteraceae (CL500-29), Microtrichaceae, and Sporichthyaceae (hgcI_clade) were the prevalent families. Results of bioinformatics and cluster analysis for V3–V4 showed that in the coastal zone of Lake Baikal actinobacteria diversity and composition were similar until 2014 since the major phylotypes were common for water and sponges.

Actinobacterial diversity has transformed significantly and increased in diseased sponges by 2015. Some phylotypes of CL500-29 and hgcI_clade groups were negligible or eliminated from the *L. baikalensis* microbial community. The new phylotypes of CL500-29, Microtrichaceae and Microbacteriaceae, unclassified Acidimicrobiia, genera *Ilumatobacter* and *Iamia* in the sponge microbial community have become the dominant groups, comprising from 60% to 90% of the actinobacterial sequences. For the first time, two rare genera *Gaiella* and *Parviterribacter* (Thermoleophilia) were identified in diseased *L. baikalensis*. The nearest homologues (98%–99% similarity) were highly similar to Actinobacteria from the benthic community and bottom sediments, soils, groundwater, wastewaters and activated sludge reactors, and from reservoirs with high anthropogenic load. The new settled actinobacterial groups in diseased *L. baikalensis* seem to have different metabolic activities: having various enzyme activities, taking part in the nitrogen and phosphorus cycles, in bioremediation from the pollution with polycyclic aromatic and aliphatic hydrocarbons, decomposition of polymers, carbohydrates and esters. Furthermore, Microbacteriaceae exhibit fungicidal activity, suppressing the pathogens of aquatic and terrestrial animals. These Actinobacteria are likely able to protect diseased sponges from additional infection with aquatic fungal pathogens.

Some sequences from the shallow and pelagic water of Lake Baikal were homologous with the cultured opportunistic genera *Actinomyces*, *Corynebacterium*, *Lawsonella*, *Rothia*, *Mycobacterium*, and *Gardnerella*. These actinobacteria had an anthropogenic origin and could be potentially dangerous for people with weakened immunity.

4. Conclusion

In summary, the negative changes in the coastal zone of Lake Baikal caused the appearance of benthic and soil-derived representatives in the microbial community of the endemic sponge *L. baikalensis*. We assume that Actinobacteria associated with anthropogenic activities that have settled on sponges are likely to contribute to the longer existence of diseased *L. baikalensis*, since they decompose decaying sponge bodies and biofouling and protect weakened sponges from the effects of pathogenic fungi. This study highlights the ecological role of actinobacteria not only in the water column and in sponges but also in the environment as a whole.

Acknowledgements

The authors would like to thank Irkutsk Supercomputer Center of SB RAS for providing access to HPC-cluster «Akademik V.M. Matrosov» (<http://hpc.icc.ru>). Our thanks to Igor V. Khanaev for sampling. The study was carried out within the framework of State Task No. 0345-2020-0003 (AAAA-A16-116122110061-6). This work was supported by RFBR grant no. 18-54-05005_arm_a.

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