**RESEARCH ARTICLE**

**Abundance and diversity of aerobic anoxygenic phototrophic bacteria in saline lakes on the Tibetan plateau**

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**Keywords**

pufL-M; AAP bacteria; abundance; diversity; Qinghai Lake.

**Abstract**

Aerobic anoxygenic phototrophic (AAP) bacteria are heterotrophic prokaryotes that are capable of utilizing light as an energy source but are not capable of producing molecular oxygen. Recently, multiple studies have found that AAP bacteria are widely distributed in oceans and estuaries and may play an important role in carbon cycling. However, AAP bacteria in inland lake ecosystems have not been investigated in depth. In this study, the abundance and diversity of the pufL-M genes, encoding photosynthetic reaction centers of AAP bacteria, were determined in the oxic water column and anoxic sediments of saline lakes (Qinghai, Erhai, and Gahai Lakes) on the Tibetan Plateau, China. Our results indicated that AAP bacteria were abundant in inland lakes, with the proportion of AAP bacteria (in total bacteria) comparable to those in the oceans, but with a lower diversity. Salinity and pH were found to be potential factors controlling the AAP bacterial diversity and community composition. Our data have implications for a better understanding of the potential role of AAP bacteria in carbon cycling in inland lake ecosystems.

**Introduction**

Aerobic anoxygenic phototrophic (AAP) bacteria are a group of heterotrophic prokaryotes capable of utilizing light as a primary energy source but not capable of producing molecular oxygen (Yurkov & Beatty, 1998a, b; Beatty, 2002). Studies have revealed that AAP bacteria are widely distributed in various habitats, including marine environments, acidic mine drainage, and hot springs (Yurkov & Beatty, 1998b), rivers and estuaries (Waidner & Kirchman, 2005, 2007, 2008), as well as inland lakes (Yurkova et al., 2002; Karr et al., 2003; Page et al., 2004; Gich & Overmann, 2006; Masin et al., 2008). AAP bacteria can account for a large portion (several percent to more than half) of the total bacterial community in various aquatic ecosystems (Kolber et al., 2001; Du et al., 2006; Jiao et al., 2007; Waidner & Kirchman, 2007; Masin et al., 2008), that is, several to about 10% of the total bacterial community in the ocean (Kolber et al., 2001), up to 34% in estuaries (Waidner & Kirchman, 2007), and more than half in freshwater mountain lakes (Masin et al., 2008). AAP bacterial biomass showed significant spatial and temporal variations in the global oceans (Jiao et al., 2007; Zhang & Jiao, 2007) and freshwater mountain lakes (Masin et al., 2008).

Phylogenetic analysis based on the pufL-M genes suggests that most, if not all, AAP bacteria belong to the alpha-, beta-, and gamma-subclasses of Proteobacteria (Yutin & Beja, 2005), and that AAP bacterial community composition varies with respect to different ecosystems. Alphaproteobacterial and gammaproteobacterial AAP bacteria are abundant in saline water bodies (such as oceans, lakes and estuaries), whereas betaproteobacterial AAP bacteria are predominant in freshwater bodies (Yurkova et al., 2002; Allgaier et al., 2003; Karr et al., 2003; Kobližek et al., 2003; Oz et al., 2005; Waidner & Kirchman, 2005, 2007, 2008; Cho et al., 2007; Jiao et al., 2007). Light, dissolved organic carbon (DOC), salinity, and association with particles may control the AAP bacterial abundance and distribution in estuaries and oceans (Jiao et al., 2007; Zhang & Jiao, 2007; Waidner & Kirchman, 2008).

Despite the fact that saline lakes account for almost half of the total volume of all terrestrial aquatic ecosystems (William, 1996), little is known about the diversity and abundance of AAP bacteria in such ecosystems. Are AAP
bacteria in saline lakes more abundant and/or diverse than the oceans? How does the AAP bacteria community respond to salinity gradient? In this study, we strive to answer these questions by investigating the abundance and diversity of AAP bacteria in three saline lakes on the Tibetan Plateau in NW China: Qinghai Lake (36° 32′–37° 15′ N, 99° 36′–100° 47′ E) and two of its daughter lakes (Gahai Lake and Erhai Lake). Multiple methods were used including aqueous geochemistry, time-series observation-based infrared epifluorescence microscopic (TIREM) counting of AAP bacteria, quantitative PCR (qPCR) determination of functional genes (pufL-M), and functional gene-based diversity analysis.

Qinghai Lake is the largest (4300 km²) saline (12.5 practical salinity unit, PSU) and alkaline (pH 9.3) lake on the Tibetan Plateau (Dong et al., 2006), and it represents a relatively pristine environment, where perturbations from human activities have been small. Because of the continuous drying, Gahai Lake (c. 35 km²) and Erhai Lake (c. 5 km²) have been isolated from Qinghai Lake since the Holocene. Because of different water dynamics, these lakes have developed into a salinity gradient (Gahai Lake > Qinghai > Erhai Lake) but their water chemistry is similar (CAS, 1979). Thus, studies of AAP bacteria abundance and diversity in these lakes can fill the aforementioned knowledge gap.

**Materials and methods**

**Description of the study site**

Qinghai Lake is a perennial lake located in a structural intermontane basin at the northeastern corner of the Tibetan Plateau (Fig. 1). The average water depth is 19.2 m, and the maximum is 28.7 m. The altitude of the lake is 3196 m above sea level (m.a.s.l.), and the surrounding mountains rise to above 5200 m.a.s.l. (Dong et al., 2006). The water column of Qinghai Lake is fairly dynamic, with limited seasonal stratification (CAS, 1979; Dong et al., 2006). The main (80%) water supplies to Qinghai Lake are inflowing rivers, which are mostly distributed on the southwest, northwest, and north of the lake. The remaining 20% of the water supplies are springs at the bottom of Qinghai Lake and rainfalls (CAS, 1979). The evaporation of the lake (c. 1400 mm year⁻¹) is in excess of the mean annual water replenishment (c. 400 mm year⁻¹), resulting in the development of a saline lake with a salinity of 12.5 PSU (Shen et al., 2005; Dong et al., 2006) and formation of isolated daughter lakes on the eastern edge of the lake. Gahai Lake and Erhai Lake are two representative daughter lakes. The area of Gahai and Erhai is c. 47.2 and c. 5 km² and the maximum water depth is 9 and 2.0 m, respectively.

**Sample collection and processing**

In July 2007, three sites (36° 735′ N/100° 355′ N, 36° 992′ N/100° 611′ E, and 36° 546′ N/100° 722′ E) from Qinghai Lake, Gahai Lake, and Erhai Lake, respectively) were selected for in situ measurement of lake water chemistry and sampling. The Qinghai Lake site was also selected for sediment core collection (Fig. 1). In situ measurements of water geochemistry were performed using a multiple parameter probe set (Horiba (Japan). Water depth was 23, 0.5, and 0.5 m at the sampling sites of Qinghai Lake, Erhai Lake, and Gahai Lake, respectively. A 56-cm-long sediment core (QL abbreviation of Qinghai Lake) was retrieved by a gravity-coring device and immediately frozen in dry ice. At the site of Qinghai Lake, depth profiles of lake water geochemistry were measured every 2 m from a large boat. For Gahai Lake and Erhai Lake, only the surface water was selected for in situ water geochemical analyses and sample collection. The geochemical parameters included pH, conductivity, turbidity, dissolved oxygen (DO), temperature, salinity, total dissolved solids, density, oxidation–reduction potential, and concentrations of nitrate, chloride, and ammonia (H. Jiang et al., unpublished data). Based on these measurements, four depths (0, 5, 12, and 23 m) of the Qinghai Lake water column were selected for molecular microbiology by pumping lake water using a submersible pump. At the same depths, water samples were collected for analysis of dissolved

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Fig. 1. A geographic map showing the Tibetan Plateau (Qaidam Basin) and the locations of Qinghai Lake, Erhai, and Gahai lakes, NW China. The sampling sites in all three lakes are shown (the symbol sizes are not proportional to the sizes of the lakes). The site on the edge of Qinghai Lake was sampled in November 2007. The one in the center of Qinghai Lake was sampled in summer.
inorganic and organic carbon (DIC and DOC). DIC samples were collected in 20-mL Teflon cap glass bottles and preserved with 200 mL saturated HgCl₂ to stop any biological activity. DOC samples were filtered through < 0.45-μm filters and collected into 20-mL Teflon cap glass bottles. DOC samples were immediately frozen in dry ice. Microbial samples were collected by on-line filtering of c. 500 mL of lake water through 0.2-μm filters (Millipore), and the filters were immediately frozen in dry ice. In November 2007, triplicate water samples were collected from the edge of Qinghai Lake (36°34′N, 100°42′E) (Fig. 1).

All the samples for molecular work were stored in liquid nitrogen from the date of collection until analysis. In < 1 week, the samples were taken to China University of Geosciences in Beijing, where they were stored in a −80 °C freezer. The sediment core was dissected according to our established procedure (Jiang et al., 2008). Sediment subsamples were collected every 10–20 cm with a total of five for the QL core (56 cm in length).

**Geochemical analyses**

DOC and DIC samples were analyzed at the State Key Laboratory of Marine Environmental Science at Xiamen University, China. DOC was measured from acidified samples using a high-temperature catalytic oxidation analyzer (Shimadzu TOC-V) (Sharp et al., 1995, 2002). DIC concentration was determined using a Li-Cor® nondispersive IR detector (Li-6252) after the sample was acidified (Wang & Cai, 2004). Total nitrogen (TN) and Total phosphorus (TP) were measured according to standard methods (Jin & Tu, 1990). Sediment pore water samples were collected and analyzed for pH, anions, and cations in the laboratory according to our previously established protocols (Jiang et al., 2008). Major anions and cations were combined to calculate salinity (PSU).

**AAP bacterial cell counts**

Water samples were fixed for 15 min with paraformaldehyde (2%, final concentration) and then stained with 4’6-diamidino-2-phenylindole (5 μg mL⁻¹, final concentration) for 30 min in the dark. Cells were filtered onto 0.2 μm pore-size black polycarbonate membranes (Whatman) for counting. The abundance of AAP bacteria was determined by the TIREM protocol as described previously (Jiao et al., 2006, 2007).

**DNA isolation, PCR amplification, and phylogenetic analysis**

Six water samples (four from Qinghai Lake, at 0, 500, 1200, and 2300 cm depth, respectively, and one each from Gahai Lake and Erhai Lake, respectively) and two sediment subsamples (0 and 52 cm from the QL core) were subjected to community DNA extraction using the FastDNA® SPIN Kit for soil (Qbiogene Inc., Irvine, CA) according to the protocol of the manufacturer. The partial pufL-M genes of the extracted DNA samples were amplified using the primer set of pufL forward (5’-CTK TTC GAC TTC TGG GTS GG-3’) and pufM reverse (5’-CCA TSG TCC AGC GCC AGA A-3’) (Nagashima et al., 1997; Beja et al., 2002) using the FailSafe™ PCR System (Epicentre Inc.). PCR conditions were as follows: 94 °C for 5 min; 40 cycles of denaturing (30 s at 94 °C), annealing (30 s at 55 °C), and extension (2 min at 72 °C); and a final extension at 72 °C for 7 min. The PCR products were purified using the GeneClean® Turbo kit (Qbiogene Inc.) according to the manufacturer’s protocol (Jiang et al., 2006). Eight pufL-M gene clone libraries (Erhai Lake-W; Gahai Lake-W; QL-0-W at 0 cm of the Qinghai Lake water column; QL-500-W, 500 cm; QL-1200-W, 1200 cm; and QL-2300-W, 2300 cm; QL-0-S, at 0 cm of the sediment core; QL-52-S, 52 cm) were constructed according to previous procedures (Jiang et al., 2006). Approximately 30 randomly selected colonies per sample were analyzed for insert pufL-M gene sequences. Plasmid DNA of randomly selected clones was extracted using the QuickClean 5M Miniprep Kit (GenScript Corporation, Piscataway, NJ). The pufL-M gene fragments of selected clones were sequenced using pufM reverse primer with the BigDye Terminator version 3.1 chemistry (Applied Biosystems, Foster City, CA). The pufL-M gene sequences were determined using an ABI 3730 automated sequencer. Nucleotide sequences were assembled and edited using SEQUENCER v.4.1 (GeneCodes, Ann Arbor, MI). Clone sequences showing 97% similarity or higher were considered to be the same group (Jiao et al., 2007). One sequence from each group was then selected as a representative for phylogenetic analysis. Neighbor-joining phylogenies were constructed from dissimilar distance and pairwise comparisons with the Jukes–Cantor distance model using the MEGA (molecular evolutionary genetics analysis) program, version 4.1. Shannon–Weaver diversity indices of all clone libraries were then calculated at the group level using the PAST program (http://folk.uio.no/ohammer/past/). Coverage (C) was calculated as follows: C = 1 − (n₁/N), where n₁ is the number of phylotypes that occurred only once in the clone library and N is the total number of clones analyzed. The sequences determined in this study were deposited in the GenBank database under accession numbers EU371072–EU371104.

**qPCR**

Copies of the pufL-M genes and bacterial/archaeal 16S rRNA gene in selected samples were determined by qPCR according to the protocols of Du et al. (2006) and our previous study (Jiang et al., 2007), respectively. Amplification conditions were 50 °C for 2 min, and then 95 °C for 15 min, followed by 45 cycles (15 s at 94 °C, 30 s for annealing at 53 °C, and 30 s at 72 °C) in a reaction volume of 25 μL, containing 12.5 μL of QuantitTec SYBR-Green Master Mix.
(QIAGEN, Valencia, CA) and 2.5 pmol of each primer. Purified plasmid DNA of Clone QL-0-WP18 (obtained in this study) was used as a standard, which, with serial dilutions, was used to yield the standard curve. In order to evaluate the ratio of AAP to total bacteria in the samples, total bacterial 16S rRNA gene copies were also determined by qPCR with the use of the primer set of Bac331F (5'-TCC TAC GGG AGG CAG CAG T-3')/Bac797R (5'-GGA CTA CCA GGG TCT AAT CCT GTT-3') (Nadkarni et al., 2002) according to the conditions outlined in our previous study (Jiang et al., 2007). All qPCR reactions were performed in duplicate. \( R^2 \) values of the standard curves were 0.999 and 0.997 for the \( pufM \) and total bacterial 16S rRNA genes, respectively. All qPCRs were performed using a Rotor-Gene TM3000 real-time thermal cycler (Corbett Life Science, Sydney, Australia). Melting curve analysis was performed to determine the melting point of the amplification products and to assess reaction specificity. After the qPCR reaction was complete, the temperature was ramped from 72 to 95 °C, increasing by 0.1 °C each step, waiting for 45 s at the first step, and then 5 s for each subsequent step. The melting curve had only one peak, indicating that the SYBR green signals were not from primer–dimer artifacts and/or non-specific DNA contamination.

**Results**

**Lake water chemistry**

Both pH and salinity increased from Erhai Lake to Qinghai Lake to Gahai Lake (Table 1). The major cations and anions were \( \text{Na}^+ \) and \( \text{Cl}^- \) for all three lakes (data not shown). Qinghai Lake was fairly dynamic, without stratification in the water column (data not shown; H. Jiang et al., unpublished data). In Qinghai Lake, the DIC concentration, ranging from 20.9 to 22.4 mmol L\(^{-1}\), was fairly constant throughout the water column, while the DIC concentrations for Erhai and Gahai were lower than those in Qinghai Lake (Table 1), but DOC concentrations were much higher than those in Qinghai Lake. The pH and salinity value for the pore water of the sediment core at 52 cm depth was 9.50 and 8 PSU, respectively.

**AAP bacteria abundance determined by TIREM and qPCR**

The TIREM and qPCR data were consistent with each other (Table 2). Among the three studied lakes, Qinghai Lake had the highest AAP bacteria abundance, followed by Erhai Lake and Gahai Lake. The Qinghai Lake sample collected in November 2007 had the highest AAP bacteria abundance. The number of AAP bacteria \( pufL-M \) gene copies in the...
Qinghai Lake sediments was below the detection limit (< $10^2$ copies g$^{-1}$ sediment).

**Diversity of AAP bacteria**

A total of 210 AAP bacteria pufL-M gene clone sequences (102, 26, and 25 clones from the water samples of Qinghai Lake, Gaahi Lake, and Erhai Lake, respectively; 57 clones from the Qinghai Lake sediments) were subjected to sequence similarity analysis (Table 3). These sequences belonged to *Alphaproteobacteria* and *Gammaproteobacteria* (Fig. 2). The *Alphaproteobacteria* group could be classified into eight clusters: *Loktanella*-like, *Roseobacter*-like, *Sphingomonas*-like, *Rubritepida*-like, *Porphyrobacter*/*Erythrobacter*-like sequences, and three unknown subgroups (Fig. 3). The group affiliation analyses of these sequences showed distinct differences in their relative abundance among either the three lakes or different depths of the Qinghai Lake water column (Fig. 3). In Erhai Lake, the AAP bacteria community was composed of four groups: *Gammaproteobacteria*-like, *Sphingomonas*-like, *Rubritepida*-like, and the unknown group-3, among which the *Gammaproteobacteria*-like sequences were predominant. In contrast, AAP bacteria pufL-M gene clone sequences in Gaahi Lake consisted of only one major group: *Loktanella*-like, which was absent in Erhai Lake (Fig. 3).

In Qinghai Lake, whose salinity (c. 12.5 PSU) and pH (9.4–9.5) were between Erhai Lake and Gaahi Lake, the AAP bacteria community was mainly composed of the *Loktanella*-like group, with a few other groups in smaller proportions: *Roseobacter*-like, *Porphyrobacter*/*Erythrobacter*-like, *Gammaproteobacteria*-like, and the unknown group-1. Although the water column of Qinghai Lake was not stratified, the AAP bacteria community composition did not appear to be completely mixed (Fig. 3): at the depth of 0 cm (near the surface of the water column), the AAP bacteria community consisted of *Loktanella*-like, *Roseobacter*-like, *Porphyrobacter*/*Erythrobacter*-like, and unknown group-1 sequences. At the depth of 500 cm, the AAP bacteria community was composed of *Loktanella*-like, *Roseobacter*-like, and unknown group-1 sequences. The *Loktanella*-like

Table 2. AAP bacteria abundance in saline lakes from Tibetan Plateau as determined by qPCR and TIREM

<table>
<thead>
<tr>
<th>Sample</th>
<th>qPCR</th>
<th>TIREM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAP bacteria (cell mL$^{-1}$)</td>
<td>AAP bacteria$^{*,1}$ (%)</td>
</tr>
<tr>
<td>Erhai Lake</td>
<td>1.13E+04 (± 1173)</td>
<td>1.57 ± 0.23</td>
</tr>
<tr>
<td>Gaahi Lake</td>
<td>9.60E+03 (± 589)</td>
<td>0.95 ± 0.09</td>
</tr>
<tr>
<td>QL-0-W</td>
<td>4.60E+04 (± 2371)</td>
<td>5.23 ± 0.91</td>
</tr>
<tr>
<td>QL-500-W</td>
<td>4.34E+04 (± 2935)</td>
<td>4.24 ± 0.67</td>
</tr>
<tr>
<td>QL-1200-W</td>
<td>4.06E+04 (± 1877)</td>
<td>5.35 ± 0.43</td>
</tr>
<tr>
<td>QL-2300-W</td>
<td>4.16E+04 (± 2199)</td>
<td>4.52 ± 0.59</td>
</tr>
<tr>
<td>QL surface water$^6$</td>
<td>Not determined</td>
<td>Not determined</td>
</tr>
<tr>
<td>QL-0-S1</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
<tr>
<td>QL-52-S1</td>
<td>Not detectable</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>

$^*$Average of triplicates.
$^1$AAP bacteria %: the ratio of AAP bacteria to total bacteria.
$^2$With TIREM, abundance of AAP bacteria and total bacteria were counted separately and SDs were not applied to AAP bacteria %.
$^6$Samples collected in November 2007.

The unit is cells per gram of sediments (dry weight).

Table 3. Diversity indices of AAP bacterial pufL-M gene clone libraries from the three saline lakes in the Tibetan Plateau

<table>
<thead>
<tr>
<th>Clone libraries</th>
<th>Total clones sequenced</th>
<th>Coverage (%)</th>
<th>No. of groups</th>
<th>SW$^*$-diversity index</th>
<th>pH</th>
<th>Salinity (PSU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erhai Lake-W</td>
<td>25</td>
<td>96.0</td>
<td>6</td>
<td>1.42</td>
<td>9.83–9.78</td>
<td>9.8</td>
</tr>
<tr>
<td>Gaahi Lake-W</td>
<td>26</td>
<td>100.0</td>
<td>2</td>
<td>0.49</td>
<td>9.32–9.34</td>
<td>32.0</td>
</tr>
<tr>
<td>QL-0-W</td>
<td>29</td>
<td>96.6</td>
<td>5</td>
<td>1.10</td>
<td>9.46</td>
<td>14.3</td>
</tr>
<tr>
<td>QL-500-W</td>
<td>26</td>
<td>96.2</td>
<td>4</td>
<td>0.85</td>
<td>9.50</td>
<td>14.3</td>
</tr>
<tr>
<td>QL-1200-W</td>
<td>20</td>
<td>100.0</td>
<td>1</td>
<td>0</td>
<td>9.50</td>
<td>14.3</td>
</tr>
<tr>
<td>QL-2300-W</td>
<td>27</td>
<td>96.3</td>
<td>4</td>
<td>0.87</td>
<td>9.39</td>
<td>14.3</td>
</tr>
<tr>
<td>QL-0-S</td>
<td>29</td>
<td>96.6</td>
<td>5</td>
<td>1.35</td>
<td>9.39</td>
<td>14.3</td>
</tr>
<tr>
<td>QL-52-S1</td>
<td>28</td>
<td>96.4</td>
<td>6</td>
<td>1.40</td>
<td>9.50</td>
<td>8.0</td>
</tr>
</tbody>
</table>

$^*$Shannon–Weaver index.

The groups were made at the 97% similarity level (Jiao et al., 2007).
Fig. 2. Neighbor-joining tree (partial sequences, c. 490 bp) showing the phylogenetic relationships of AAP bacterial pufL-M gene sequences cloned from the samples studied to closely related sequences from the GenBank database. One representative clone type within each phylotype is shown, and the number of clones within each phylotype is shown at the end. If there is only one clone sequence within a given phylotype, the number ‘1’ is omitted. Clone sequences from this study are coded as follows for the example of QL-500-WP14: AAP bacterial pufL-M clone number 14 from the depth of 500 cm in the water column of Qinghai Lake. W, water; P, AAP bacteria. Scale bars indicate the Jukes–Cantor distances. Bootstrap values of > 50% (for 1000 iterations) are shown. Clone sequences obtained in this study are in bold.
sequences were the only phylogenetic group at the depth of 1200 cm. Three groups (Gammaproteobacteria-like, Loktanella-like, and Roseobacter-like) were predominant at the depth of 2300 cm.

The sequenced clones in each library covered 96–100% diversity (Table 3). The Shannon–Weaver indices for the Erhai Lake water/Qinghai Lake sediments, the Qinghai Lake water column, and the Gahai Lake water were c. 1.4, c. 0.9–1.1 (except for the depth of 1200 cm), and c. 0.5, respectively (Table 3). The diversity indices of the pufL-M gene clone libraries in the studied lake waters appeared to be in inverse correlation with salinity.

**Discussion**

AAP bacteria are able to supplement their aerobic respiration with photosynthesized ATP, thus conserving organic carbon (Kolber et al., 2000, 2001). Because of such a unique role in global carbon cycling, AAP bacteria have increasingly drawn researchers’ attention since the last decade. However, little is known about the abundance and diversity of AAP bacteria in high-elevation saline lakes.

**AAP bacteria abundance**

The abundance of AAP bacteria varies considerably in aquatic environments (Kolber et al., 2001; Schwalbach & Fuhrman, 2005; Cottrell et al., 2006; Sieracki et al., 2006; Lami et al., 2007), with estuaries and mountain lakes having a high abundance (Schwalbach & Fuhrman, 2005; Waidner & Kirchman, 2007). The reasons for such a large variation are not clear, although environmental nutrient status (Kolber et al., 2001; Waidner & Kirchman, 2007; Masin et al., 2008), light intensity (Waidner & Kirchman, 2007), associations with sediment particle (Waidner & Kirchman, 2007), and chlorophyll a concentration (Jiao et al., 2007) have been hypothesized to be some variables responsible for this variation. In the three Tibetan lakes studied, the fractions of AAP bacteria (i.e. the percentage of AAP bacteria in total bacteria) were comparable to the levels detected in the Indian (3.79 ± 1.72%), the Atlantic (1.57 ± 0.68%), and the Pacific (1.08 ± 0.74%) oceans (Table 2), but much lower than those in freshwater lakes of an oligotrophic to a mesotrophic nature (7–80% depending on seasonality and nutrient levels) (Masin et al., 2008) and in estuaries (Waidner & Kirchman, 2007, 2008). The lakes with the higher DOC content (i.e. Erhai and Gahai) exhibited lower AAP bacteria abundance, consistent with results from a previous study (Masin et al., 2008).

**AAP bacterial diversity**

The overall diversity of AAP bacteria was low in the Tibetan lakes. The AAP bacterial diversity decreased as the salinity and pH increased (Fig. 3, Table 3). Among the three lakes studied, Gahai Lake with the highest salinity and pH exhibited the lowest AAP bacteria diversity, Erhai Lake with the lowest salinity and pH had the highest diversity, and Qinghai Lake with an intermediate salinity and pH showed an intermediate diversity. Of the limited environmental factors examined, salinity and pH appeared to be important in controlling AAP bacteria diversity. Interestingly, the sediment sample at the 52 cm depth of Qinhai Lake had a salinity similar to the Erhai water column (c. 8 PSU) and, correspondingly, the AAP bacteria diversity for the QL-52-S sample was similar to that for Erhai (Table 3 and Fig. 3), further demonstrating that salinity might be one of the important factors in controlling AAP bacteria diversity in these lakes. However, this salinity–diversity relationship did not appear to hold true across different ecosystems. For example, the salinity in all three lakes studied was lower than that of seawater, but the AAP bacteria diversity was...
Diversity and abundance of AAP bacteria in saline lakes

qualitatively lower than that in oceans. This complication suggests that a number of other factors could drive AAPB diversity (such as light availability, N and P availability, and UV irradiance) (Jiao et al., 2007 and references therein). In addition, ecosystem size and evolution time may be important factors accounting for this complication, but they were not examined in this study.

Despite recent advances in studies of AAP bacteria in many different environments, the relationship between environmental controlling factors and AAP bacterial community composition is still poorly understood. It appears that in saline water bodies (such as oceanic and coastal waters, lakes, and the saline side of estuaries), AAP bacteria belonging to Alphaproteobacteria and Gammaproteobacteria are dominant (Yurkova et al., 2002; Allgaier et al., 2003; Kobližek et al., 2003; Oz et al., 2005; Waidner & Kirchman, 2005, 2007, 2008; Cho et al., 2007; Jiao et al., 2007). In freshwater lakes and the freshwater side of estuaries, Betaproteobacteria are predominant (Karr et al., 2003; Waidner & Kirchman, 2005, 2008). This distribution pattern of AAB bacteria is similar to that defined by the 16S rRNA gene, i.e. with increased salinity, the relative abundance of Alphaproteobacteria and Gammaproteobacteria increases, but that of Betaproteobacteria decreases (Wu et al., 2006; Dong & Yu, 2007; references therein).

Consistent with this distribution pattern, our phylogenetic analysis revealed that the AAP bacterial community structure in the Tibetan saline lakes was dominated by Alphaproteobacteria and Gammaproteobacteria, broadly similar to the AAP bacterial community structure in oceans. Indeed, Yurkova et al. (2002) observed a close phylogenetic relation of some strains purified from a meromictic lake (Mahoney Lake in British Columbia) to species obtained from marine environments. This observation implies that the similar environmental conditions of saline lakes (including Mahoney Lake) and the oceans (i.e. pH, salinity, and water chemistry) may have been responsible for the similar AAP bacterial community structure. Interestingly, Jiang et al. (2008) observed archaeal biogeography where similar environmental conditions between Qinghai Lake and the world oceans resulted in a similar archaean community structure. However, a closer examination at a finer phylogenetic resolution reveals differences in the AAP bacterial community structure between the oceans and the Tibetan lakes studied. For example, Roseobacter- and Erythrobacter-related AAP bacterial clone sequences of Alphaproteobacteria were ubiquitous in the major ocean regimes (Jiao et al., 2007), but Loktanella-like sequences of Alphaproteobacteria were predominant in the Tibetan lakes (this study). Considering that AAP bacteria require light for energy, it is not surprising to observe such a difference, because of drastically different elevations and thus light intensities between Qinghai Lake and the oceans.

It is remarkable to note that the AAP bacterial community structure of Erhai Lake was completely different from that of either Qinghai or Gahai Lake. This difference could not be solely ascribed to salinity, because the salinity contrast between Erhai Lake and Qinghai Lake/Gahai Lake was not as great as between freshwater and seawater in the Delaware estuary (Waidner & Kirchman, 2008). The AAP bacterial community structure from Erhai Lake (with the lowest salinity, 9.8 PSU) was not dominated by freshwater Betaproteobacteria-like, but by saline water Gammaproteobacteria-like AAP bacterial sequences. An important difference between Erhai Lake and Qinghai/Gahai Lake was turbidity (Table 1). Whereas Erhai Lake was turbid, Qinghai and Gahai Lakes were clear. Thus, it was reasonable to speculate that the AAP bacteria detected in Erhai may largely be associated with sediment particles, whereas AAP bacteria in Qinghai/Gahai Lakes were mostly free-living. Previous studies observed that particle-attached and free-living bacterial community composition is fundamentally different (DeLong et al., 1993; Crump et al., 1999; Phillips et al., 1999; Schweitzer et al., 2001; Acinas et al., 2005). It has been shown that most AAP bacteria are associated with particles, and this attachment apparently increases their competitiveness in estuary environments (Yutin et al., 2007; Waidner & Kirchman, 2008).

Although the Qinghai Lake water column was vertically mixed with only slight stratification of geochemical variables (Table 1) and there was no systematic decline in the AAP bacteria abundance with increased depth, the AAP bacteria community composition did vary with depth. This variation may be partly caused by decreased light intensity with depth. It is well established that light intensity affects pigment formation necessary for photosynthesis (Aagaard & Sistrom, 1972; Drews & Golecki, 1995). Blue light inhibits photosynthetic pigment accumulation in Roseobacter denitrificans (Iba & Takamiya, 1989; Takamiya et al., 1992). The fact that the Roseobacter-like pufL-M sequences at the bottom were more abundant than the top of the water column in Qinghai Lake (Fig. 3) appeared to be consistent with this inhibition effect. Because of high elevation, UV light intensity is expected to be much stronger than at sea level (Askew, 2002).

AAP bacteria in anoxic sediments: fossil or light-independent AAP bacteria?

Retrieval of AAP bacteria pufL-M gene sequences from the anoxic and dark sediments of Qinghai Lake was unexpected (Fig. 2). Although the surface sediment of Tokyo Bay was used previously for AAP bacteria isolation work (Yurkov & Beatty, 1998b), AAP bacteria were never isolated successfully. We postulated that two possibilities could explain the presence of the AAP bacteria pufL-M genes in the sediments:
(1) they were derived from dead AAP bacteria cells deposited from the overlying water column (fossil DNA); (2) they were derived from active cells (of unknown metabolic pathways) that may have the \( pufL-M \) genes. The water column and sediments of Qinghai Lake had some AAP bacteria groups in common, such as the \( \text{Loktanella-like, Roseobacter-like, } \text{Porphyrobacter/Erythrobacter-like, } \) and unknown group-1 sequences. Excluding the unknown group-1, none of the known AAP bacteria in the \( \text{Loktanella-like, Roseobacter-like, } \) and \( \text{Porphyrobacter/Erythrobacter-like } \) groups has either been cultured from sediments or shown the ability to survive in anoxic environments, because they all require light for growth. Thus, the AAP bacteria-originated \( pufL-M \) gene sequences in the sediments might have been of fossil origin. The fossil DNA of obligate phototrophic algae and their lipid derivatives have been well studied in the context of paleoecology, archaeology, and paleontology (Coolen & Overmann, 1998; Coolen et al., 2004, 2006). However, no similar studies have been performed on AAP bacteria-derived fossil DNA.

Despite the similarity of some cloned gene sequences between the water column and the sediments of Qinghai Lake, multiple AAP bacterial \( pufL-M \) gene sequences retrieved from the water–sediment interface (i.e. the unknown group-2, Figs 2 and 3) were not found in the water column. This observation also indicated two possibilities. First, these sequences may have been present in the water column but were overlooked using our sampling scheme. In this scenario, these sequences may represent fossil DNA from the water column (see the discussion above). Second, these AAP bacteria \( pufL-M \) gene sequences might be unique to the sediments and possibly derived from indigenous cells. Xiong et al. (2000) and Beatty (2002) proposed that certain purple photosynthetic bacteria might have lost their photosynthetic functions in evolution. According to such a theory, Jiao et al. (2007) speculated that some AAP bacteria species could have lost their photosynthetic genes and gradually developed heterotrophic metabolic pathways. If this evolution pathway were true, AAP bacteria at the transition might have first developed heterotrophic metabolic pathways before they became completely independent of light (i.e. loss of \( pufL-M \) genes). The AAP bacteria cells, from which the \( pufL-M \) gene sequences in the unknown group-2 originated, could have represented this transition stage. Although these AAP bacteria could still possess the \( pufL-M \) genes because they may be deposited from the oxic water column within years (according to a sedimentation rate of 1.25 mm year\(^{-1} \)), CAS, 1979), the anoxic and dark environments in the Qinghai Lake sediments would not allow them to depend on light for growth. Such AAP bacteria could have developed a distinct heterotrophic pathway to cope with the conditions in the sediments. However, future work would be necessary for verification of this evolution hypothesis.

**Conclusion**

Molecular work performed in this study indicated that AAP bacteria were abundant in saline lakes on the Tibetan Plateau, NW China, with the AAP bacteria percentage levels comparable to those in the oceans. Out of a limited number of environmental factors examined (salinity, pH, DO, conductivity, turbidity, TN, TP, and DIC and DOC), salinity, and pH were found to be potential factors in controlling the AAP bacteria diversity and community structure. Whereas certain AAP bacteria may be preserved as fossil DNA in anoxic and dark lake sediments, others might be able to survive independent of light but may still possess the \( pufL-M \) genes.

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**References**


ocean revealed by epifluorescence microscopy and quantitative PCR. *Limnol Oceanogr* **50**: 385–393.


