Influence of Salinity on the Bacterial Community Composition in Lake Bosten, a Large Oligosaline Lake in Arid Northwestern China

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Salinity was found to be the dominating contributor controlling bacterial community composition (BCC) and the abundance of *Betaproteobacteria* in the oligosaline Lake Bosten. The high percentage of unclassified bacteria inhabiting this unique habitat highlights the potential ecological importance of BCC in the early stage of lake salinization and eutrophication.

Currently, most of our knowledge about lake bacterial ecology originates from lowland freshwater lake systems (9, 20, 28, 31, 36) and from saline and hypersaline habitats (8, 11, 29, 30, 33). We still know very little about the bacterial community compositions (BCC) in oligosaline lakes in arid regions. Lake Bosten was the largest inland freshwater lake in China before the 1960s. In the past 50 years, however, it has evolved from a freshwater to an oligosaline lake (2, 32). It represents an interesting ecosystem featuring a complex hydrology and an intrasystem salinity gradient, as well as a nutrition gradient from oligotrophic to mesotrophic (32).

To scrutinize the BCC and the related environmental factors of Lake Bosten in arid northwestern China, surface water (the top 50 cm) samples from 17 sites of the large-lake area (LLA; surface area, $\approx 950 \text{ km}^2$, and mean depth, $\approx 7 \text{ m}$) and 6 sites of the small-lake area (SLA) were collected during 11 to 13 June 2010 (Fig. 1A). Biotic and abiotic variables of sampled water (see Table S1 in the supplemental material) were measured using standard methods (12). Then, a subsample of water (250 ml) for 16S rRNA gene analysis was filtered through a 0.2- μm -pore-size polycarbonate filter in the field, and genomic DNA was extracted (26, 35). For denaturing gradient gel electrophoresis (DGGE) analysis, PCR amplification was carried out using a touchdown program with the primers 341F and 534R (19, 26).

Cluster analysis of environmental parameters, including total dissolved solids (TDS) revealed distinct spatial differences (Fig. 2A): samples with low salinity ($\text{TDS} < 0.4 \text{ g liter}^{-1}$) and high salinity ($\text{TDS} > 1.1 \text{ g liter}^{-1}$) were separated from each other and grouped into two respective arbitrarily defined clusters. Cluster analysis of BCC based on the DGGE data also revealed distinct spatial heterogeneity (Fig. 2B), and the dendrogram of BCC was similar to the matrices based on environmental parameters. Redundancy analysis (RDA) performed with the software CANOCO 4.5 (27) indicated that salinity (TDS) was the most important selector in Lake Bosten (Fig. 2C), solely accounting for 17.8% of the variation in BCC. Our results agree with previous findings that salinity is the major factor relating microbial communities (13, 18, 23, 30, 33).

To reveal the influence of salinity on bacterial taxonomic differentiation in detail, DNA extracted from six samples (sites 2, 7, 14, 15, 21, and 22) was selected for construction of the 16S rRNA

gene clone libraries based on the DGGE profiles (6, 21, 25). In total, 99 operational taxonomic units (OTUs, $\geq 97\%$ similarity) were acquired from 374 nonchimeric partial 16S rRNA gene sequences (see Table S2 in the supplemental material). *Betaproteobacteria* were the most abundant bacterial group in the clone libraries of sites 2, 7, and 15, accounting for 45.9%, 48.4%, and 52.6% of the clones, respectively (Fig. 1B). *Bacteroidetes* accounted for 73.4% of the clones in the library of site 22. Consistent with our result, previous studies also showed that *Betaproteobacteria* and *Bacteroidetes* are often the numerically dominant groups inhabiting the upper waters and organic particles in freshwater lakes (1, 9, 14, 20, 25, 36). However, detailed analysis of the BCC revealed high percentages of unidentified bacteria in the libraries (see Fig. S1 and S2 in the supplemental material). For example, 62.7% of *Betaproteobacteria* and 82.1% of *Bacteroidetes* could not be affiliated to known bacteria at the genus level, indicating that potential new bacterial ribotypes inhabit the unique habitat of Lake Bosten.

Salinity also affects the abundance of *Betaproteobacteria*. The high betaproteobacterial abundance in freshwater habitats is in sharp contrast to their relative scarcity in oceans and hypersaline lakes (3, 30, 36), indicating the physiological barrier posed to this group by high salinity (5, 13, 17). Within the salinity gradient from 0.2 g liter^{-1} to 1.7 g liter^{-1} in Lake Bosten, however, we found that betaproteobacterial abundance was positively correlated with salinity ($r = 0.954$, $P = 0.003$, $n = 6$) (see Fig. S3 in the supplemental material). This is consistent with the findings of *Betaproteobacteria* peaking at salinities of around 1 g liter^{-1} in an estuary (4). In contrast, Wu et al. found a negative correlation of betaproteobacterial abundance with a salinity range from 0.2 g liter^{-1} to 3.6 g liter^{-1} in high mountain lakes (30). The differences may result from different levels of nutrients in Lake Bosten (mainly mesotro-

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FIG 1 (A) Map of Lake Bosten, showing the sampling sites and the concentrations of total dissolved solids (TDS) in the surface water. Site annotations were derived from those of long-term monitoring sites used by the Institute of Lake Bosten. (B) Bacterial community composition of the six representative sampling sites in Lake Bosten. Numbers of sequenced clones and operational taxonomic units (OTUs, $\geq 97\%$ identity) are indicated above the major taxonomic group distribution pie chart for each site. Clones affiliated with *Actinobacteria*, *Acidobacteria*, *Chlorobi*, *Cyanobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*, *Firmicutes*, *Planctomycetes*, and unclassified bacteria are included in "Others." L, liter.

phic) and in the Tibetan Plateau lakes (oligotrophic). Bacterial growth in lakes is determined by the trophic status of the systems (7). In Lake Bosten, the increase in salinity is often coupled with increasing nutrient concentrations due to human activities (32, 34). And *Betaproteobacteria* have the ability to degrade complex organic macromolecules that come from input of pollutants (10, 20).

Statistical analysis demonstrated significant spatial heterogeneity of BCC in Lake Bosten, as determined by β -Libshuff comparisons (22, 24) of the six clone libraries (see Table S3 in the supplemental material). Two main aspects have shaped and are still shaping the distribution patterns of salinity in Lake Bosten and, thus, structure the spatial heterogeneity of BCC. The first is continuous input of saline water from salt leaching in the process of agricultural production since the 1960s. About 220,000 tons of salt are discharged from the drainage channels

(especially the Huangshui River) (Fig. 1A) on the northwestern side of Lake Bosten every year (34), which influenced the BCC in most of the sampling sites (except sites 13 and 14) in LLA. The second is the hydrology of Lake Bosten and the subsequent local environments of different sampling sites (32). Kaidu River supplies an average of 85% of the runoff into the lake. Divided by the Baolangsumu diversion gate (Fig. 1A), about 70% of the volume of the Kaidu River flows into the LLA through the eastern branch, and the water is removed by two pumping stations at the southwestern margin. Because of the short water residence time, the input of allochthonous riverine bacteria would be an important reason for the significant difference of BCC between site 14 and other sites (15, 16). The western branch of the Kaidu River flows into the reed-covered SLA (Fig. 1A). Accordingly, the BCC in site 22 seemed to be mainly influenced by the abundant submersed macrophytes

FIG 2 Similarity matrices and redundancy analysis (RDA) of the studied samples from Lake Bosten. (A) Water column chemistry and environmental parameters as determined by a similarity analysis (distance) in Primer-E. (B) Dendrogram of eubacterial communities generated from DGGE band presence-absence data using the software GelCompar II. Samples from both matrices can be grouped into two arbitrary clusters: cluster I presents high-TDS samples, and cluster II presents low-TDS samples. (C) RDA biplot showing different bacterial communities in relation to the four significant environmental factors. Site numbers are adjacent to the symbols. TDS, TP, Turb and DO refer to total dissolved solids, total phosphorus, turbidity, and dissolved oxygen, respectively.

(31). In addition to salinity, the anaerobic condition is considered to be the strongest determinant of BCC in site 21.

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