The prevalence of Atribacteria affiliated with JS1 in the sediment core of Lake Sayram, the largest alpine lake, China

Keqiang Shao,[1] Tunasheng Ba,[2] Boqiang Qin,[1] Jianying Chao,[3] Guang Gao[1*]

[1]Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing; [2]Environmental monitoring station of Bayingolin Mongolia Autonomous Prefecture, Korle; [3]Ministry of Environmental Protection, Nanjing Institute of Environmental Sciences, Nanjing, China

ABSTRACT

Atribacteria play an important role in global carbon cycling. Little is known, however, about the Atribacteria communities found in alpine lakes. The presence and diversity of Atribacteria in the sediment core of alpine Sayram lake in China were investigated using Illumina MiSeq sequencing in this study. According to the findings, Atribacteria affiliated with JS1 were dominant in the sediment core of Sayram Lake, with a relative abundance of 0.19 to 10% (average 3.75%) of all bacterial sequences. Furthermore, the relative abundance of Atribacteria associated with JS1 increased with sediment depth. As a result, this study significantly contributes to our understanding of Atribacteria community habitat.

Human activities continue to release carbon into bodies of water, the land, and the atmosphere (Biddanda, 2017). Water bodies, despite accounting for less than 4% of the earth's surface, play an important role in the global carbon cycle due to their high rates of carbon respiration and fixation (Cole *et al.*, 2007). The amount of carbon emitted by inland waters equals the net amount of carbon absorbed by organisms on Earth's land surface and in the ocean (Biddanda, 2017).

Atribacteria are strictly anoxic bacteria that may play an important role in the global carbon cycle (Liu *et al.*, 2021). As a result, studying lake Atribacteria communities is critical for gaining a better understanding of carbon cycling in bodies of water. Atribacteria are found in a variety of environments, including the terrestrial and marine subsurface (Vick *et al.*, 2010), anaerobic marine sediments (Webster *et al.*, 2004), oil reservoirs (Wang *et al.*, 2014), and brackish waters (Rinke *et al.*, 2013). However, knowledge of Atribacteria communities in alpine lake sediment, particularly in arid and semi-arid regions, is limited.

Sayram Lake is the largest oligotrophic alpine and cold-water inland lake in China's Xinjiang Uygur autonomous region and is known as a "pearl" of the Silk Road. The lake's transparency ranges between 8 and 13 meters, its maximum depth is 90 meters, and its average depth is 46 meters (Zeng *et al.*, 2014). Using Illumina MiSeq sequencing, we assessed the presence and diversity of Atribacteria communities in the sediment core of the alpine Sayram lake. On August 16, 2018, we collected a 100-cm-long sediment core from Sayram Lake's lake center (44.597312°N and 81.206671°E; Fig. 1). The sediment core was sub-sampled in situ at 4 cm intervals. Wet sediment samples were kept at 4°C until they were freeze-dried. These 25 sediment samples were identified as S1-S25, respectively. Sediment samples for total organic carbon (TOC) analysis were treated with HCl (~3 N) to remove carbonates and then rinsed with deionized water to remove remaining chloride. TOC analysis was performed using a CE-440 elemental analyzer (EAI Company, USA). The results showed that the TOC values of 25 different layer sediment samples in the sediment core of Sayram lake varied from 4.15 to 8.32% (average 6.49%) (Fig. 2).

Sediment DNA was extracted by the PowerCleanTM DNA Clean-Up Kit for Soil (Mo Bio, USA) according to the procedures of the manufacturer. Detection of DNA concentration shows that the purification and recovery

rate of this Kit is 88%-95%, using a NanoDrop ND-1000 UV/Vis spectral photometer. The bacterial V3-V4 hyper-variable regions were amplified using the primer set 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Sequencing was performed using the Illumina MiSeq platform by Guangzhou Gene Denovo Biotechnology (Co. Ltd.), China. The sequencing data have been deposited at the Sequence Read Archive database of the National Center for Biotechnology Information (NCBI) under accession number PRJNA853917. After importing and merging paired-end raw reads, a standard quality control process was implemented, comprising trim off adapters and primers as well as remove low quality reads (total expected errors >1) (Christensen, 2018). Chimeras were identified and removed with the program USEARCH (version 10) (Edgar, 2010). The bacterial phylotypes were identified and assigned to operational taxonomic units
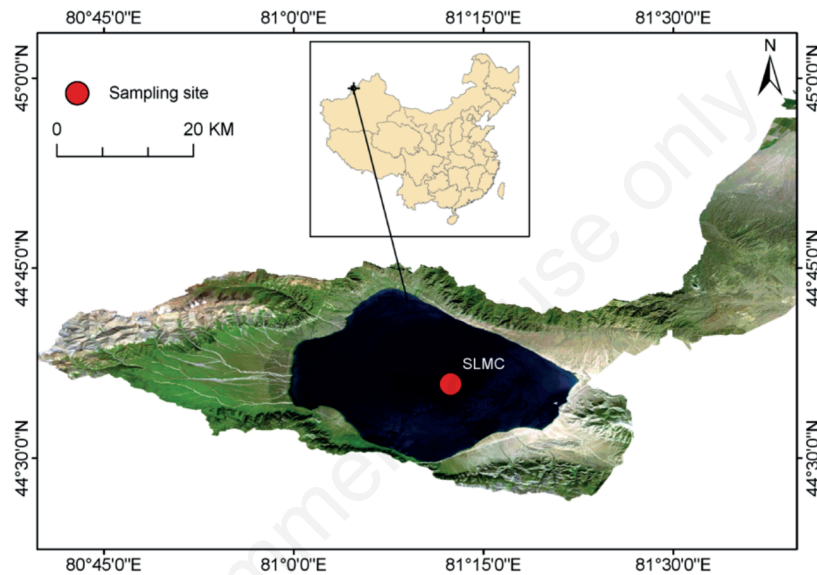

Fig. 1. Satellite images of alpine Sayram lake basin, China, showing the location of sampling site.
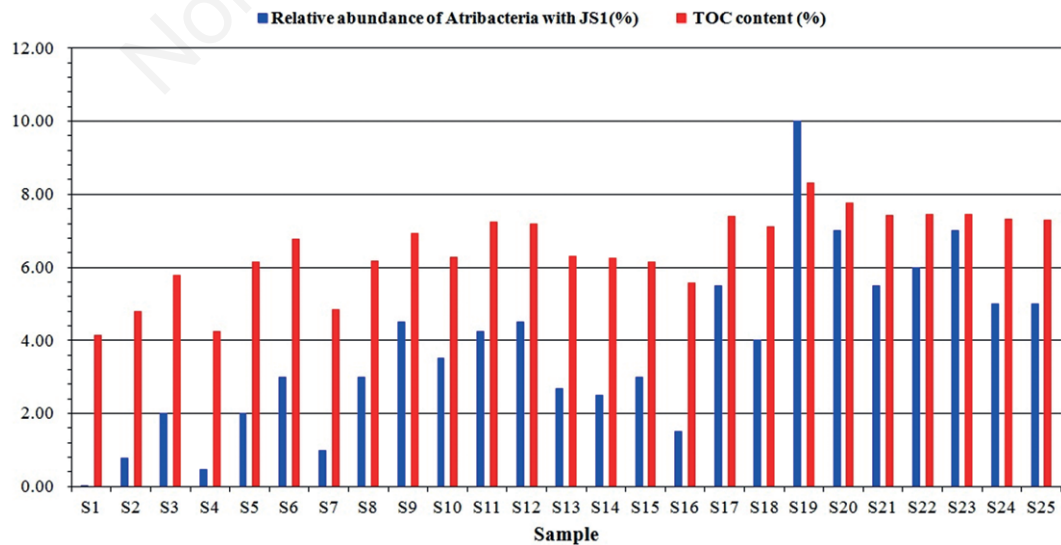

Fig. 2. The relative abundance of Atribacteria affiliated with JS1 (blue column) and the content of total organic carbon (TOC) (red column) in different layer samples at the sediment core in the alpine Sayram lake, China.

(OTUs) based on 97% similarity threshold using UP-ARSE (v7.0.1090) (Edgar, 2013). The low abundance OTUs (<10 reads) was filtered out to minimize the random sequencing error. Taxonomic classification was analyzed using the databases of the SILVA small subunit rRNA (SSU) Database Project v132 with a confidence threshold of 80%.

The sequencing results showed that Atribacteria was one of the dominant bacterial phylum in the sediment core of Sayram lake, and had a relative abundance of 0.19 to 10% (average 3.75%) of total sequences among 25 different layer sediment samples (Fig. 2). Our unexpected findings within the high abundance of Atribacteria of the sediment core in Sayram lake agreement with those of many previous studies, reporting that Atribacteria dominant in various environments enriched with hydrocarbon, including oil reservoirs (Hernández-Torres *et al.*, 2015), methane hydrates (Vigneron *et al.*, 2017), marine hydrocarbon seeps (Kleindienst *et al.*, 2014) and tailing ponds of oil sands (Toth *et al.*, 2018). Atribacteria was a candidate phylum including the OP9 and JS1 lineages (Nobu *et al.*, 2016). Our results showed that the Atribacteria from the alpine Sayram lake sediment only included one subgroup: JS1 (Fig. 2). The JS1 lineage was originally also found in the Japan Sea sediment, and was especially predominant in anaerobic organic-rich environments (Lee *et al.*, 2018).

The presence of Atribacteria in high abundance in the sediment core of the alpine Sayram lake may be related to the higher TOC content of the sediment. Spearman correlation analysis using SPSS 20.0 revealed that TOC content was significantly positively correlated with Atribacteria relative abundance (r=0.9268, p<0.001). Previous research has shown that Atribacteria can degrade sugars, organic compounds, and short chain n-alkanes (Liu *et al.*, 2021), and that they are dominant in anaerobic, organic carbon-rich, and methane-rich marine environments (Hoshino *et al.*, 2020). Notably, the vertical distribution of Atribacteria in the alpine Sayram lake sediment revealed that the relative abundance of Atribacteria increased with depth in the sediment, ranging from 0.19 percent in surface sediment to 10% in deeper sediment. This is consistent with the findings of Carr *et al.* (2015), who found that the relative abundance of Atribacteria increased with depth in Antarctic marine sediment.

In summary, Illumina MiSeq sequencing revealed a high relative abundance of Atribacteria associated with JS1 in the sediment core of the alpine Sayram lake, accounting for 3.75% of total sequence reads and being the dominant phylum. This study adds to our understanding of the habitat of Atribacteria communities in arid and semi-arid ecosystems, which is currently under threat.

REFERENCES

Biddanda BA, 2017. Global significance of the changing freshwater carbon cycle. Eos 98.

Carr SA, Orcutt BN, Mandernack KW, Spear JR, 2015. Abundant atribacteria in deep marine sediment from the Adélie Basin, Antarctica. Front Microbiol 6:872.

Christense H, 2018. Introduction to bioinformatics in microbiology. Springer, Cham: 213 pp.

Cole JJ, Prairie YT, Caraco NF, Mcdowell WH, Tranvik LJ, Striegl RG, et al., 2007. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. Ecosystems 10:172-185.

Edgar RC, 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26: 2460-2461.

Edgar RC, 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10:996-998.

Hernández-Torres J, Castillo Villamizar GA, Salgar-Chaparro SJ, Silva-Plata BA, Serna Daza OD, Martínez-Pérez F, et al., 2015. Prokaryotic community characterization in a mesothermic and waterflooded oil reservoir in Colombia. Geomicrobiol J 33:110-7.

Hoshino T, Doi H, Uramoto GI, Wörmer L, Adhikari RR, Xiao N, et al., 2020. Global diversity of microbial communities in marine sediment. P Natl Acad Sci USA 117:27587-27597.

Kleindienst S, Herbst FA, Stagars M, von Netzer F, von Bergen M, Seifert J, et al., 2014. Diverse sulfate-reducing bacteria of the Desulfosarcina/Desulfococcus clade are the key alkane degraders at marine seeps. ISME J 8:1-16.

Lee YM, Hwang K, Lee JI, Kim M, Hwang CY, Noh HJ, et al., 2018. Genomic insight into the predominance of candidate phylum Atribacteria JS1 lineage in marine sediments. Front Microbiol 9:2909.

Liu ZL, Liu YF, Lin DD, Zhou L, Mu BZ, 2021. [Environmental distribution and functions of the Atribacteria].[Article in Chinese]. Acta Microbiol Sinica 61:1416-1427.

Nobu MK, Dodsworth J, Murugapiran SK, Rinke C, Gies E, Webster G, et al., 2016. Phylogeny and physiology of candidate phylum 'Atribacteria' (OP9/JS1) inferred from cultivation-independent genomics. ISME J 10:273-286.

Rinke C, Schwientek P, Sczyrba A, Ivanova NN, Anderson IJ, Cheng JF, et al., 2013. Insights into the phylogeny and coding potential of microbial dark matter. Nature 499:431-437.

Toth CRA, Gieg LM, 2018. Time course-dependent methanogenic crude oil biodegradation: dynamics of fumarate addition metabolites, biodegradative genes, and microbial community composition. Front Microbiol 8:1-16.

Vick TJ, Dodsworth JA, Costa KC, Shock EL, Hedlund BP, 2010. Microbiology and geochemistry of Little Hot Creek, a hot spring environment in the Long Valley Caldera. Geobiology 8:140-154.

Vigneron A, Alsop EB, Cruaud P, Philibert G, King B, Baksmaty L, et al., 2017. Comparative metagenomics of hydrocarbon and methane seeps of the Gulf of Mexico. Sci Rep 7:16015.

Wang LY, Ke WJ, Sun XB, Liu JF, Gu JD, Mu BZ, 2014. Comparisonof bacterial community in aqueous and oil phases of water-flooded petroleum reservoirs using pyrosequencing and clone library approaches. Appl Microbiol Biotechnol 98:4209-4221.

Zeng J, Deng LJ, Lou K, Zhang T, Yang HM, Shi YW, et al., 2014. Molecular characterization of the planktonic microorganisms in water of two mountain brackish lakes. J Basic Microbiol 54:509-520.