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ORIGINAL PAPER

Planktonic actinobacterial diversity along a salinity gradient of a river and five lakes on the Tibetan Plateau

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Abstract The diversity and community structure of planktonic Actinobacteria in a freshwater river and five fresh/saline/hypersaline lakes on the Tibetan Plateau, China were investigated with a combination of geochemical and 16S rRNA gene phylogenetic analyses. A total of 387 actinobacterial 16S rRNA gene clones were sequenced, and they could be classified into *Actinobacteridae*, *Acidimicrobidae*, and unclassified Actinobacteria. The *Actinobacteridae* sequences were distributed into five suborders (e.g., *Corynebacterineae*, *Frankineae*, *Micrococcineae*, *Propionibacterineae*, and *Streptosporangineae*) and unclassified Actinobacteridae. Some actinobacterial members (specifically *Micrococcineae*) were present in a wide range of salinities (from freshwater to NaCl saturation). Statistical analysis showed that salinity and salinity-related environmental variables (such as ions and total nitrogen) significantly ($r > 0.5$; $P < 0.05$) influenced the distribution of planktonic actinobacterial community in the investigated aquatic biotopes. Our data have implications for a better understanding of the distribution of Actinobacteria in high-elevation lakes.

Keywords *Actinobacteria* · Diversity · Salinity · Tibetan Plateau

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Introduction

Actinobacteria, one of the largest taxonomic units within the domain *Bacteria*, are ubiquitous in both terrestrial and aquatic ecosystems (Embley and Stackebrandt 1994). Culture-dependent and -independent studies showed that *Actinobacteria* can tolerate or are adapted to a wide range of environmental gradients, such as temperature [from cold (Liu et al. 2009; Mevs et al. 2000) to hot (Mohagheghi et al. 1986; Song et al. 2009)], pH [from acidic (Mendez et al. 2008; Mohagheghi et al. 1986) to alkaline (Tiago et al. 2004)], and salinity [from freshwater (Allgaier and Grossart 2006; Hahn et al. 2003; Hahn 2009; Holmfeldt et al. 2009; Newton et al.; 2007; Warnecke et al. 2004; Zwart et al. 2002) to saline (Dong et al. 2006) and hypersaline (Jiang et al. 2006; Mancinelli 2005; Stach and Bull 2005; Wu et al. 2009)]. However, it is still poorly known how diversity and community structure of planktonic actinobacterial community in lakes respond to these environmental gradients (e.g., temperature, pH, and salinity).

As the largest and highest plateau on Earth, the Tibetan Plateau has thousands of lakes, which possess multiple environmental gradients such as salinity (from 0.1 to 426.3 g/L) and pH (5.4–10.2) (Zheng 1995). These lakes represent pristine environments, where perturbations from human activities are minimal. Therefore, the effect of

environmental conditions on actinobacterial communities can be discerned. The objective of this study was to investigate the response of the planktonic actinobacterial diversity and community structure to environmental changes in waters collected from one freshwater river and five fresh/saline/hypersaline lakes on the Tibetan Plateau with an integrated approach including geochemical and bacterial 16S rRNA gene phylogenetic analyses.

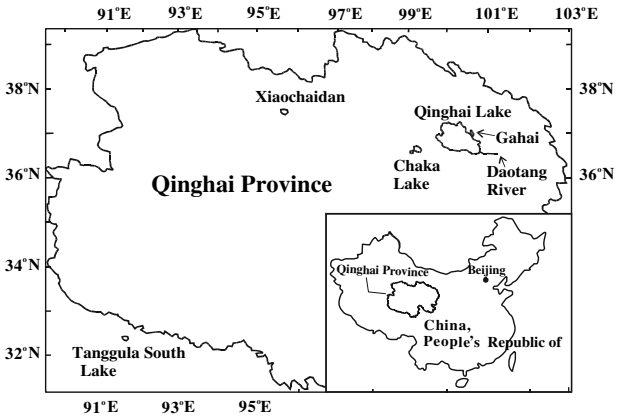


Fig. 1 A geographic map showing the locations of Daotang River and studied saline/hypersaline lakes on the Tibetan Plateau, NW China

Materials and methods

Description of the study sites

Daotang River and five fresh/saline/hypersaline lakes (Tanggula South, Qinghai, Gahai, Xiaochaidan, and Chaka Lakes) on the Tibetan Plateau were selected for this study (Fig. 1; Table 1). Daotang River, located in the southeast of Qinghai Lake, is one perennial inflowing river of Erhai Lake, a daughter lake of Qinghai Lake (Jiang et al. 2009b). Daotang River is around 40 km long, and its water column is clear (Wang and Dou 1998). Tanggula South Lake is located on the southern slope of Tanggula Mountain, and it is a glacial lake. Qinghai Lake is the largest saline and alkaline lake on the Tibetan Plateau in NW China (Jiang et al. 2009b). Qinghai Lake has a well-mixed water column (Jiang et al. 2008). Gahai Lake is another daughter lake of Qinghai Lake (Jiang et al. 2009b; Wang and Dou 1998). Xiaochaidan Lake and Lake Chaka are hypersaline lakes located near the northeastern edge and in the southern corner of Qaidam Basin, respectively (Jiang et al. 2006; Wang and Dou 1998).

Field sampling

Water samples were collected from the surface of the river and lakes (top 20–50 cm) with a 5-l Schindler sampler.

Table 1 Geochemical and geographic parameters of the Daotang River and five saline/hypersaline lakes on the Tibetan Plateau

Sample	Daotang River	Tanggula South Lake	Qinghai Lake	Gahai Lake	Xiaochaidan Lake	Chaka Lake
Area (km^2)	NA	0.10	4340	47.2	71.5	116.1
Sampling location (N/E)	100.74/36.577	91.522/32.405	36.607/100.509	37.006/100.580	37.275/95.280	36.420/99.071
Elevation (m)	3210	4987	3196	3196	3172	3214
Maximum water depth (m)	1.7	NA	27	9.5	0.69	0.25
Sampling water depth (m)	0.5	0.5	0.5	0.5	0.2	0.2
Water temperature (°C)	15.7	10.9	13.5	15.9	21	16.8
Water type	Cl–Na	S–Na–II	Cl–Na	SO_4–Na	Cl–Na	Cl–Na
Concentration of major ions (mg L^{-1})						
K^+	6.94	5	151.30	295.39	920	2089.3
Na^+	7.36	315.03	3531.65	9610.95	54691.24	107460.3
Ca^{2+}	21.66	27.58	77.28	77.60	625.22	823.2
Mg^{2+}	38.12	50.21	590.86	692.83	2588.66	9740.8
Cl^-	150.34	83.41	4907.48	8246.13	72063.44	187633.2
SO_4^{2-}	96.59	674.31	2000.41	12513.9	28735.16	17099.8
HCO_3^-	149.01	27.12	826.25	419.31	366.08	153.1
CO_3^{2-}	86.61	93.36	414.76	143.91	380.09	0.0
Salinity (g L^{-1})	0.1	1.28	12.5	32	160	325
pH	8.7	9.2	9.3	9.2	9.2	7.4
TN (mg L^{-1})	1.09	1.523	0.94	3.48	4.38	8.31
TP (mg L^{-1})	0.024	0.034	0.0	0.034	0.004	0.0

NA not available

Bacterioplankton samples (250–500 mL water) for molecular analyses were collected onto 0.2-μm Isopore filters, which were subsequently stored in liquid nitrogen until analysis. Untreated water samples (2–3 l) were transported to the laboratory for immediate geochemical analysis.

Measurements of physical and chemical parameters

The pH was measured in the field with a portable pH meter (PT-10, SARTORIUS, Germany). In the laboratory, concentrations of the eight major ions, i.e., potassium, sodium, calcium, magnesium, chloride, sulfate, carbonate, and bicarbonate, were analyzed by inductively coupled plasma mass spectrometry. The concentrations of total nitrogen (TN) and total phosphorus (TP) were determined with alkaline persulfate digestion followed by colorimetric analysis (Hosomi and Sudo 1986). The salinity (salt concentration) was determined by summing up the concentrations of the measured ions in the unit of g L^{-1}.

DNA extraction, PCR, and phylogenetic analyses

Genomic DNA was extracted from microbial biomass-containing filters as described previously (Dong et al. 2006; Jiang et al. 2006). The actinobacterial 16S rRNA gene from the extracted DNA samples was amplified using the Actinobacteria-specific primer set of S-C-Act-0235-a-S-20 (5′-CGC GGC CTA TCA GCT TGT TG-3′) and S-C-Act-0878-a-A-19 (5′-CCG TAC TCC CCA GGC GGG G-3′) (Stach et al. 2003) with the same PCR conditions as those described previously (Song et al. 2009). Successful PCR products were purified according to our previously described procedure (Jiang et al. 2009b). Actinobacterial 16S rRNA gene clone libraries were constructed, and clones were RFLP-screened according to Song et al. (2009). Unique RFLP types were identified with a combination of visual inspection and rarefaction analysis (http://www.uga.edu/strata/software/Software.html). The RFLP screening was stopped when the rarefaction curves were saturated. Unique clones (one from each RFLP type) were sequenced with an ABI 3100 automated sequencer using universal primers: M13F (5′-GTT TTC CCA GTC ACG AC-3′) at Shanghai Sangon Biotech. The raw sequences were trimmed by using Sequencher 4.8. Potential chimeric sequences were examined with Bellerophon (http://foo.maths.uq.edu.au/~huber/bellerophon.pl), and if discovered, they were discarded. Operational taxonomic units (OTUs) were determined using DOTUR (Schloss and Handelsman 2005) with a 97% cutoff value. Neighbor-joining phylogenetic trees 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. Bootstrap replications of 1000 were assessed. The sequences determined in this study were deposited in the GenBank database under accession numbers FJ498808–FJ498865.

Statistical analysis

Coverage of the clone libraries was calculated with the equation $C = 1 - n/N$, where n is the number of unique RFLP types and N is the total number of RFLP-screened clones (Dong et al. 2006; Jiang et al. 2008). The actinobacterial gene clone sequences were used to construct biotic similarity matrices, and environmental variables (ionic concentrations, salinity, pH, temperature, TN, and TP) were used to construct environmental similarity matrices. The Mantel test was performed to reveal any correlation between these two sets of matrices (biotic vs. environmental) by using the *zt* software (http://www.psb.ugent.be/~erbon/mantel/) according to procedures as previously described (Jiang et al. 2009a).

Results

Water chemistry of the studied lakes

The salinity was 0.1, 1.3, 12.5, 32, 160, and 325 g L^{-1} for Daotang River, Tanggula South, Qinghai, Gahai, Xiaochaidan, and Chaka Lakes, respectively (Table 1). The water type of the lakes was Na–Cl except for Tanggula South and Gahai Lakes (Na–SO_4). The pH was from slightly to moderately alkaline (Table 1). Total nitrogen of the sampled waters ranged from 1 to 8.3 mg L^{-1} with very low to undetectable levels of total phosphorus.

Phylogenetic diversity of *Actinobacteria*

Six clone libraries (DTR, TSL, QL, GHL, XCDL, and LCK for Daotang River, Tanggula South, Qinghai, Gahai, Xiaochaidan, and Chaka Lakes, respectively) were constructed. A total of 522 (64, 47, 84, 91, 92, and 144 for DTR, TSL, QL, GHL, XCDL, and LCK, respectively) actinobacterial 16S rRNA gene clones were RFLP-screened, and a total of 85 (12, 7, 5, 9, 16, and 36 for DTR, TSL, QL, GHL, XCDL, and LCK, respectively) OTUs were identified. Fifty-eight (12, 7, 5, 9, 4, and 21 for DTR, TSL, QL, GHL, XCDL, and LCK, respectively) of the retrieved OTUs belonged to *Actinobacteria*. These sequences were affiliated with the *Actinobacteridae* (e.g., suborders of *Corynebacterineae*, *Frankineae* and *Micrococcineae*, *Propionibacterineae*, *Streptosporangineae*, and unclassified *Actinobacteridae*), *Acidimicrobidae*, and unclassified *Actinobacteria* (Table 2; Fig. 2a). The major

Table 2 Ecological estimates and major group affiliation of clone sequences retrieved from Daotang River and five saline/hypersaline lakes on the Tibetan Plateau

Community	DTR	TSL	QL	GHL	XCDL	LCK
Library size (no. of clones)	64	47	84	91	92	144
Coverage (%)	98.4	95.7	98.8	96.7	98.9	98.6
No. of observed OTUs	12 (12)[a]	7 (7)[a]	5 (5)[a]	9 (9)[a]	16 (4)[a]	36 (21)[a]
Major group affiliation	No. of clones (relative percentage in each actinobacterial clone library)					
Actinobacteria						
Actinobacteridae						
Corynebacterineae	7 (10.9)					3 (5.4)[b]
Frankineae					9 (20.0)[b]	6 (10.7)[b]
Micrococcineae	52 (81.3)	12 (25.5)	83 (98.8)	91 (100)	36 (80.0)[b]	27 (48.2)[b]
Propionibacterineae						5 (8.9)[b]
Streptosporangineae						1 (1.8)[b]
Unclassified *Actinobacteridae*	5 (7.8)	35 (74.5)	1 (1.2)			
Acidimicrobidae						5 (8.9)[b]
Unclassified Actinobacteria						9 (16.1)[b]
Non-Actinobacteria						
α-Proteobacteria						3
ε-Proteobacteria						8
Gemmatimonadetes					2	
Synergistetes					7	
Uncultured Candidate division OP11						25
Uncultured Candidate division OD1					38	18
Unclassified bacteria						34

[a] Numbers in the parenthesis indicated the number of clones affiliated with *Actinobacteria*

[b] The non-actinobacterial clone sequences were excluded for the calculation of the relative percentage of each major group in the clone libraries

group composition and the relative abundance of each group varied among the samples (Table 2). The *Micrococcineae* group was the predominant group in all samples except for TSL. The GHL clone library was the least diverse and consisted of only one group, *Micrococcineae*, whereas the clone library of the hypersaline lake, LCK, was the most diverse (Table 2).

The other 27 (12 and 15 from XCDL and LCK, respectively) OTUs retrieved in this study belonged to non-*Actinobacteria* and were affiliated with *Alpha-* and *Epsilon-proteobacteria*, *Gemmatimonadetes*, *Synergistetes*, uncultured Candidate Division OP11 (Hugenholtz et al. 1998) and OD1 (Harris et al. 2004), and unclassified bacteria (Fig. 2b; Table 2), suggesting that the Actinobacteria-specific primers may not be inclusive of all actinobacterial sequences.

Statistical analysis

The number of sequenced clones represented 96.7–98.8% coverage for each clone library (Table 2). The Mantel test showed that at the species level (97% OTU), HCO_3^- ion concentration was the significant ($P < 0.05$) variable influencing actinobacterial community structure, and at the major group (as defined in Table 2) level, salinity, TN, and concentration of HCO_3^- and major ions (i.e., K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2+}) were significant variables influencing actinobacterial community structures. In contrast, pH, temperature, and TP did not show significant effect on the actinobacterial community structure.

Discussion

Planktonic actinobacterial community in freshwater biotopes on the Tibetan Plateau

Several studies have investigated actinobacterial 16S rRNA genes (a total of ~3000 sequences were examined) in waters collected from a variety of freshwater habitats (rivers, reservoirs, and lakes), and they found that planktonic freshwater *Actinobacteria* could be grouped into

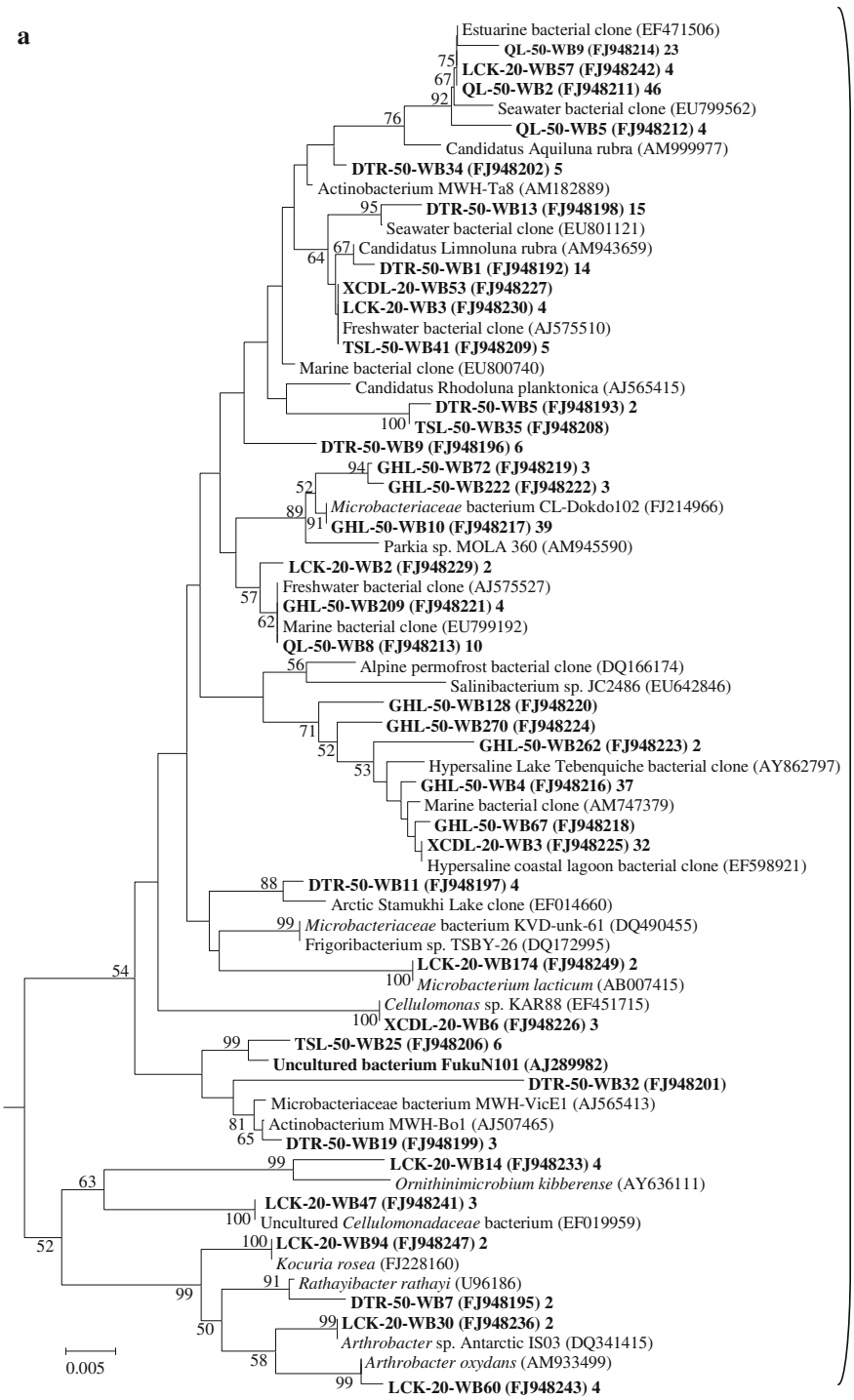
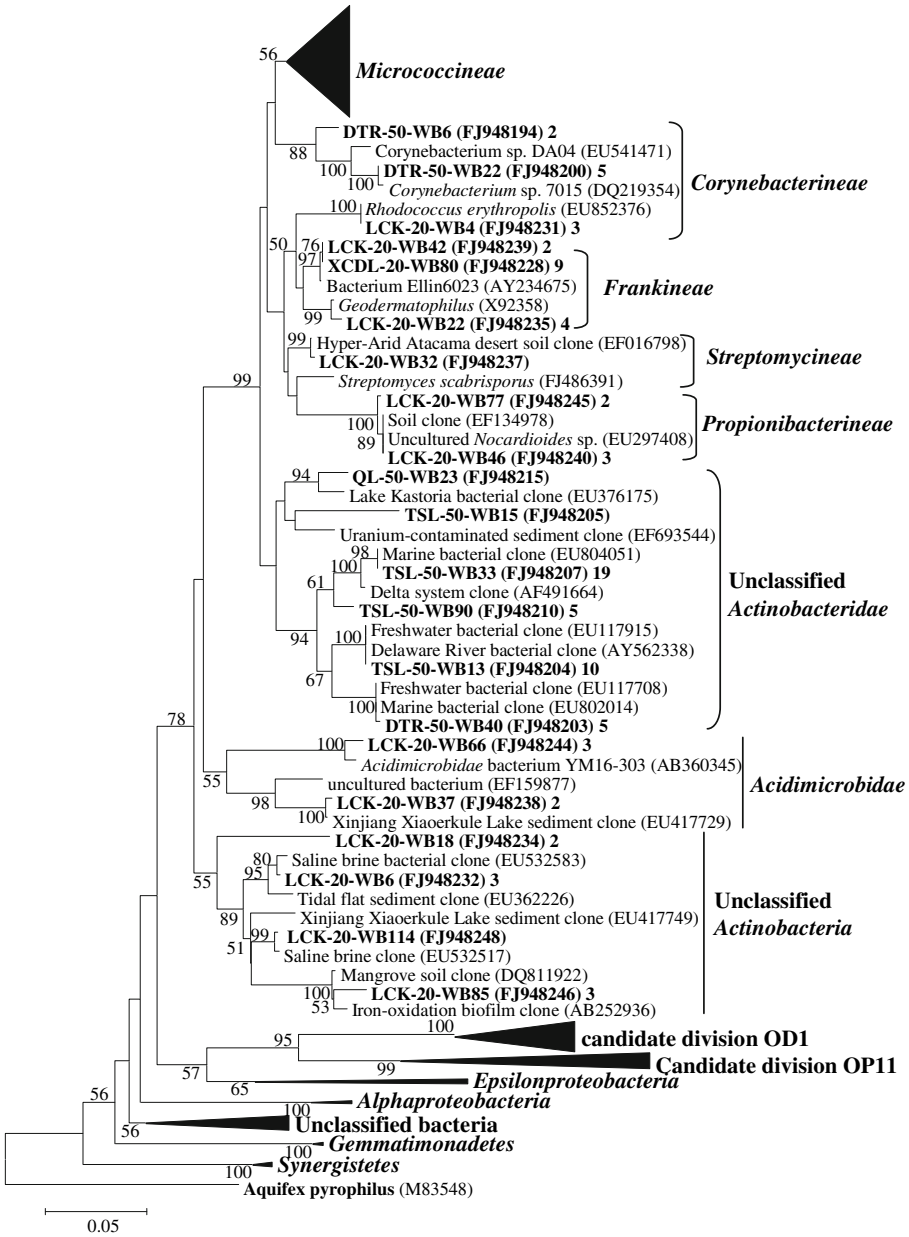


Fig. 2 Neighbor-joining tree (partial sequences, ~640 bp) showing the phylogenetic relationships of 16S rRNA gene sequences cloned from the studied samples to closely related sequences from the GenBank database. One representative clone type within each phylotype is shown, and the number of clones within each OTU is shown at the end. If there is only one clone within a given phylotype, the number "1" is omitted. Clone sequences from this study are coded as follows for the example of GHL-50-WB128: bacterial 16S rRNA gene clone number 128 from the depth of 50 cm in the water column of Gahai Lake. *W* water, *B* bacteria. *Scale bars* indicate the Jukes-Cantor distances. Bootstrap values of >50% (for 1000 iterations) are shown. **a** The actinobacterial 16S rRNA gene clones; **b** the non-actinobacterial clones

several major clusters: *ac*I, *ac*II and *ac*III, *ac*IV, *ac*STL, Microthrix, Soil I, Soil II and Soil III, and Luna-1 and 2 (Allgaier and Grossart 2006; Hahn et al. 2003; Hahn 2009; Holmfeldt et al. 2009; Newton et al. 2007; Warnecke et al. 2004). Due to lack of cultured representatives of these freshwater actinobacterial clusters, their physiological properties and ecological roles are still unknown (Allgaier and Grossart 2006; Warnecke et al. 2004). In order to determine the affiliation relationship between these proposed freshwater actinobacterial groups and the clone sequences obtained in this study, phylogenetic analysis was performed on pooled sequences from available habitats (including the clone sequences retrieved in this study and those determined previously) (Allgaier and Grossart 2006; Hahn 2009; Hahn et al. 2003; Holmfeldt et al. 2009; Warnecke et al. 2004) (Fig. 3). The results showed that

Fig. 2 continued

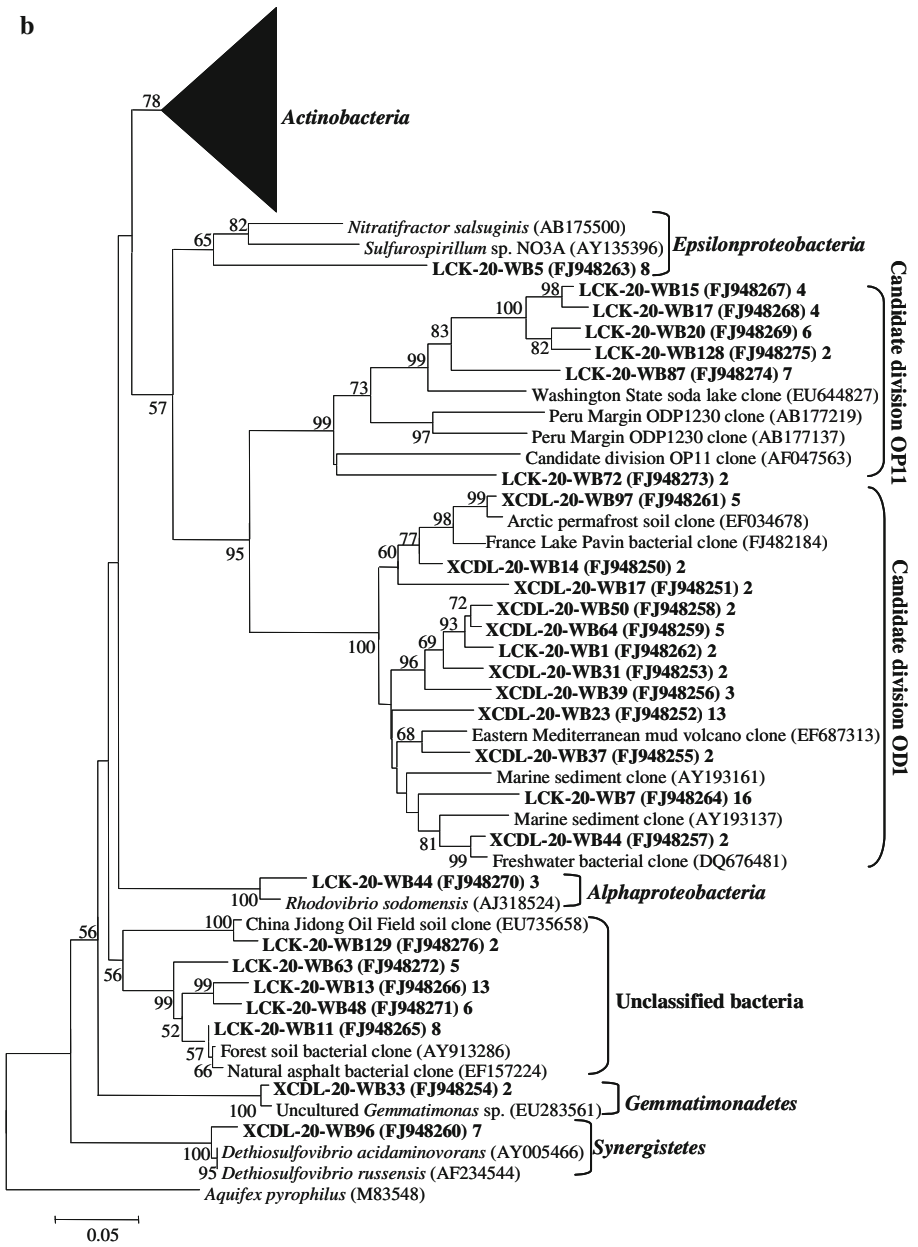


those previously proposed freshwater actinobacterial groups (Allgaier and Grossart 2006; Hahn et al. 2003; Warnecke et al. 2004) can cover all of the actinobacterial 16S rRNA gene clone sequences retrieved from TSL (freshwater biotope) (Fig. 3), but not those from DTR (also freshwater biotope). For example, the TSL actinobacterial 16S rRNA gene clone sequences were affiliated with *ac*I, *ac*II, *ac*STL, and Luna 2. Whereas most clone sequences (54 out of 64) from DTR were affiliated with *ac*I, *ac*II, *ac*STL, and Luna 2, the other sequences in DTR were not grouped with any previously known freshwater actinobacterial groups. This observation suggested that the Tibetan freshwater aquatic ecosystems could harbor many unknown actinobacterial groups. This observation was true not only for actinobacterial sequences, but also for bacteria in general (Dong et al. 2010).

Response of planktonic actinobacterial community to salinity gradient

Our phylogenetic analysis (Fig. 2a) and major group affiliation (Table 2) showed that the groups of *ac*I and *ac*II were dominant in all studied lakes except for the hypersaline lake (Lake Chaka), consistent with the previous studies in freshwater systems (Warnecke et al. 2004, 2005; Allgaier and Grossart 2006; Hahn 2009). Among the environmental factors examined, the pH did not appear to be a significant factor. This lack of significance could be

Fig. 2 continued



attributed to the narrow pH range (7.4–9.3) of the lakes investigated in this study. In contrast to pH, salinity (including salinity-related major ions) and TN significantly affected actinobacterial community structures in the studied aquatic biotopes (Tables 2, 3). Salinity was positively correlated with the diversity of the actinobacterial community (Table 3). This observation appeared to be in contrary to the general ecological principle that more extreme environments decrease diversity (Frontier 1985; Hacine et al. 2004). However, a closer examination revealed that salinity was positively correlated with TN ($R^2 = 0.931$, figure not shown). High TN content may be a result of lake evaporation in that evaporation of saline lakes would result in high concentration of salts, total organic carbon (TOC), dissolved organic carbon (DOC), and TN (Hammer 1986). Therefore, the apparent positive correlation between the actinobacterial diversity and salinity may actually reflect a positive relationship between the diversity and TN/TOC contents.

Salinity not only affected the diversity but also community composition. Our affiliation analysis (Fig. 3) showed that the *ac*I group was composed of sequences from freshwater habitats only, and that no sequences retrieved from saline lakes were affiliated with this group, as consistent with a previous study (Warnecke et al. 2004). However, a total of 146 clones retrieved from saline and

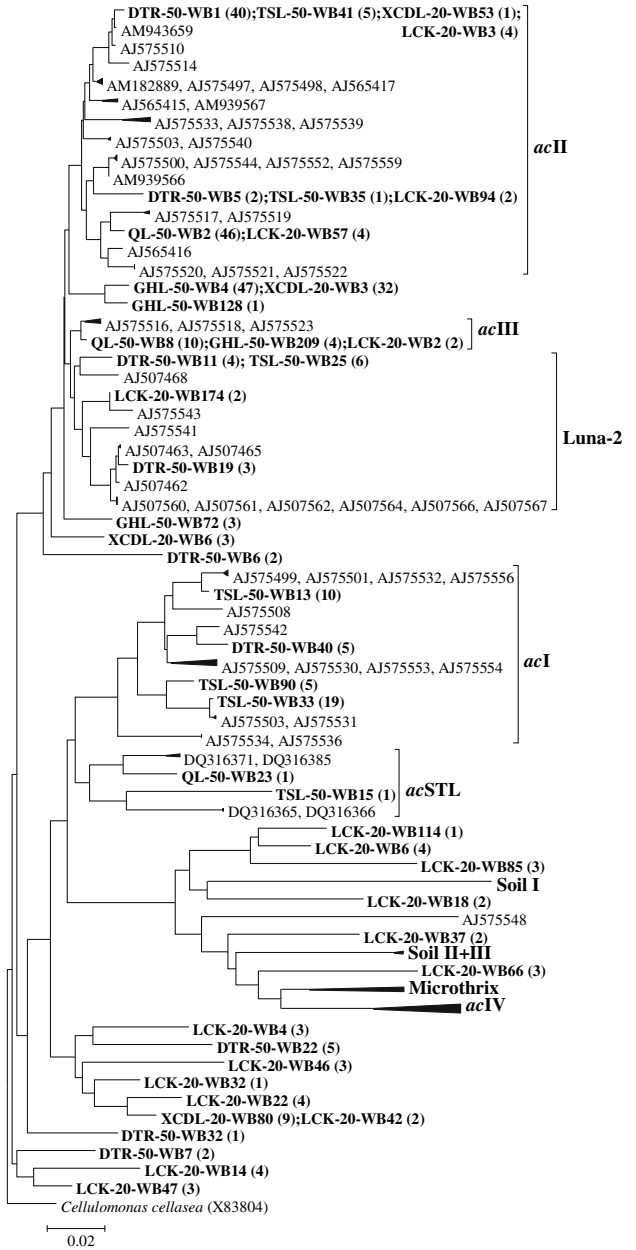


Fig. 3 Neighbor-joining tree (partial sequences, ~640 bp) showing the phylogenetic affiliation of the actinobacterial 16S rRNA gene sequences cloned from the studied samples with the typical freshwater actinobacterial clusters reported by Allgaier and Grossart (2006), Hahn et al. (2003), Hahn (2009), and Warnecke et al. (2004). The same algorithms as for Fig. 2 are used. *Cellulomonas cellasea* (X83804) was used as an outer group. One representative clone type within each OTU is shown, and the number of clones within each OTU is shown at the end

hypersaline lakes belonged to the *ac*II group (accounting for 52.9% of total actinobacterial clone sequences in these four lakes), suggesting that some members in the *ac*II group have a wider range of salinity tolerance than previously thought (Warnecke et al. 2004; Holmfeldt et al. 2009). The *ac*III group contained sequences from saline

Table 3 Simple Mantel test for similarity between environmental variables and actinobacterial community in the investigated saline lakes

	OTU level		Major group level[a]	
	r	*P* value	*r*	*P* value
K^+	+0.0444	0.3208	*+0.7984*	*0.0083*
Na^+	+0.0072	0.3472	*+0.8077*	*0.0056*
Ca^{2+}	+0.0128	0.2819	*+0.7416*	*0.0083*
Mg^{2+}	+0.0186	0.3500	*+0.7371*	*0.0083*
Cl^-	−0.0039	0.6472	*+0.7809*	*0.0083*
SO_4^{2-}	+0.0486	0.3847	*+0.5540*	*0.0389*
HCO_3^-	*+0.4336*	*0.0167*	*−0.4440*	*0.0333*
CO_3^{2-}	−0.0958	0.4556	−0.0707	0.4694
Salinity	+0.0120	0.3417	*+0.8081*	*0.0056*
pH	−0.0170	0.6417	+0.6371	0.0833
Temperature	−0.1518	0.1819	+0.0844	0.3972
TN	+0.0391	0.3819	*+0.8229*	*0.0083*
TP	−0.0666	0.4556	−0.1660	0.2333

Non-actinobacterial clones were not included in the Mantel test analysis

The correlation is significant when the *P* value is less than 0.05 (italicized)

r the correlation value; + and − positive and negative correlation, respectively

[a] Major groups were defined as shown in Table 2

and hypersaline lakes, broadly consistent with the observations by Warnecke et al. (2004). It was unexpected to observe the complete absence of the *ac*IV group in our studied river and lakes as this group has been commonly observed in freshwater rivers and lakes, estuaries, and hypersaline soda lakes (Warnecke et al. 2004; Holmfeldt et al. 2009). These two previous studies have implied that the *ac*IV group could tolerate higher salinity than the *ac*I and *ac*II groups, but lower than *ac*III. Because of a limited number of environmental factors examined in this study, possible reasons for the absence of the *ac*IV group remained unknown.

The actinobacterial diversity of Lake Chaka retrieved in this study was apparently higher than that of the same lake studied previously (Jiang et al. 2006). In this study, the actinobacterial community was composed of *Actinobacteridae* (including *Corynebacterineae*, *Frankineae*, *Micrococcineae*, *Propionibacterineae*, and *Streptosporangineae*), *Acidimicrobidae*, and unclassified Actinobacteria. However, only a few actinobacterial clone sequences were retrieved in our previous study (Jiang et al. 2006), and they were grouped into the *Micrococcineae* of *Actinobacteridae*. Such difference could be explained by the primers used for PCR amplification of the 16S rRNA gene: the general bacterial primers of Bac27F and U1492R were used in Jiang et al. (2006), while the Actinobacteria-specific primers of

S-C-Act-0235-a-S-20 and S-C-Act-0878-a-A-19 were employed in this study. This observation was consistent with a previous proposal that some specific bacterial groups would be under-represented in 16S rRNA clone libraries generated with general bacterial primers (Cottrell and Kirchman 2000). Thus, group-specific primers are more effective than general primers when characterizing diversity and community composition of specific groups of bacteria.

Non-specificity of the Actinobacteria-specific primers employed

The primer set (S-C-Act-235-a-S-20 and S-C-Act-878-a-A-19) employed in this study was originally designed to be diagnostic for the *Actinobacteria* 16S rRNA gene based upon all known actinobacterial strains available in the GenBank database at the time (Stach et al. 2003). Subsequently, this primer set has been proven its efficiency for characterizing diversity of actinobacterial communities in freshwater and marine environments (Allgaier and Grossart 2006; Newton et al. 2007; Piao et al. 2008; Warnecke et al. 2004). However, this primer set may produce non-specific amplification when used for hot spring samples (Song et al. 2009) and hypersaline lakes (this study). In Song et al. (2009), a large percent of clone sequences (32%) were grouped with uncultured Candidate Division OP10 which was first defined in Obsidian Pool (OP), a 75–95°C hot spring on the northern flank of the Yellowstone National Park caldera (Hugenholtz et al. 1998). In this study, a total of 135 clones were grouped with *Alpha-* and *Episilonproteobacteria*, *Gemmatimonadetes*, *Synergistetes*, uncultured Candidate Division OP11, uncultured Candidate Division OD1, and unclassified bacteria (Fig. 2b; Table 2), suggesting that the primer set of S-C-Act-235-a-S-20 and S-C-Act-878-a-A-19 has limitations when employed in characterizing actinobacterial community in hot springs and hypersaline lakes. This observation may not be surprising because the pool of actinobacterial clone sequences used for developing the primers (Stach et al. 2003) included few members from hot springs and hypersaline lakes. Therefore, we conclude that with this primer set the actinobacterial diversity may be underestimated. More work is required in saline/hypersaline environments to update the actinobacterial 16S rRNA gene sequence pool in order to design new *Actinobacteria*-specific primer sets covering highly diverse actinobacterial communities.

Conclusion

The actinobacterial communities in the studied freshwater biotopes on the Tibetan Plateau were predominated by the *ac*I and *ac*II groups with different compositions than those from other freshwater ecosystems. A large number of clone sequences retrieved in this study were not affiliated with any previously defined freshwater actinobacterial groups. Salinity was an important environmental parameter shaping the diversity and the community structure of *Actinobacteria*, but certain groups exhibited a higher salinity range than previously thought.

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