Ann. Limnol. - Int. J. Lim. 52 (2016) 27–32
© EDP Sciences, 2016
DOI: 10.1051/limn/2015035

Available online at:
www.limnology-journal.org

Comparison of bacterial diversity and species composition in three endemic Baikalian sponges

Eun-Young Seo[1], Dawoon Jung[1], Olga I. Belykh[2], Natalia A. Bukshuk[2], Valentina V. Parfenova[2], Yochan Joung[3], Il Chan Kim[4], Joung Han Yim[4] and Tae-Seok Ahn[1]*

[1] Department of Environmental Science, Kangwon National University, Chuncheon 200-701, Republic of Korea
[2] Limnological Institute, Siberian Branch, Russian Academy of Sciences, Irkutsk 664033, Russia
[3] Department of Biological Sciences, Inha University, Incheon 402-751, Republic of Korea
[4] Polar BioCenter, Korea Polar Research Institute, KOPRI, Incheon 406-840, Republic of Korea

Received 10 July 2015; Accepted 23 November 2015

Abstract – Baikalian sponges are unique organisms with many species harboring symbiotic microbes that produce novel bioactive compounds. To investigate bacterial diversity of three species of Baikalian sponges, specimens of *Lubomirskia baicalensis*, *Baikalospongia intermedia* and *Swartschewskia papyracea* collected from Lake Baikal were processed by pyrosequencing. We found differences in the species composition and diversity in bacteria among these sponges. Cyanobacteria accounted for the highest proportion and the second group was Proteobacteria in three sponges. The bacterial communities in *B. intermedia* and *L. baicalensis* were highly similar but less similar to the bacterial community associated with *S. papyracea*. Diversity of the bacterial community in *S. papyracea* was higher than in *L. baicalensis* and *B. intermedia*. In particular, the relative abundance of *Actinobacteria* was higher in *S. papyracea*. Bacterial species in phyla *Acidobacteria* and *Gemmatimonadetes* were only found in *S. papyracea*.

Key words: Bacterial community / bacterial diversity / freshwater sponges / Lake Baikal / pyrosequencing

Introduction

Lake Baikal is an ancient rift-valley freshwater ecosystem containing many endemic species, including sponges (Moore *et al.*, 2009). Sponges are regarded as one of the oldest metazoans and are known to host diverse symbiotic microorganisms (Taylor *et al.*, 2007). Sponges are filter feeders capable of pumping a large volume of water through their vascularized canal system and expelling sterile water (Reiswig, 1974). They play the role of host to tremendously dense and diverse microbial communities, which comprise up to 40% of sponge volume and help in many aspects of sponge biology (Hentschel *et al.*, 2002). These symbionts have ecologically important functions, they are sources of bioactive compounds (Dunlap *et al.*, 2007), and produce diverse pharmacological metabolites (Vogel, 2008).

Among the various species of sponges, there are species with high microbial abundance (HMA; 10^{8-10} cells.g^{-1}) and low microbial abundance (LMA; $< 10^{6}$ cells.g^{-1}) (Kamke *et al.*, 2010). The microbial diversity of marine sponges has been studied in great detail (Schmitt *et al.*, 2012), but the symbiont communities associated with freshwater sponges have been studied far less. It is difficult to compare microbial communities in marine and freshwater sponges because the microbial community in most freshwater sponges has not been thoroughly studied (Gernert *et al.*, 2005). Many symbionts associated with sponges are highly host-specific and some species have been found in other environments (Schmitt *et al.*, 2012). Different species of sponges inhabiting the same location can greatly differ in the abundance of their associated bacteria (Cleary *et al.*, 2013). However, many microbial phylotypes were repeatedly detected in different sponges. Therefore, the respective microbes were defined as sponge-specific (Kamke *et al.*, 2010). To understand the ecological importance of sponge–microbe associations, microbial diversity by molecular techniques must be examined.

Among Baikalian sponges, the genera *Lubomirskia*, *Baikalospongia* and *Swartschewskia* are widely distributed and are generally abundant (Weinberg *et al.*, 2003). According to the latest taxonomic revision of the Baikalian sponges, 13 species of sponges are endemic and belong to the Lubomirskiidae family and five species are

*Corresponding author: ahnts@kangwon.ac.kr

more cosmopolitan and belong to the Spongillidae family (Masuda, 2009). Lubomirskiidae are a diverse and widespread group of sponges in Lake Baikal. Spongillidae are not as abundant and inhabit mainly the shallow bays in Lake Baikal. The Lubomirskiidae include species such as *Lubomirskia baicalensis*, *Baikalospongia bacillifera*, *Baikalospongia intermedia*, *Baikalospongia martinsoni* and *Swartschewskia papyracea*. *L. baicalensis* and *Baikalospongia* sp. have been studied in regards to their symbiont microbial community (Parfenova *et al.*, 2008; Gladkikh *et al.*, 2014) but almost no studies have focused on bacterial symbionts associated with *S. papyracea*. In this study, we compared the bacterial community in three endemic sponges, *L. baicalensis*, *B. intermedia* and *S. papyracea* to understand the symbiont community harbored in these Baikalian sponges.

Materials and methods

Sample collection

Sponge samples were collected in the southern area of Lake Baikal near the town of Lystviyanka (51°53.2855′N, 105°03.5243′E) on August 16, 2012. Three endemic species, *B. intermedia, L. baicalensis* and *S. papyracea* were selected for this study. The specimens were collected at 7, 10 and 42 m depth by scuba diving. All samples were kept at 4 °C and DNA was extracted within 2 h and the extracted DNA was then stored at −80 °C until the analysis was performed in the laboratory.

DNA extraction and amplicon pyrosequencing

Subsamples (0.25 g) were used for DNA extraction. Nucleic acid extraction was carried out following the manufacturer's guidelines (MoBio Laboratories, Carlsbad, CA). The extracted genomic DNA was amplified as previously described (Chun *et al.*, 2010). DNA sequencing was performed by Chunlab Inc. (Korea) using the 454 GS FLX Titanium Sequencing System (Roche). Each pyrosequencing read was taxonomically assigned using the CL community™ program, Version 3.10.

General analysis of the pyrosequencing-derived dataset

To avoid overestimating rare phylotypes, we performed clustering and diversity estimates at genetic divergences of ≥ 3% (Kunin *et al.*, 2010). Rarefaction analysis was used to determine whether the operational taxonomic units (OTUs) were present in the bacterial community. Biodiversity indices were estimated with various calculators using Mothur software (Schloss *et al.*, 2009). Bacterial richness (Chao 1 estimator) and Simpson's diversity indices were used to estimate species dominance and evenness. To detect specific taxa in selected sponges, the Cluster Database at High Identity with Tolerance method was used and sequence similarity was defined across all samples (including < 1% of all sequences). Similarities among bacterial community structure were evaluated with the unweighted pair-group method using arithmetic averages (UPMGA) dendrogram.

Results

Bacterial community

A total of 26 091 reads of 16S rRNA genes with an average length of 400 bp was obtained by pyrosequencing. The retrieved reads were 7496, 13 654 and 4941 reads from *B. intermedia*, *L. baicalensis* and *S. papyracea*, respectively. The reads were adjusted to 4941 reads to compare precisely. Because each rarefaction curve approached a plateau, the bacterial communities within these samples seem well represented. The rarefaction curves indicate that the OTUs increased across the sampling sites and phylotypes ranging from 194 to 758 were found in each sponge (Fig. 1). By applying the averaged rarefaction curves for our data, each 4941 sequence reads would correspond to 161, 162 and 253 reads for species associated with *B. intermedia*, *L. baicalensis* and *S. papyracea*, respectively. The calculated Shannon index was 0.153, 0.058 and 0.027 for *B. intermedia*, *L. baicalensis* and *S. papyracea*. The Simpson index was 2.793, 3.957 and 4.881 for *B. intermedia*, *L. baicalensis* and *S. papyracea*. Chao 1 richness was 318, 1231 and 1904, respectively, for *B. intermedia*, *L. baicalensis* and *S. papyracea* (Fig. 2).

Bacterial diversity within sponges

A total of 14 828 reads was successfully assigned from the species up to the phylum level (phyla level, cut off < 1.0). The six phyla groups *Cyanobacteria*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Planctomycetes* and *Verrucomicrobia* were found in the

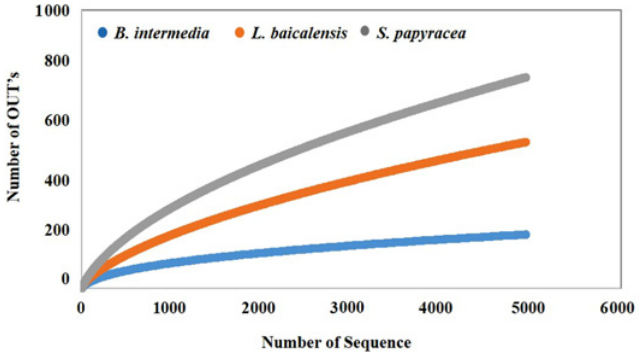

Fig. 1. Species curves of the number of OTUs using bacterial 16S rRNA gene sequences from Baikalian sponges.

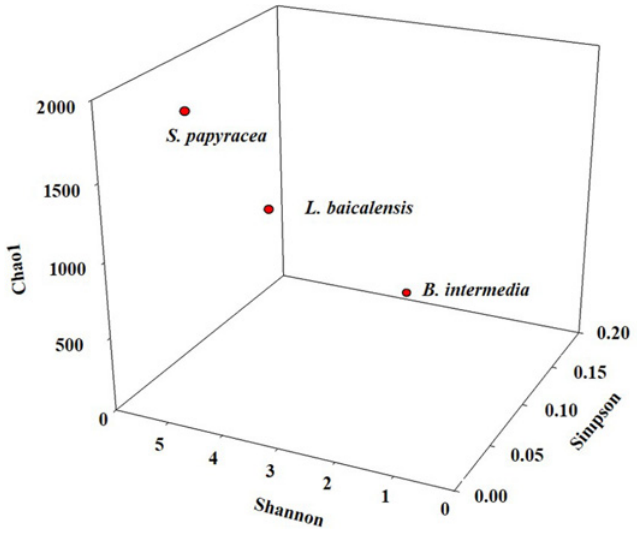

Fig. 2. Indices of species richness and diversity in Baikalian sponges.

sponges and of all sequence reads analyzed represented 70, 12, 3, 2, 4 and 5% for *B. intermedia,* 78, 9, 3, 2, 5 and 1% for *L. baicalensis* and 43, 25, 13, 8, 3 and 5% for *S. papyrace* (Fig. 3). Among these six phyla, *Cyanobacteria* encompassed the majority of sequences (42–78%) and *Proteobacteria* was the next most predominant group and other phyla comprised a minor portion of the overall bacterial community in each sponge.

Cyanobacteria and Actinobacteria

Cyanobacteria accounted for the highest proportion (< 43 to < 78%) in these three sponges. All reads grouped as *Cyanobacteria* were affiliated with order *Synechococcales*, *Chroococcales* and *Nostocales*. *Nostocales* was identified only in *B. intermedia* and *S. papyracea* whereas *Chroobacteria* was found in *L. baicalensis* and *S. papyracea*. In particular, *Prochlorococcus marinus* was found only in *S. papyracea*. Taxonomic groups in the genus *Prochlorococcus* accounted for between < 40 and 74% of the total bacteria in all sponges (Fig. 4).

The relative abundance of *Actinobacteria* in *S. papyracea* (13.4%) was three times higher than the abundance in *L. baicalensis* (3.46%) and *B. intermedia* (3.87%). Common to all three species were species in orders *Acidimicrobiales*, *Frankiales* and *Planktophila*. Ten reads in the orders *Micrococcales*, *Bacteroidales*, *Verrucomicrobiales* and *Solirubrobacterales* were found only in *S. papyracea* (Fig. 5).

Comparison of bacterial community structure

The results from the UPMGA analysis show that the bacterial communities in *B. intermedia* and *L. baicalensis* were highly similar but less similar to the bacterial community associated with *S. papyracea* (Fig. 6).

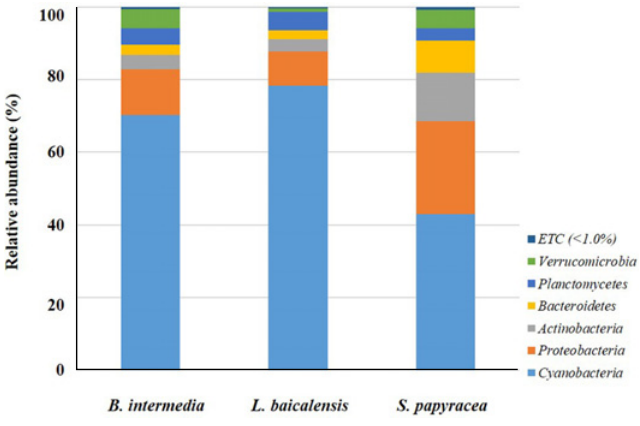

Fig. 3. Relative abundance of the most abundant bacterial classes (phyla) in Baikalian sponges.

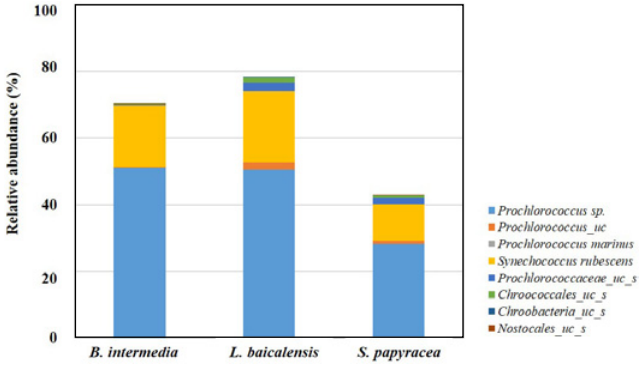

Fig. 4. Relative abundance of order level based on phylum *Cyanobacteria* in Baikalian sponges.

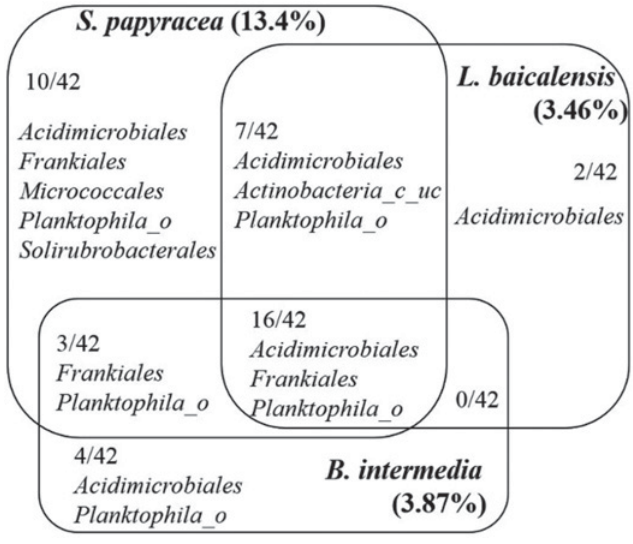

Fig. 5. Venn diagram showing the OTU numbers for order *Actinobacteria* for Baikalian sponges.

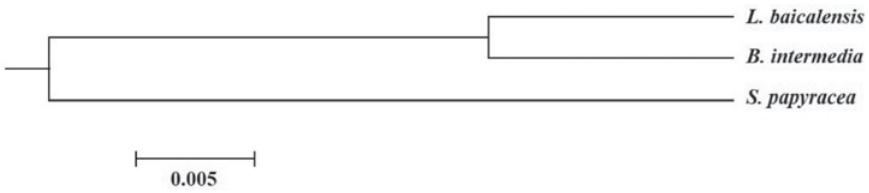

Fig. 6. Similarity among bacterial communities in Baikalian sponges.

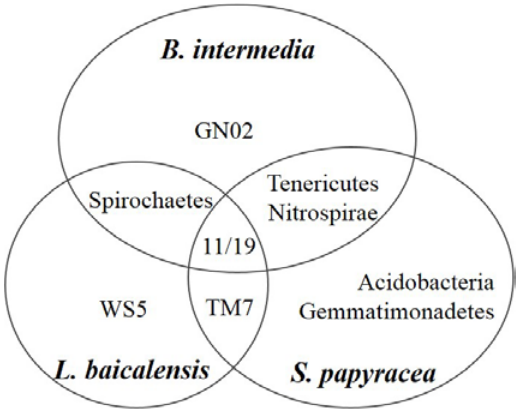

Fig. 7. Distribution of phyla between the three Baikalian sponges in this study.

Eleven phyla, including *Cyanobacteria*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Planctomycetes*, *Verrucomicrobia*, *Chloroflexi*, *OD1*, *Armatimonadetes*, *Bacteria_uc* and *Chlorobi* were common to the three sponges. *Spirochaetes* phylum was associated with *B. intermedia* and *L. baicalensis*, whereas *Tenericutes* and *Nitrospirae* were found with *B. intermedia* and *S. papyracea*. TM 7 was found in *L. baicalensis* and *S. papyracea*. Phyla GN02 and WS5 were found in *B. intermedia* and *L. baicalensis*. Members of *Acidobacteria and Gemmatimonadetes* were only found in *S. papyracea* (Fig. 7).

Discussion

Comparison of bacterial composition and diversity within sponges

The bacterial community species composition in *S. papyracea* differed from the species composition in the other two sponges. We also found that bacterial diversity in *S. papyracea* was higher than that in *B. intermedia* and *L. baicalensis*. Until now, very few studies have been conducted on the bacterial symbionts in *S. papyracea*. This likely reflects the challenges of collecting samples of *S. papyracea* in the field. Firstly, the small size of *S. papyracea* (1–4 mm in diameter) presents difficulties for sampling and handling in the field. Secondly, the usual habitat of *S. papyracea* is in secluded areas under ledges. Thirdly, *S. papyracea* is only found in deeper areas in Lake Baikal where dives cannot easily reach (Masuda, 2009). Because *B. intermedia* and *L. baicalensis* inhabit shallow zones, these sponges are much more easily found in Lake Baikal. The results of this study show that the bacterial diversity in *S. papyracea* was highest among the three sponges and that the bacterial species composition in *S. papyracea* differs from that in *B. intermedia* and *L. baicalensis*.

We propose two hypotheses on the diversity of bacterial symbionts in *S. papyracea*. First, the bacterial community within *S. papyracea* is sponge-specific. A unique microbial community with high sponge-specificity has been shown in previous studies (Taylor *et al.*, 2004; Webster and Blackall, 2009). By comparing the bacterial richness and species composition of two sponge species inhabiting both isolated saline lakes and an adjacent open system, some bacteria were found to be host-specific and others habitat-specific (Cleary *et al.*, 2013). Sponge–bacteria interactions could differ according to nutrient acquisition strategy, stability of the sponge skeleton and metabolic differences (Vacelet and Boury-Esnault, 1995; Weisz *et al.*, 2007). However, in the case of sympatric sponges, the microbial community is known to have a higher tendency toward a "generalist association" (Alex *et al.*, 2013) and the bacterial species in different sponges are more closely related to each other (Schmitt *et al.*, 2012). Secondly, *S. papyracea* seems to be affected by environmental factors of its own unique bacterial community as a "rare biosphere" compared with other co-occurring sponges. *S. papyracea* was collected only at the deeper depth (42 m), where the higher pressure, water temperature and nutrient poor conditions are different from shallower depths. Though no direct relationship between sponge-symbionts and the bacterial community in the ambient water exists, the ambient conditions can be a trigger for building community structure within sponges (Burja and Hill, 2001). Therefore, the microbial community in *S. papyracea* might be adapted for specific environmental conditions, such as low light levels or higher pressure. We found that only members of *Acidobacteria* and *Gemmatimonadetes* (two phyla, 28 genera, 88 species) were associated with *S. papyracea*. These phyla are ubiquitous in some soils (DeBruyn *et al.*, 2011), but little is known of their ecology in freshwater ecosystems. We suggest that the microbial symbiont community within sponges is strongly affected by environmental conditions or gradients across water depths in Lake Baikal.

Cyanobacteria and Actinobacteria

Among the three sponges, *Cyanobacteria*, *Proteobacteria* and *Actinobacteria* were identified as major components in the microbial symbiont community. These groups have frequently been reported both in marine and freshwater sponges (Li *et al.*, 2006). In our study, the relative *Cyanobacteria* abundance decreased dramatically at the deepest depth (42 m). For *Cyanobacteria*, light intensity is a major factor controlling cyanobacterial growth (Bryant and Frigaard, 2006) and darkness can cause a loss of cyanobacterial symbionts (Regoli *et al.*, 2000). Considering the transparency of our sampling sites, *B. intermedia* and *L. baicalensis* were collected at shallower areas with efficient light (Hampton *et al.*, 2008). Gladkikh *et al.*, (2014) reported that the proportion of *Cyanobacteria* was only 1.4 and 1.3% in *B. intermedia* and *L. baicalensis*, respectively. Another study showed that the highest proportion of cyanobacteria was 61.4 and 21.4% in summer and winter, in sponges, respectively (Erwin *et al.*, 2012). We assume that *Cyanobacteria* are more abundant at shallow depths with higher light intensities and warmer temperatures. Within the Cyanobacteria, members of *Prochlorococcus* were abundant in all three sponges. However, *P. marinus* was present only in *S. papyracea* (42 m depth). *P. marinus* can absorb blue light in deep water (Ralf and Repeta, 1992), and is known as a major primary producer in the ocean (Munn, 2011). To date, little is known about this bacterium in freshwater sponges. We found a new member of *Prochlorococcus* in Baikalian sponges. The relative abundance of *Actinobacteria* increased with water depth and the species diversity was highest in *S. papyracea*. *Actinobacteria* is recognized as the producer of many bioactive metabolites, including antibacterials (Mahajan and Balachandran, 2011). We found a specific *Actinobacteria* which is associated with *S. papyracea* but not with other species of sponges studied here.

Based on our results, further research should focus on functional gene diversity to understand more specific ecological function and isolation of these symbionts for biotechnical application. Specifically, we suggest that a new technique, such as the I-tip method (Jung *et al.*, 2014) should be applied to isolate uncultured symbionts associated with *S. papyracea*, which might lead to novel useful isolates as these were more diverse and specific.

Acknowledgements. This work was supported by the Basic Research Project No. 55.1.3. "The structure, formation, dynamics and metabolic potential of microbial communities and phages in biofilms of freshwater reservoirs", and was supported by a 2014 Research Grant from Kangwon National University (Grant no. 120140152). We thank J. S. Owen for help in editing.

References

Alex A., Silva V., Vasconcelos V. and Antunes A., 2013. Evidence of unique and generalist microbes in distantly related sympatric intertidal marine sponges (Porifera: Demospongiae). *PLoS ONE*, *8*(11), 1–10, doi: 10.1371/journal.pone.0080653

Bryant D.A. and Frigaard N.-U., 2006. Prokaryotic photosynthesis and phototrophy illuminated. *Trends Microbiol.*, *14*, 488–496.

Burja A.M. and Hill R.T., 2001. Microbial symbionts of the Australian Great Barrier Reef sponge, *Candidaspongia flabellata*. *Hydrobiologia*, *461*, 41–47.

Chun J., Kim K.Y., Lee J.-H. and Choi Y., 2010. The analysis of oral microbial communities of wild-type and toll-like receptor 2-deficient mice using a 454 GS FLX Titanium pyrosequencer. *BMC Microbiol.*, *10*, 101–108.

Cleary D.F., Becking L.E., de Voogd N.J., Pires A.C., Polónia A.R., Egas C. and Gomes N.C., 2013. Habitat-and host-related variation in sponge bacterial symbiont communities in Indonesian waters. *FEMS Microbiol. Ecol.*, *85*, 465–482.

DeBruyn J.M., Nixon L.T., Fawaz M.N., Johnson A.M. and Radosevich M., 2011. Global biogeography and quantitative seasonal dynamics of *Gemmatimonadetes* in soil. *Appl. Environ. Microbiol.*, *77*, 6295–6300.

Dunlap W.C., Battershill C.N., Liptrot C.H., Cobb R.E., Bourne D.G., Jaspars M., Long P.F. and Newman D.J., 2007. Biomedicinals from the phytosymbionts of marine invertebrates: a molecular approach. *Methods*, *42*, 358–376.

Erwin P.M., López-Legentil S. and Turon X., 2012. Ultrastructure, molecular phylogenetics, and chlorophyll a content of novel cyanobacterial symbionts in temperate sponges. *Microb. Ecol.*, *64*, 771–783.

Gernert C., Glöckner F.O., Krohne G. and Hentschel U., 2005. Microbial diversity of the freshwater sponge *Spongilla lacustris*. *Microb. Ecol.*, *50*, 206–212.

Gladkikh A., Kalyuzhnaya O.V., Belykh O., Ahn T.S. and Parfenova V., 2014. Analysis of bacterial communities of two Lake Baikal endemic sponge species. *Microbiology*, *83*, 787–797.

Hampton S.E., Izmest'eva L.R., Moore M.V., Katz S.L., Dennis B. and Silow E.A., 2008. Sixty years of environmental change in the world's largest freshwater lake–Lake Baikal, Siberia. *Glob. Change Biol.*, *14*, 1947–1958.

Hentschel U., Hopke J., Horn M., Friedrich A.B., Wagner M., Hacker J. and Moore B.S., 2002. Molecular evidence for a uniform microbial community in sponges from different oceans. *Appl. Environ. Microbiol.*, *68*, 4431–4440.

Jung D., Seo E.-Y., Epstein S.S., Joung Y., Han J., Parfenova V.V., Belykh O.I., Gladkikh A.S. and Ahn T.S., 2014. Application of a new cultivation technology, I-tip, for studying microbial diversity in freshwater sponges of Lake Baikal, Russia. *FEMS Microbiol. Ecol.*, *90*, 417–423.

Kamke J., Taylor M.W. and Schmitt S., 2010. Activity profiles for marine sponge-associated bacteria obtained by 16s rRNA vs 16s rRNA gene comparisons. *ISME J.*, *4*, 498–508.

Kunin V., Engelbrektson A., Ochman H. and Hugenholtz P., 2010. Wrinkles in the rare biosphere: Pyrosequencing errors can lead to artificial inflation of diversity estimates. *Environ. Microbiol.*, *12*, 118–123.

Li Z.-Y., He L.-M., Wu J. and Jiang Q., 2006. Bacterial community diversity associated with four marine sponges from the South China Sea based on 16s rDN-DGGE fingerprinting. *J. Exp. Mar. Biol. Ecol.*, *329*, 75–85.

Mahajan G.B. and Balachandran L., 2011. Antibacterial agents from actinomycetes-a review. *Front. Biosci.*, *4*, 240–253.

Masuda Y., 2009. Studies on the taxonomy and distribution of freshwater sponges in Lake Baikal. *In*: Mueller W.E.G. and Grachev M.A. (eds.), Biosilica in Evolution, Morphogenesis, and Nanotechnology, Progress in Molecular and Subcellular Biology, Vol. 47, Springer-Verlag, Berlin, 81–110.

Moore M.V., Hampton S.E., Izmest'eva L.R., Silow E.A., Peshkova E.V. and Pavlov B.K., 2009. Climate change and the world's "sacred sea"-Lake Baikal, Siberia. *BioScience*, *59*, 405–417.

Munn C., 2011. Marine Microbiology: Ecology and Application (2nd edn,), Garland Science, New York, 50.

Parfenova V., Terkina I., Kostornova T.Y., Nikulina I., Chernykh V. and Maksimova E., 2008. Microbial community of freshwater sponges in Lake Baikal. *Biol. Bull.*, *35*, 374–379.

Ralf G. and Repeta D.J., 1992. The pigments of *Prochlorococcus marinus*: the presence of divinylchlorophyll a and b in a marine procaryote. *Limnol. Oceanogr.*, *37*, 425–433.

Regoli F., Cerrano C., Chierici E., Bompadre S. and Bavestrello G., 2000. Susceptibility to oxidative stress of the mediterranean demosponge *Petrosia ficiformis*: role of endosymbionts and solar irradiance. *Mar. Biol.*, *137*, 453–461.

Reiswig H.M., 1974. Water transport, respiration and energetics of three tropical marine sponges. *J. Exp. Mar. Biol. Ecol.*, *14*, 231–249.

Schloss P.D., Westcott S.L., Ryabin T., Hall J.R., Hartmann M., Hollister E.B., Lesniewski R.A., Oakley B.B., Parks D.H. and Robinson C.J., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.*, *75*, 7537–7541.

Schmitt S., Tsai P., Bell J., Fromont J., Ilan M., Lindquist N., Perez T., Rodrigo A., Schupp P.J. and Vacelet J., 2012. Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. *ISME J.*, *6*, 564–576.

Taylor M.W., Radax R., Steger D. and Wagner M., 2007. Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiol. Mol. Biol. Rev.*, *71*, 295–347.

Taylor M.W., Schupp P.J., Dahllöf I., Kjelleberg S. and Steinberg P.D., 2004. Host specificity in marine sponge -associated bacteria, and potential implications for marine microbial diversity. *Environ. Microbiol.*, *6*, 121–130.

Vacelet J. and Boury-Esnault N., 1995. Carnivorous sponges. *Nature*, *373*, 333–335.

Vogel G. 2008. The inner lives of sponges. *Science 320*, 1028–1030.

Webster N.S. and Blackall L.L., 2009. What do we really know about sponge-microbial symbioses. *ISME J.*, *3*, 1–3.

Weinberg E., Weinberg I., Efremova S., Tanichev A. and Masuda Y., 2003. Late Pliocene spongial fauna in Lake Baikal (from material from the deep drilling core BDP-96-1). *In*: Kashiwaya K. (ed.), Long Continental Records from Lake Baikal, Springer-Verlag, Tokyo, 283–293.

Weisz J.B., Hentschel U., Lindquist N. and Martens C.S., 2007. Linking abundance and diversity of sponge-associated microbial communities to metabolic differences in host sponges. *Mar. Biol.*, *152*, 475–483.