Extremophiles (2009) 13:623–632
DOI 10.1007/s00792-009-0245-3

ORIGINAL PAPER

Diversity of Actinobacterial community in saline sediments from Yunnan and Xinjiang, China

Jinyuan Wu · Tongwei Guan · Hongchen Jiang · Xiaoyang Zhi · Shukun Tang · Hailiang Dong · Lili Zhang · Wenjun Li

Received: 30 November 2008 / Accepted: 31 March 2009 / Published online: 7 June 2009

Abstract The diversity and community structures of actinobacteria in saline sediments collected from Yunnan and Xinjiang Provinces, China, were investigated with cultivation and 16S rRNA gene analysis. A total of 163 actinobacterial isolates were obtained, and they were affiliated with the order *Actinomycetales* (distributed into five suborders: *Streptosporangineae*, *Micrococcineae*, *Streptomycineae*, *Pseudonocardineae*, and *Glycomycineae*). A total of 748 actinobacterial 16S rRNA gene clones were examined, and they could be classified into *Actinomycetales*, *Acidimicrobiales*, and unclassified actinobacteria. The *Actinomycetales* sequences were distributed into nine suborders: *Streptosporangineae*, *Glycomycineae*, *Micromonosporineae*, *Pseudonocardineae*, *Corynebacterineae*, *Frankineae*, *Propionibacterineae*, *Streptomycineae*, and *Micrococcineae*. The unclassified actinobacteria contained three new clusters at the level of subclass or order. Our 16S rRNA gene phylogenetic data indicated that actinobacterial communities were very diverse in the investigated saline sediments (salinity 0.4–11.6%) and some actinobacterial members may be halotolerant or halophilic. The actinobacterial community structures in the saline sediments were different from those in marine and freshwater environments. Our data have implications for a better understanding of the distribution of *Actinobacteria* in saline environments.

Keywords Saline sediments · Salinity · *Actinobacteria* · Diversity

Communicated by F. Robb.

Electronic supplementary material The online version of this article (doi:10.1007/s00792-009-0245-3) contains supplementary material, which is available to authorized users.

J. Wu · X. Zhi · S. Tang · W. Li (✉)
The Key Laboratory for Microbial Resources of the Ministry of Education, Laboratory for Conservation and Utilization of Bio-resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, China
e-mail: wjli@ynu.edu.cn

J. Wu
e-mail: ufogio@hotmail.com

T. Guan · L. Zhang
Key Laboratory of Protection and Utilization of Biological Resources, Tarim Basin of Xinjiang Production and Construction Corps, Tarim University, Alar, Xinjiang 843300, China

H. Jiang · H. Dong
Geomicrobiology Laboratory, State Key Laboratory of Geological Processes and Mineral Resources, School of Earth Sciences and Resources, China University of Geosciences, Beijing 100083, China

H. Dong
Department of Geology, Miami University, Oxford, OH 45056, USA

Introduction

The *Actinobacteria* phylum, one of the main phyla within the *Bacteria*, contains four classified subclasses: *Rubrobacteridae*, *Coriobacteridae*, *Acidimicrobidae*, and *Actinobacteridae*, and some unclassified *Actinobacteria* (Boone et al. 2001; Hugenholtz and Stackebrandt 2004; Gao and Gupta 2005). Due to their biotechnological applications, *Actinobacteria* are drawing increasing attention from microbiologists (Ward and Bora 2006). *Actinobacteria* are ubiquitous in soils (Cho et al. 2006; Gremion et al. 2003; Piao et al. 2008; and references therein), marine environments (Bull et al. 2005; Goodfellow and Haynes 1984; Maldonado et al. 2005; Stach and Bull 2005; Stach et al. 2003a; Ward and Bora 2006), and freshwater

ecosystems (Allgaier and Grossart 2006; Glockner et al. 2000; Nielsen et al. 2006; Warnecke et al. 2004, 2005; Zwart et al. 2002; and references therein). These previous studies showed that actinobacterial diversity was variable in different environments. For example, the actinobacterial community in deep-sea sediments was composed of members of the suborders *Corynebacterineae*, *Frankineae* and *Streptomycineae* in the *Actinomycetales* and some unclassified marine actinobacterial groups (Stach et al. 2003a). In contrast, freshwater actinobacteria (e.g., reservoirs, lakes, and estuaries) could be grouped into four main clusters: *acI* (affiliated with *Frankineae*), *acII* and *acIII* (affiliated with *Micrococcineae*), and *acIV* (related to uncultured marine actinobacterial clones) (Warnecke et al. 2004). Some of these groups (i.e., *acI* and *acII*) were almost exclusively composed of clone sequences retrieved from freshwaters and clearly separated from soil/marine actinobacterial lineages (Warnecke et al. 2004).

Actinobacteria also occur in saline and hypersaline environments. For example, actinobacterial 16S rRNA gene clone sequences were retrieved in saline and hypersaline environments (Dong et al. 2006; Humayoun et al. 2003; Jiang et al. 2006; Mancinelli 2005; Mesbah et al. 2007; Oren 2002; and references therein), and several novel actinobacterial members have been successfully characterized (Cai et al. 2008; Guan et al. 2009; Li et al. 2006, 2008; Syed et al. 2008; Tang et al. 2008). Despite these findings, relative to our knowledge of these organisms in other environments, little is known about the diversity and community structure of *Actinobacteria* in saline environments. Saline and hypersaline habitats (e.g., lakes, soils, salterns, and salt rocks) are widespread all over the world (Dong and Yu 2007). The ecological characteristics (i.e., diversity, distribution, and composition) of halophiles in such ecosystems have been extensively investigated (Jiang et al. 2007; Mancinelli 2005; Oren 2002; and references therein), but actinobacteria have never been specifically studied as halophilic or halotolerant organisms.

The main objective of this research was to study the actinobacterial diversity and community structure in saline sediments collected from two distantly separated provinces in China with culture-dependent and culture-independent techniques (i.e., actinobacterial 16S rRNA gene phylogenetic analysis).

Materials and methods

Site description and sample collection

The sampling sites are located in Yunnan and Xinjiang Provinces in China (Figs. 1, 2, Electronic supplementary material). In Yunnan Province, three saline sediment samples were obtained from HM2 (25°22.366′N, 101°44.568′E) (an abandoned salt mine), HM10 (25°23.567′N, 101°45.105′E) (a natural hypersaline spring), and JM (22°35.069′N, 101°50.034′E) (an abandoned salt mine). In Xinjiang Province, two saline sediment samples were collected from XL (37°5.283′N, 81°31.178′E) (Lake Xiaoerkule) and YT (40°7.581′N, 77°21.085′E) (Lake Yutian), Xiaoerkule and Yutian Lakes are, respectively, located in the northwest and south of Tarim Basin, an inland dry basin in Xinjiang Province. Saline sediment samples were collected at 10–30 cm depth at each sampling site and stored in sterile 50 mL Falcon centrifuge tubes. Samples for cultivation and molecular analysis were stored in regular ice and dry ice, respectively. The pH and salinity of the spring water at HM10 were measured in the field. In the laboratory, samples for cultivation were processed as described below; and those for molecular analysis were stored at −80°C until further analysis. The pH and salinity were measured with portable meters after the sediments were dissolved into distilled water. The concentrations of major cations and trace elements in the dissolved sediments were measured according to Yakimov et al. (2002).

Isolation of microorganisms

Five saline sediment samples (HM2, HM10, JM, XL, and YT) were chosen for cultivation of *Actinobacteria*. In order to isolate moderately halophilic and/or halotolerant actinobacteria, the sediments (2 g wet weight) were dispersed into 18 mL sterilized NaCl brine (con. 10%, w/v) and incubated at 37°C for 30 min with shaking at 150 rpm. The resulted slurry was then serially diluted with sterilized NaCl brine (con. 10%, w/v). Aliquots (0.2 mL) of each dilution were spread onto petri dishes using four different media (g L^{-1}): cellulose–casein multi-salt medium (Tang et al. 2008), modified ISP media 4 & 5 (Shirling and Gottlieb 1966), and one home-made medium containing (g L^{-1}, w/v): mannitol, 10.0; K_2HPO_4, 0.2; $MgSO_4{\cdot}7H_2O$, 0.2; $CaSO_4{\cdot}2H_2O$, 0.1; $CaCO_3$, 5.0; KCl, 50.0; and agar, 20.0. All the agar plates were supplemented with 10% (w/v) NaCl (Shirling and Gottlieb 1966). In order to suppress the growth of non-actinobacterial bacteria and fungi, the solidified media were supplemented with potassium dichromate (15 mg L^{-1}). The petri dishes were incubated at 37°C for 4–6 weeks. Based on the size and color, colonies were picked and further purified on inorganic salts–starch agar supplemented with 10% (w/v) NaCl (Shirling and Gottlieb 1966).

Identification of actinobacterial isolates

The total genomic DNA of isolated microorganisms were extracted using the method previously described (Cui et al. 2001), and then used as template in the diagnostic PCR

identification for *Actinobacteria*. PCR amplification was performed with actinobacterial 16S rRNA gene-specific forward primer S-C-Act-0235-a-S-20 (5′-CGC GGC CTA TCA GCT TGT TG-3′) and reverse primer S-C-Act-0878-a-A-19 (5′-CCG TAC TCC CCA GGC GGG G-3′) (Stach et al. 2003b). PCR reaction was made in a total volume of 50 μL containing PCR buffer (1×) with 7.5 μM $MgCl_2$, deoxynucleoside triphosphates (200 μM dNTP), 0.5 μM (each) S-C-Act-0235-a-S-20 and S-C-Act-0878-a-A-19 primers, and DNA template (ca. 80 ng). Amplification was made using a "Hot-start" protocol, which consisted of an initial denaturation at 95°C for 4 min, a brief pause (30 s) for adding *Taq* DNA polymerase (1.0 U) and 30 cycles of denaturation at 95°C for 45 s, annealing at 70°C for 45 s, and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. The PCR products were examined on regular agarose gel (conc. 1%).

For sequencing, genomic DNA of the identified actinobacterial strains was PCR amplified with 16S rRNA gene-specific primers A (5′-CAG AGT TTG ATC CTG GCT-3′) and B (5′-AGG AGG TGA TCC AGC CGC A-3′), with the same conditions as previously described (Cui et al. 2001). The amplified PCR products were purified using a TaKaRa DNA fragment purification kit (Ver. 2.0) and sequenced using an ABI 3100 automated sequencer with primer A at Shanghai Sangon Biotech, Shanghai, China.

Extraction of community DNA from the saline sediments

The total community DNA from the saline sediments were extracted using a method modified from that of Zhou et al. (1996) and Jackson et al. (1997): about 5 g of each wet-sediment sample was added to 12 mL of extraction buffer (0.1 mol L^{-1} Na_2HPO_4, 0.1 mol L^{-1} EDTA, 0.1 mol L^{-1} Tris base) and 50 μL of lysozyme (10 mg mL^{-1}) in 50 mL sterile centrifuge tubes. The samples were mixed for 2 min and incubated for 30 min at 37°C. One and a half milliliters of 20% CTAB and 70 μL of proteinase K (20 mg mL^{-1}) were added and incubated for 30 min at 37°C. After this, 1.5 mL of 10% SDS was added to the tubes and incubated for 2 h at 65°C with periodical (every 10 min) mixing. The samples were centrifuged (15 min, 7,500*g*, room temperature) and the supernatant was decanted into a new sterile tube followed by the addition of 12 mL of phenol:chloroform:isoamyl alcohol (25:24:1, pH 8.0). The samples were mixed and centrifuged (15 min, 13,000*g*, room temperature), and then the aqueous supernatant was transferred into a new sterile tube. The above steps were repeated and after the final step 0.6 volume (final) of cold isopropanol was added to the supernatant solutions. The samples were mixed, and the nucleic acids were precipitated by centrifugation (20 min, 13,000*g*, room temperature). The precipitate was washed with ice-cold 70% ethanol and dissolved in 300 μL TE buffer. Total DNA (1–2 μg) was further purified with a UNIQ-10 Column DNA Gel Extraction Kit (Shanghai Sangon) according to the manufacturer's instructions.

Clone library construction

Extracted genomic DNA was PCR amplified with the primer set of S-C-Act-0235-a-S-20 and S-C-Act-0878-a-A-19 using the same conditions as those for the isolates. The PCR products were purified using the PCR purification kit (Shanghai Sangon) and ligated into the pMD18-T Easy vector system according to the manufacturer's protocol (TaKaRa Biotech.). Plasmid DNA was transformed into *Escherichia coli* strain DH5α with a standard transformation protocol (Sambrook and Russell 2001). Transformed *E. coli* cells were grown on Luria–Bertani plates containing 100 μg mL^{-1} ampicillin. Around 120–180 colonies per sample were randomly picked for RFLP analysis. Plasmid DNA of the selected clones was re-amplified using primers S-C-Act-0235-a-S-20 and S-C-Act-0878-a-A-19 with the same conditions as described above. The PCR product of each clone was digested at 37°C for 3 h with a *Hha*I and *Hae*III mix (TaKaRa, Dalian, China) for RFLP analysis. The digests were analyzed on 15% polyacrylamide gel electrophoresis. Each RFLP group was preliminarily defined as an operational taxonomic unit (OTU).

Phylogenetic analysis of actinobacterial 16S rRNA gene sequences

Unique RFLP patterns were identified visually and representatives of each restriction pattern were selected for 16S rRNA gene sequencing. Clone sequences were determined 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 assembled and edited by using Sequencher, version 4.1 (Gene Codes, Ann Arbor, MI). The potential chimeric sequences were examined with the CHECK_CHIMERA program (Larsen et al. 1993) and discarded. Operational taxonomic units (OTUs) were identified using DOTUR 1.53 (Schloss and Handelsman 2005). The sequences of ≥99% identity were clustered into one OTU (Stach et al. 2003a; and references therein). One sequence was selected from each OTU for phylogenetic analyses. The selected sequences (for both isolates and clones) were aligned with ClustalX (Thompson et al. 1994). Phylogenetic tree (neighbor-joining, maximum parsimony, maximum likelihood) was constructed using the MEGA 4 from dissimilar distances and pairwise comparisons with the Kimura 2-parameter model (Tamura et al. 2007). Rarefaction analysis was performed using software available online at http://www.uga.edu/~strata/software/Software.html.

The ecological diversity within each community (clone library) was estimated using SPADE v2.1 (http://chao.stat.nthu.edu.tw/softwareCE.html). Assessment of the difference or similarity between a pair of microbial communities was calculated using ARLEQUIN v3.1 (Excoffier et al. 2005). The similarity or difference between two microbial communities was assessed using pairwise F statistics (Mathur et al. 2007). LIBSHUFF analysis (Singleton et al. 2001) was performed in the same way as described by Jiang et al. (2006). Mantel test was carried out using the R program (Team 2007).

Nucleotide sequence accession numbers

The sequences reported in this study have been deposited in the GenBank Database (Accession no. EU652923–EU652931, EU652936–EU652938 and FJ425033–FJ425055 for the obtained actinobacterial isolates; accession no. EU532492–EU532596, EU417729–EUEU417751 and FJ214060–FJ214097 for the actinobacterial 16S rRNA gene clone sequences).

Result

Sediment geochemistry

Combined field and laboratory measurements showed that the sample HM10 had the highest salinity (11.6%), and pH was nearly neutral for all the samples (Table 1, Electronic supplementary material). The sodium concentrations ranged from 3.88 g kg^{-1} (HM2) to 114.68 g kg^{-1} (HM10).

Phylogenetic analysis of *Actinobacteria* isolates

A total of 163 (19, 43, 10, 49, and 42 from HM2, HM10, JM, XL, and YT, respectively) isolates were obtained and subjected to 16S rRNA gene phylogenetic analysis (Table 2, Electronic supplementary material). All these isolates were affiliated with the order *Actinomycetales* and distributed into five suborders: *Streptosporangineae*, *Micrococcineae*, *Streptomycineae*, *Pseudonocardineae*, and *Glycomycineae* (Fig. 1a). Most (90%) isolates belonged to two suborder: *Streptosporangineae* and *Pseudonocardineae* (Table 2, ESM).

Phylogenetic analysis of environmental actinobacterial community

A total of 748 actinobacterial 16S rRNA gene clones (157, 146, 151, 123, and 171 from library JM, HM2, HM10, YT, and XL, respectively) were subjected to RFLP analysis, and a total of 219 (48, 34, 51, 42, and 44 from JM, HM2, HM10, YT, and XL, respectively) clones (RFLP types) were sequenced. Out of these clone sequences, a total of 164 (34, 25, 40, 26, and 39 for JM, HM2, HM10, YT, and XL, respectively) OTUs were identified and subjected to phylogenetic analysis (Table 3, Electronic supplementary material). All actinobacterial clone sequences retrieved in this study could be classified into *Actinomycetales*, *Acidimicrobiales*, and unclassified *Actinobacteria* (Fig. 1b).

In the *Actinomycetales*, more than a half of clones (59%, 439 out of 748) belonged to nine suborders: *Streptosporangineae*, *Glycomycineae*, *Micromonosporineae*, *Pseudonocardineae*, *Corynebacterineae*, *Frankineae*, *Propionibacterineae*, *Streptomycineae*, and *Micrococcineae* (Table 3, ESM; Fig. 1b). About 11% (88 out of 748) of the actinobacterial clones were affiliated with the *Acidimicrobiales* (Table 3, ESM; Fig. 1b). Most clones in the *Actinomycetales* and the *Acidimicrobiales* were related (identity 94–99%) to cultured actinobacterial members. About 30% (221 out of 748) of the actinobacterial clones were grouped into unclassified *Actinobacteria*, the saline sediment groups (SSGs), which were clearly separated from all other *Actinobacteria* clusters. The SSGs contained sequences from HM2, HM10, JM, and XL. No sequence from YT was affiliated with this cluster (Table 3, ESM; Fig. 1b).

Diversity index analysis

Rarefaction curves indicated that the diversity of clone libraries JM and XL was highest, and that of YT and HM2 was lowest (Fig. 3, ESM). This trend was consistent with diversity indices of Shannon (Chao and Shen), Shannon (Jackknife), gene diversity, nucleotide diversity, and θ (π) determined by SPADE and Arlequin (Table 4, Electronic supplementary material). The LIBSHUFF results showed significant difference between YT and other samples, and between HM2 and HM10/JM (P values <0.05; Table 5, Electronic supplementary material). Mantel test showed that OTU richness was not significantly correlated with salinity ($r^2 = 0.3495$, $P = 0.1812$).

Discussion

Diversity of actinobacterial 16S rRNA gene clone sequences

To our knowledge, this research was the first to specifically study actinobacterial diversity and community structures in saline sediments. Although rarefaction curves and calculations of diversity indices of the clone libraries showed some difference in diversity among the two groups of samples from Yunnan and Xinjiang Provinces (thousands of kilometers away from one another), there

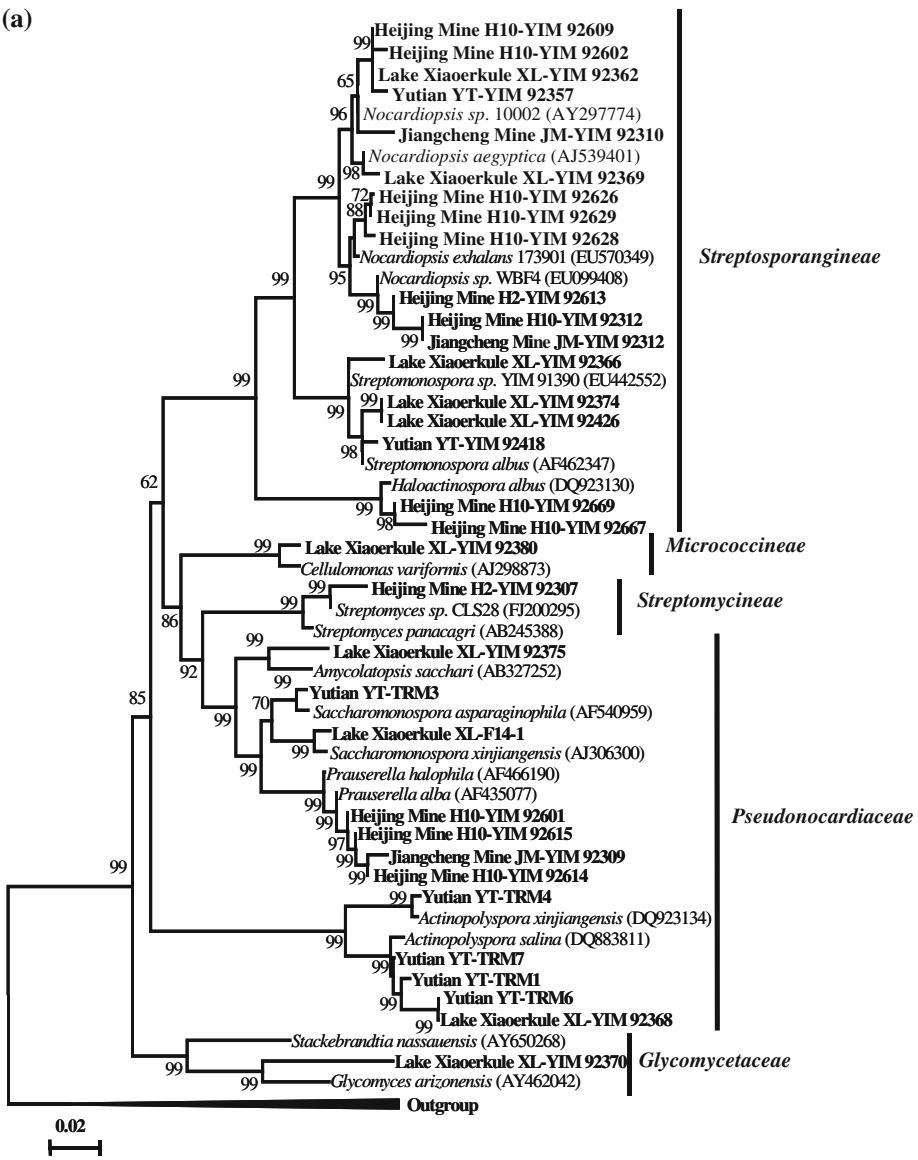


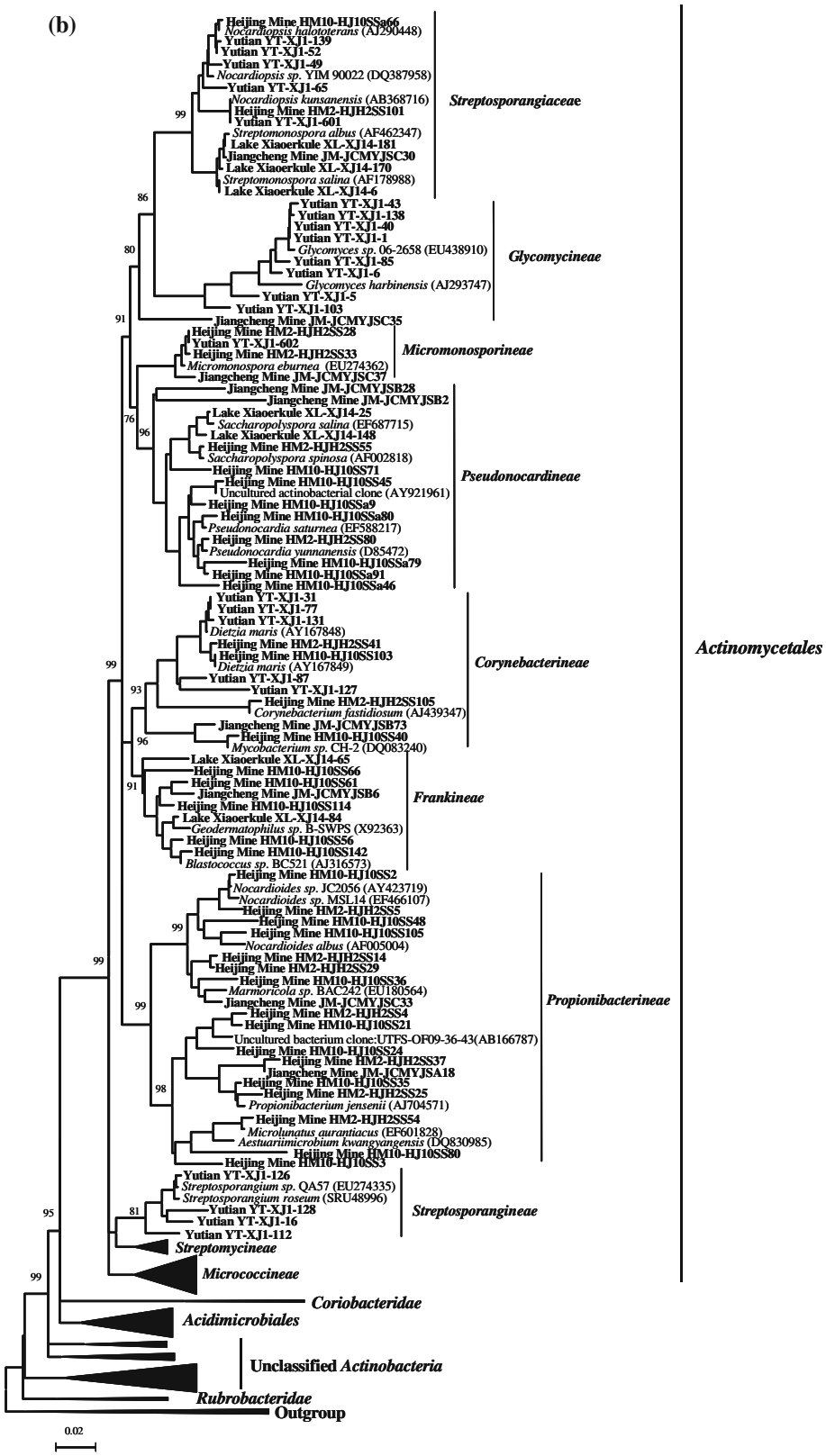
Fig. 1 Neighbor-joining tree showing the phylogenetic trees of partial actinobacterial 16S rRNA gene sequences of obtained isolates (**a**) and clones (**b**) from saline sediments collected from Yunnan and Xinjiang, China. The partial sequences determined in this study are in *bold*. The most closely related sequences found in the GenBank database are also included. The scale bar represents 2% nucleotide sequence difference. Bootstrap values of >50% (for 1000 iterations) are shown

was no biogeographical grouping among the clone sequences (F_{st}, data not shown; Fig. 1; Table 5, ESM), suggesting that geographic distance was not an important factor controlling actinobacterial community structures. This observation was consistent with the ubiquitous nature of actinobacteria in natural environments (Warnecke et al. 2005).

Our results suggested that actinobacterial communities in the saline sediments from Yunnan and Xinjiang Provinces of China were more diverse at the level of both orders and suborders than those in marine and freshwater ecosystems. Previous studies have shown that actinobacterial communities in marine and freshwater ecosystems were mainly composed of the *Actinomycetales* (including four suborders: *Corynebacterineae*, *Frankineae, Micrococcineae,* and *Streptomycineae*) and uncultured marine actinobacterial groups (Maldonado et al. 2005; Stach et al. 2003b; Warnecke et al. 2004, 2005; and references therein). All actinobacterial clone sequences retrieved in this study could be affiliated with the *Actinomycetales* (but with different suborders including: *Streptosporangineae*, *Glycomycineae*, *Micromonosporineae*, *Pseudonocardineae*, *Corynebacterineae*, *Frankineae*, *Propionibacterineae*, *Streptomycineae*, and *Micrococcineae*), *Acidimicrobiales*, and unclassified *Actinobacteria*. The *Acidimicrobiales* was an important group (12%, 88 out 748) in the investigated saline sediments (Table 3, ESM; Fig. 1b), but is usually absent in marine and freshwater ecosystems (Maldonado et al. 2005; Stach et al. 2003a; Warnecke et al. 2004, 2005; and references therein).

Growing in media with a high NaCl concentration (10%), there is no doubt that the obtained actinobacterial

Fig. 1 continued



isolates were halotolerant or halophilic. For example, one isolate (Lake Xiaoerkule XL-YIM 92370 in Fig. 1a) showed no growth in the absence of NaCl, grew optimally in the presence of NaCl [8–12% (w/v)], and can tolerate up to 25% of NaCl in the growth media (Guan et al. 2009).

Fig. 1 continued

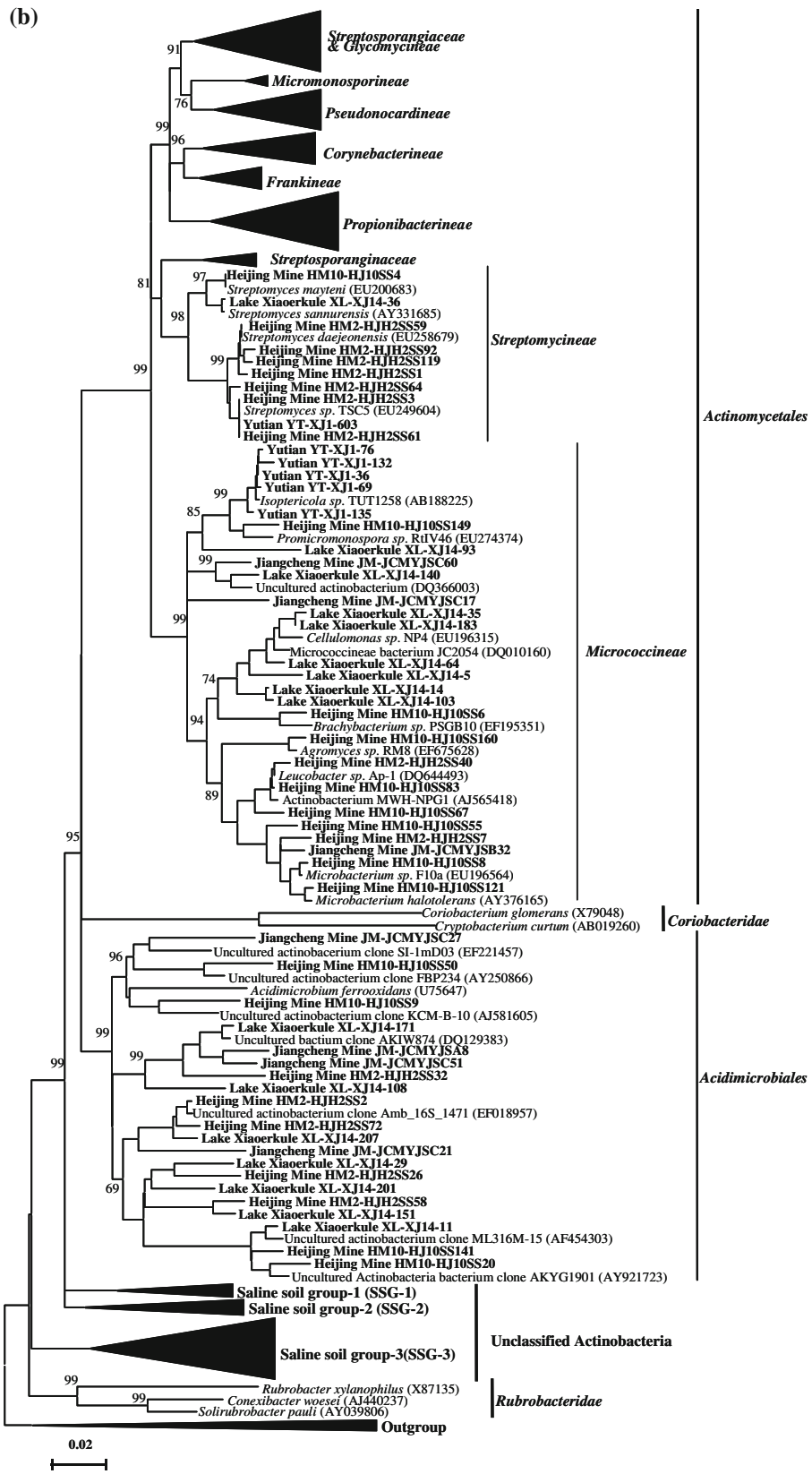
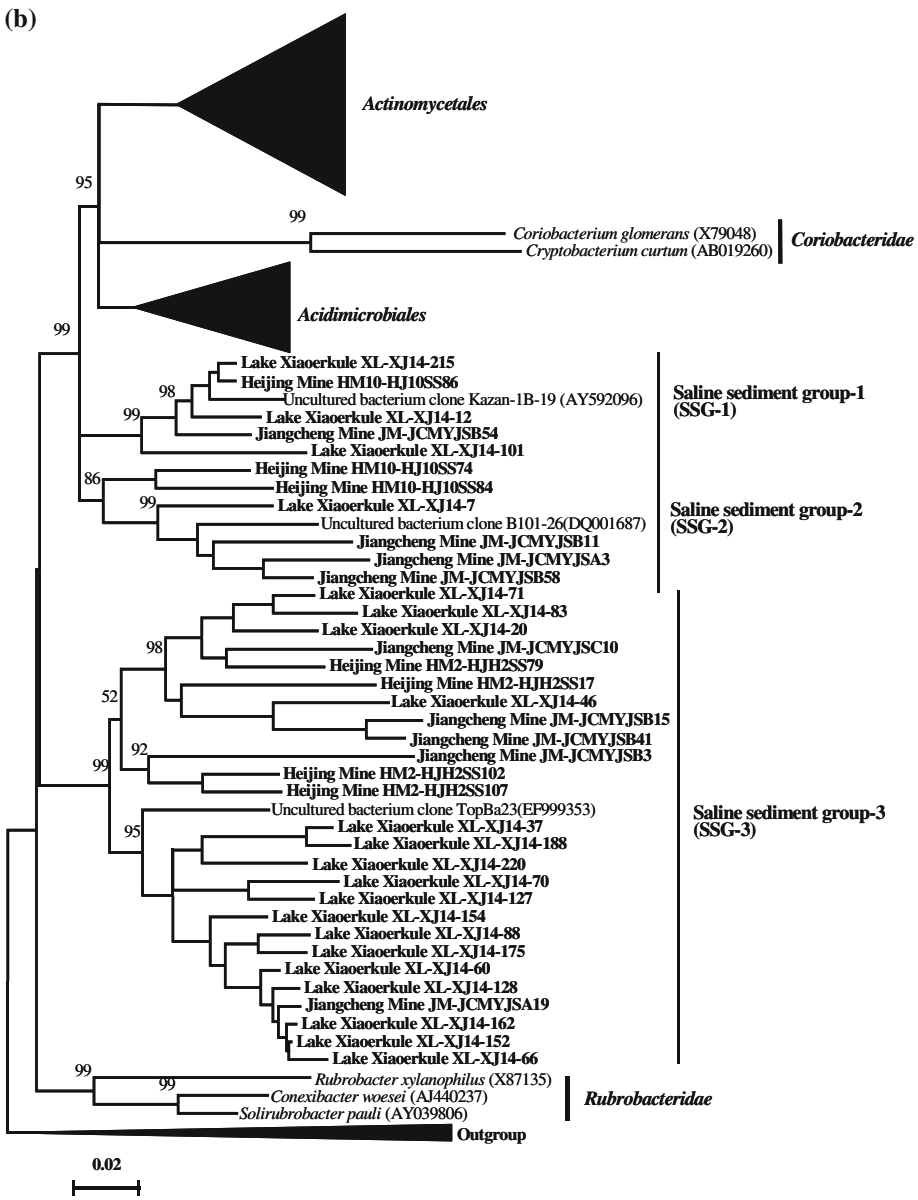


Fig. 1 continued



The clusters, termed as *SSG-1*, *SSG-2*, and *SSG-3* in the unclassified *Actinobacteria* (Fig. 1b), remained stable in different types of tree constructions (neighbor-joining, maximum parsimony, maximum likelihood). They were clearly separated from other classified actinobacterial lineages. No clone sequences in these unclassified actinobacterial clusters were closely related to any characterized actinobacterial members, and were only related to a very limited number of clone sequences in the GenBank database (i.e., AY592096). In the light of the branching position in the phylogenetic tree, these unclassified clusters may represent new subclass(es) or order(s) in the *Actinobacteria*.

Response of actinobacterial community structure to salinity

There was no significant correlation between the actinobacterial diversity (OTU richness) and salinities (0.4–11.6%) (Tables 3, 4, ESM; Mantel test results: $r^2 = 0.3495$, $P = 0.1812$), which was inconsistent with the general ecological principles that more extreme environments decrease diversity (Hacine et al. 2004; and references therein). Possible reasons for such inconsistency could be twofold: i) actinobacteria may tolerate a large range of salinity, ii) actinobacteria may be attached to sediment particles. Most, if not all, microorganisms in the

sediments may be attached to mineral particle surfaces, so that the measured salinity may not represent the true salinity of the micro-niches where the actinobacterial sequences were detected.

However, certain members of the *Actinobacteria* responded to salinity change, but this response was not sufficient to cause any phylogenetic groupings according to salinity. For example, the order *Acidimicrobiales*, and suborders *Micromonosporineae* and *Streptosporangineae* were present at low-salinity sediments and absent at high salinities (Table 3, ESM), suggesting that these organisms were only halotolerant. In contrast, the suborder *Frankineae* was only present in the high-salinity samples (XL and HM10), suggesting that some members of this suborder were halophilic.

Conclusion

Our data showed that the actinobacterial communities in saline sediments possessed different compositions from marine and freshwater ecosystems. Actinobacterial communities in the investigated saline sediments were very diverse, and consisted of *Actinomycetales* (distributed into nine suborders: *Streptosporangineae*, *Glycomycineae*, *Micromonosporineae*, *Pseudonocardineae*, *Corynebacterineae*, *Frankineae*, *Propionibacterineae*, *Streptomycineae* and *Micrococcineae*), *Acidimicrobiales*, and unclassified actinobacteria. The unclassified actinobacteria comprised some as-yet unknown deep-branching groups, and some actinobacterial members were halotolerant or halophilic.

Acknowledgments This research was supported by the National Basic Research Program of China (No. 2004CB719601), the Key Project of Chinese Ministry of Education (No. 206139), the National Natural Science Foundation of China (No. 30600001, 30660005, 30860002, 30870005), and the Yunnan Provincial Natural Science Foundation (project no. 2007C167 M). W.-J. Li was supported by Program for New Century Excellent Talents in Yunnan University. We are grateful to two anonymous reviewers for their constructive comments that greatly improved the quality of the manuscript.

References

Allgaier M, Grossart HP (2006) Diversity and seasonal dynamics of *Actinobacteria* populations in four lakes in northeastern Germany. Appl Environ Microbiol 72:3489–3497

Boone DR, Castenholz RW, Garrity GM (2001) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York

Bull AT, Stach JE, Ward AC, Goodfellow M (2005) Marine *Actinobacteria*: perspectives, challenges, future directions. Anton Van Leeuwenhoek 87:65–79

Cai M, Zhi XY, Tang SK, Zhang YQ, Xu LH, Li WJ (2008) *Streptomonospora halophila* sp. nov., a halophilic actinomycete isolated from a hypersaline soil. Int J Syst Evol Microbiol 58:1556–1560

Cho S, Han J, Seong CN, Kim SB (2006) Phlyogenetic diversity of acidophilic sporoactinobacteria isolated from various soils. J Microbiol 44:600–606

Cui XL, Mao PH, Zeng M, Li WJ, Zhang LP, Xu LH, Jiang CL (2001) *Streptomonospora salina* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae*. Int J Syst Evol Microbiol 51:357–363

Dong H, Yu B (2007) Geomicrobiological processes in extreme environments: a review. Episodes 30:202–216

Dong H, Zhang G, Jiang H, Yu B, Leah RC, Courtney RL, Fields MW (2006) Microbial diversity in sediment of saline Qinghai Lake, China: linking geochemical controls to microbial ecology. Microb Ecol 51:65–82

Excoffier L, Laval LG, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47–50

Gao B, Gupta RS (2005) Conserved indels in protein sequences that are characteristic of the phylum Actinobacteria. Int J Syst Evol Microbiol 55:2401–2412

Glockner FO, Zaichikov E, Belkova N, Denissova L, Pernthaler J, Pernthaler A, Amann R (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of *Actinobacteria*. Appl Environ Microbiol 66:5053–5065

Goodfellow M, Haynes JA (1984) Actinomycetes in marine sediments. In: Yakoleff V (ed) Biochemical and biomedical aspects of Actinomycetes. Academic Press, London, pp 453–472

Gremion F, Chatzinotas A, Harms H (2003) Comparative 16S rDNA and 16S rRNA sequence analysis indicates that *Actinobacteria* might be a dominant part of the metabolically active bacteria in heavy metal-contaminated bulk and rhizosphere soil. Environ Microbiol 5:896–907

Guan T, Tang S, Wu J, Zhi X, Xu L, Zhang L, Li W (2009) *Haloglycomyces albus* gen. nov., sp. nov., a novel halophilic filamentous actinomycete of the family *Glycomycetaceae*. Int J Syst Evol Microbiol (in press)

Hacine H, Rafa F, Chebhouni N, Boutaiba S, Bhatnagar T, Barratti JC, Ollivier B (2004) Biodiversity of prokaryotic microflora in E1 Golea Salt lake, Algerian Sahara. J Arid Environ 58:273–284

Hugenholtz P, Stackebrandt E (2004) Reclassification of *Sphaerobacter thermophilus* from the subclass Sphaerobacteridae in the phylum Actinobacteria to the class Thermomicrobia (emended description) in the phylum Chloroflexi (emended description). Int J Syst Evol Microbiol 54:2049–2051

Humayoun SB, Bano N, Hollibaugh JT (2003) Depth distribution of microbial diversity in Mona Lake, a mermictic soda lake in California. Appl Environ Microbiol 69:1030–1042

Jackson CR, Harper JP, Willoughby D, Roden EE, Churchill PF (1997) A simple, efficient method for the separation of humic substances and DNA from environmental samples. Appl Environ Microbiol 63:4993–4995

Jiang H, Dong H, Zhang G, Yu B, Chapman LR, Fields MW (2006) Microbial diversity in water and sediment of Lake Chaka: an athalassohaline lake in northwestern China. Appl Environ Microbiol 72:3832–3845

Jiang H, Dong H, Yu B, Li Y, Ji S, Liu X, Zhang C (2007) Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan Plateau. Environ Microbiol 9:2603–2621

Larsen N, Olsen GJ, Maidak BL, McCaughey MJ, Overbeek R, Macke TJ, Marsh TL, Woese CR (1993) The ribosomal data base project. Nucleic Acids Res 21:3021–3023

Li W, Kroppenstedt RM, Wang D, Tang S, Lee JC, Park DJ, Kim CJ, Xu L, Jiang C (2006) Five novel species of the genus *Nocardiopsis* isolated from hypersaline soils and emended description of *Nocardiopsis salina*. Int J Syst Evol Microbiol 56:1089–1096

Li W, Zhang Y, Schumann P, Liu H, Yu L, Zhang Y, Stackebrandt E, Xu L, Jiang C (2008) *Nesterenkonia halophila* sp. nov., a moderately halophilic, alkalitolerant actinobacterium isolated from a saline soil. Int J Syst Evol Microbiol 58:1359–1363

Maldonado LA, Stach JE, Pathom-aree W, Ward AC, Bull AT, Goodfellow M (2005) Diversity of cultivable *Actinobacteria* in geographically widespread marine sediments. Anton Van Leeuwenhoek 87:11–18

Mancinelli RL (2005) Microbial life in brines, evaporites and saline sediments: the search for life on Mars. In: Tokano T (ed) Water on Mars and life. Springer, Berlin, pp 277–297

Mathur J, Bizzoco RW, Ellis DG, Lipson DA, Poole AW, Levine R, Kelley ST (2007) Effects of abiotic factors on the phylogenetic diversity of bacterial communities in acidic thermal spring. Appl Environ Microbiol 73:2612–2623

Mesbah NM, Abou-El-Ela SH, Wiegel J (2007) Novel and unexpected prokaryotic diversity in water and sediments of the alkaline, hypersaline lakes of the Wadi An Natrun, Egypt. Microb Ecol 54:598–617

Nielsen JL, Klausen C, Nielsen PH, Burford M, Jorgensen NO (2006) Detection of activity among uncultured Actinobacteria in a drinking water reservoir. FEMS Microbiol Ecol 55:432–438

Oren A (2002) Halophilic microorganisms and their environments, vol 5. Kluwer Academic Publishers, Jerusalem

Piao Z, Yang L, Zhao L, Yin S (2008) Actinobacterial community structure in soils receiving long-term organic and inorganic amendments. Appl Environ Microbiol 74:526–530

Sambrook J, Russell DW (2001) Molecular cloning, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Schloss PD, Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. Appl Environ Microbiol 71:1501–1506

Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340

Singleton DR, Furlong MA, Rathbun SL, Whitman WB (2001) Quantitative comparisons of 16S rRNA gene sequence libraries from environmental samples. Appl Environ Microbiol 67:4374–4376

Stach JE, Bull AT (2005) Estimating and comparing the diversity of marine *Actinobacteria*. Anton Van Leeuwenhoek 87:1572–9699

Stach JE, Maldonado LA, Masson DG, Ward AC, Goodfellow M, Bull AT (2003a) Statistical approaches for estimating actinobacterial diversity in marine sediments. Appl Environ Microbiol 69:6189–6200

Stach JE, Maldonado LA, Ward AC, Goodfellow M, Bull AT (2003b) New primers for the class Actinobacteria: application to marine and terrestrial environments. Environ Microbiol 5:828–841

Syed DG, Tang SK, Cai M, Zhi XY, Agasar D, Lee JC, Kim CJ, Jiang CL, Xu LH, Li WJ (2008) *Saccharomonospora saliphila* sp. nov., a halophilic actinomycete from an Indian soil. Int J Syst Evol Microbiol 58:570–573

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599

Tang S, Tian X, Zhi X, Cai M, Wu J, Yang L, Xu L, Li W (2008) *Haloactinospora alba* gen. nov., sp. nov., a halophilic filamentous actinomycete of the family *Nocardiopsaceae*. Int J Syst Evol Microbiol 58:2075–2080

Team RDC (2007) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Ward AC, Bora N (2006) Diversity and biogeography of marine *Actinobacteria*. Curr Opin Microbiol 9:279–286

Warnecke F, Amann R, Pernthaler J (2004) Actinobacterial 16S rRNA genes from freshwater habitats cluster in four distinct lineages. Environ Microbiol 6:242–253

Warnecke F, Sommaruga R, Sekar R, Hofer JS, Pernthaler J (2005) Abundances, identity, and growth state of *Actinobacteria* in mountain lakes of different UV transparency. Appl Environ Microbiol 71:5551–5559

Yakimov M, Giuliano L, Crisafi E, Chernikova T, Timmis K, Golyshin P (2002) Microbial community of a saline mud volcano at San Biagio-Belpasso, Mt. Etna (Italy). Environ Microbiol 4:249–256

Zhou J, Bruns MA, Tiedje JM (1996) DNA recovery from soils of diverse composition. Appl Environ Microbiol 62:316–322

Zwart G, Crump BC, Kamst-van Agterveld MP, Hagen F, Han S-K (2002) Typical freshwater bacteria: an analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat Microb Ecol 128:141–155