Diversity of Bacillus-like bacterial community in the sediments of the Bamenwan mangrove wetland in Hainan, China

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<td>Liu, Min; Institute of Tropical Biosciences and Biotechnology, Key Laboratory of Biology and Genetic Resources of Tropical Crops of Ministry of Agriculture, Chinese Academy of Tropical Agricultural Sciences, Cui, Ying; Institute of Tropical Biosciences and Biotechnology, Key Laboratory of Biology and Genetic Resources of Tropical Crops of Ministry of Agriculture, Chinese Academy of Tropical Agricultural Sciences, Chen, Yu-qing; Institute of Tropical Biosciences and Biotechnology, Key Laboratory of Biology and Genetic Resources of Tropical Crops of Ministry of Agriculture, Chinese Academy of Tropical Agricultural Sciences, Lin, Xiangzhi; Chinese Academy of Tropical Agricultural Sciences, Huang, Hui-qin; Institute of Tropical Biosciences and Biotechnology, Key Laboratory of Biology and Genetic Resources of Tropical Crops of Ministry of Agriculture, Chinese Academy of Tropical Agricultural Sciences, Bao, Shixiang; Institute of Tropical Bioscience and Biotechnology, Tropical Marine Biological Resources Research Center</td>
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Diversity of *Bacillus*-like bacterial community in the sediments of the Bamenwan mangrove wetland in Hainan, China

Min Liu¹, Ying Cui¹, Yuqing Chen¹, Xiangzhi Lin¹, Huiqin Huang¹, Shixiang Bao¹

¹. Institute of Tropical Biosciences and Biotechnology, Key Laboratory of Biology and Genetic Resources of Tropical Crops of Ministry of Agriculture, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, Hainan, People’s Republic of China

Correspondence: Shi-xiang Bao: E-mail: baoshixiang@itbb.org.cn; Telephone: +86(898)66891557; Hui-qin Huang: E-mail: huanghuiqin@itbb.org.cn; Telephone: +86(898)66890671

Running title: *Bacillus*-like bacterial community in mangrove sediments
Abstract: Members of the genus *Bacillus* and related spore-forming genera are ubiquitous. However, *Bacillus*-like species isolated from marine sediments have attracted less interest than their terrestrial relatives. Here, we investigated the diversity of *Bacillus*-like bacterial community in the sediments of the Bamenwan mangrove wetland in Hainan, China, using culture-dependent and culture-independent methods and present the first report on this subject. We also discovered some potential novel species from the sediment samples. Four families, Bacillaceae (58%), Paenibacillaceae (22%), Alicyclobacillaceae (15%), and Planococcaceae (5%), and nine genera, *Bacillus* (42%), *Paenibacillus* (16%), *Halobacillus* (13%), *Alicyclobacillus* (11%), *Rummeliibacillus* (5%), *Cohnella* (5%), *Tumebacillus* (4%), *Pontibacillus* (3%), and *Aneurinibacillus* (2%), were identified by pyrosequencing. In contrast, only four genera, *Bacillus* (57%), *Paenibacillus* (23%), *Halobacillus* (14%), and *Virgibacillus* (6%), were detected by the culture-dependent method. In the 16S rDNA sequencing analysis, the isolates HB12036 and HB12037 were closest to *Bacillus okuhidensis* Kh10-101<sup>T</sup> and *Paenibacillus xylanilyticus* XIL14<sup>T</sup> with similarities of 94.8% and 95.9%, respectively, indicating that these were novel species. *Bacillus* sp. HB12035 and HB12040 exhibited antimicrobial activity against *Staphylococcus aureus* ATCC25923, and *Bacillus* sp. HB12033 exhibited antimicrobial activity against *Ustilago scitaminea* Syd.

**Keywords:** Diversity, *Bacillus*-like bacteria, 454-pyrosequencing, Culture-dependent method, Mangrove sediment

Introduction

Mangrove is a unique ecosystem with one of the most important intertidal wetland environments in the tropical and subtropical zone. It plays essential roles in preventing drought and flood, purifying seawater, promoting silt accumulation, protecting land,
regulating climate and preserving the biodiversity of coastal wetlands (Alongi 2002; Thampanya et al. 2006; Alongi 2008; Bao 2011). Because of its strong reducibility and acidity, high salinity, and rich nutrient content, the mangrove habitat is an abundant and unique microbial resource (Alongi 1996). However, the diversity of microbes in mangrove sediments remains unexplored. Microorganisms in mangrove sediments play crucial roles in the mangrove ecosystem by participating in various steps of decomposition and mineralization of organic matter and, therefore, make an important contribution to the productivity and recycling of nitrogen, phosphorus, and other essential nutrients, which are used by the plants in the mangrove ecosystem (Holguin et al. 2001).

Bacteria process most of the energy and nutrients in the tropical aquatic systems (Holguin et al. 2001). Among the bacterial populations in marine sediments, _Bacillus_ is a ubiquitous genus (Miranda et al. 2008), which possesses high economical, medical, industrial, agricultural, bio-technological and environmental importance and has a wide range of applications such as in biodefense, bio-enzyme production, and biofuel production and as a decomposer (Höfte & Whiteley 1989; Kalia et al. 1994; Porwal et al. 2008). It is not known whether _Bacillus_-like organisms also play an important ecological role in biogeochemical cycles in the mangrove ecosystem. Knowledge of their diversity, composition, distribution, and ecological function in the sediments is important for understanding the organization of mangrove ecosystems and discovering new _Bacillus_-related microbial resources.

The Bamenwan mangrove wetland is located in Wenchang estuary (19°22’-19°35’N, 110°4’-110°48’E), which is famous for its abundant mangroves (Liu et al. 2011). The Bamenwan mangrove wetland connects to Gaolong Bay through the Qinglan tidal inlet and joins the coastal region of the South China Sea. Few studies have been conducted on the diversity of bacteria in the Bamenwan mangrove sediments. Here we studied the diversity of _Bacillus_-like bacterial community in Bamenwan mangrove sediments using
454-pyrosequencing and culture-dependent methods. We also characterized the biochemical properties and antibiotic activity of some isolates.

**Materials and methods**

**Sample collection**

Three sediment (top 30 cm) samples, each in triplicate, were collected from the Bamenwan mangrove wetland in Hainan, China (19°30’ N, 110°15’ E). The samples were collected from three sites: the seaward edge of the mangrove forest (transitional zone from sea to mangrove forest) and the interior of the mature mangrove forests, dominated by *Bruguiera sexangula* and *Xylocarpus mekongensis*, respectively. Bulk sediments were collected in December 2011. Each of the triplicates at each sampling site was collected approximately 5 m apart, and then pooled. The pH of sediment samples was measured *in situ* using an IQ180G Bluetooth Multi-Parameter System (Hach Company, USA) and the salinity of pore water in the sediment was measured using a YSI 556 Multiprobe System (YSI, USA).

**DNA extraction and 454 pyrosequencing**

Microbial DNA was extracted using the FastPrep® SPIN Kit for Soil (MP Biomedicals, U.S.) according to the manufacturer’s protocol. The bacterial 16S ribosomal RNA gene was amplified by polymerase chain reaction [95 °C for 2 min, followed by 25 cycles of (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s) and a final extension at 72 °C for 5 min] using 341F (5’-CCTACGGGAGGCAGCAG-3’) and 1073R (5’-GAGCTGACGACARCCCATG-3’) as primers. PCR reactions were performed in a 20 µL mixture containing 4 µL of 5× FastPfu Buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu Polymerase, and 10 ng of template DNA. After purification using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) and quantification using QuantiFluor™-ST
(Promega, U.S.), a mixture of the amplicons was used for pyrosequencing on a Roche 454 GS FLX+ Titanium platform (Roche 454 Life Sciences, Branford, CT, U.S.) according to standard protocols. The raw reads were deposited in the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP040784). The resulting sequences were processed using QIIME (version 1.17) (Caporaso et al. 2010). After removing sequences with an average quality score < 25 over a 50 bp sliding window and sequences shorter than 200 bp, with homopolymers longer than six nucleotides, and containing ambiguous base calls or incorrect primer sequences, high-quality sequences were produced. Operational Taxonomic Units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) (Yan et al. 2006; Dias et al. 2011) and chimeric sequences were identified and removed using UCHIME. The phylogenetic affiliation of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the silva (SSU115) 16S rRNA database using confidence threshold of 70% (Yan et al. 2006; Dias et al. 2011; Mendes et al. 2012). The coverage and diversity index analyses were carried out using UniFrac. After analyzing the pyrosequencing data (including quality control and filtering), sequences belonging to Bacilli were selected for further analysis.

**Isolation of Bacillus-like bacterial and 16S rDNA sequencing**

10 g sediment samples were suspended in 100 mL sterile aged seawater and then stirred for 30 min. The low-dilution (10⁻¹ to 10⁻³), heat-shocked (80 °C for 20 min) soil suspensions were spread on a petri dish for isolating Bacillus-like species. Bacterial strains were isolated by the standard dilution-plating technique on MN agar (gelatin peptone 5 g/L, beef extract 3 g/L, NaCl 5 g/L, agar 15 g/L, pH 7.5), MH agar (peptone 5 g/L, yeast extract 1 g/L, glucose 10 g/L, NaCl 5 g/L, agar 20 g/L, pH 7.5), and M1 agar (soluble
starch 2.5 g/L, peptone 1 g/L, yeast extract 0.5 g/L, glycerol phosphate disodium salt
pentahydrate 0.1 g/L, NaCl 5 g/L, agar 20 g/L, pH 7.5). Plates were incubated at 28 °C for 7
days. Single colonies were picked from the plates and purified on MN agar. The genetic
diversity and phylogeny of Bacillus-like isolates were characterized by 16S rDNA
PCR-RFLP and sequence analysis. Bacterial 16S rRNA gene (about 1500 bp) was
amplified using primers P1 (5’-AGAGTTTGATCCTGGCTCAGAACGAACGCT-3’) and
P6 (5’-TACGGCTACCTTGTTACGACTTCCACC-3’), as described before (Chen et al.
2008). The amplicons were digested with endonuclease MspI and the restriction
enzyme-digested products were fractionated on 3% agarose gels. One or two of these
isolates selected from the different genetic clusters were identified basis of the 16S
rRNA gene sequence analysis. The closest relatives of 16S rRNA gene sequences were
obtained from the GenBank/EzBioCloud (http://www.ezbiocloud.net/) databases.
Phylogenetic trees were constructed by the neighbor-joining method (Saitou & Nei 1987)
using the MEGA 5 program (Tamura et al. 2011) with bootstrap values based on 1,000
replications (Felsenstein 1985).

Biochemical characterization and determination of antibiotic susceptibility and
antimicrobial activity
The physiological and biochemical characteristics were determined by testing for antibiotic
susceptibility, carbon source utilization, gelatin liquefaction, H₂S and melanin production,
catalase and oxidase activities, nitrate reduction, and starch and cellulose decomposition as
previously described by Smibert (1994). Antibiotic susceptibility tests were performed on
TSB agar using BBL™ Sensi-Disc™ Susceptibility Test Discs with the following antibiotics
(µg per disc): acheomycin (30), ampicillin (10), chloromycetin (30), erythromycin (15),
gentamycin (10), kanamycin (30), nalidixic acid (30), neomycin (30), novobiocin (30), and
rifampicin (5).

For analysis of antibiotic activity, the bacterial strain was cultured with shaking (200 rpm) at 28 °C in a medium containing 5 g glucose, 5 g yeast extract, 15 g starch, 10 g soy meal, and 1 L sea water, at pH 7.0. After 6 days, the fermentation broth was centrifuged and the supernatant was assayed for antimicrobial activity using the paper disc diffusion method (Isnansetyo & Kamei, 2003). Supernatant aliquots were heated at 25 °C, 37 °C, 50 °C, 60 °C, 70 °C, 80 °C, 90 °C, and 100 °C in an electronic water bath, filtered through 0.45 µm and 0.2 µm cellulose nitrate filters, and analyzed for antimicrobial activity. The target pathogenic microbial strains used in the assay were *Staphylococcus aureus* ATCC25923 (American Type Culture Collection (ATCC)), *Escherichia coli* CGMCC 1.747 (China General Microbiological Culture Collection Center (CGMCC)), *Ustilago scitaminea* Syd. and *Ralstonia dolaanacearum*.

*Ustilago scitaminea* Syd. (cultured on potato dextrose agar at 28 °C) and *Ralstonia dolaanacearum* (cultured on LB agar at 28 °C) were isolated in our laboratory from sugarcane and tomato, respectively.

### Results and discussion

#### Comparison of diversity of *Bacillus*-like bacteria from Bamenwan sediment using 454-pyrosequencing and culture-dependent method

*Bacillus*-like organisms have been isolated from deep subsurface sediments (Batzke et al. 2007), deep-sea hypersaline anoxic sediments (Sass et al. 2008), and inclusions inside materials such as salt crystals (Vreeland et al. 2000) and glacial ice (Larose et al. 2013). This is the first report on *Bacillus*-like isolates from mangrove sediments of Bamenwan, and this study also discovered some potential novel species.

From the pyrosequencing data, a total of 19134 valid reads for bacteria were obtained.
from the three sediment samples. After an initial quality check, the chimeric, archaeal, and singleton reads were checked and filtered out. Finally, 8274 effective bacterial sequences were extracted to perform further bioinformatic analysis. The library coverage of the samples was 98.3% and the Shannon diversity index was 5.79. Thirty-one phyla were identified and the top 10 phyla were Proteobacteria (49.40%), Actinobacteria (16.79%), Chloroflexi (9.46%), Acidobacteria (7.20%), Firmicutes (5.09%), Gemmatimonadetes (2.74%), Nitrospirae (2.24%), Bacteroidetes (2.06%), Cyanobacteria (1.90%), and Chlorobi (1.89%) (Fig. 1). In the 421 sequences affiliated to Firmicutes, three classes were detected: Bacilli (25.42%), Clostridia (72.20%), and Erysipelotrichia (2.38%). Four families, Bacillaceae (58%), Paenibacillaceae (22%), Alicyclobacillaceae (15%), and Planococcaceae (5%) were identified. There were nine genera, Bacillus (42%), Paenibacillus (16%), Halobacillus (13%), Alicyclobacillus (11%), Rummeliibacillus (5%), Cohnella (5%), Tumebacillus (4%), Pontibacillus (3%) and Aneurinibacillus (2%), observed by the pyrosequencing method (Fig. 2a). Bacillus was the predominant genus, followed by Paenibacillus, Halobacillus, and Alicyclobacillus.

A total of 155 Bacillus-like isolates with different morphologies were obtained from the diluted and heat-shocked sediment samples cultured on three different media (MN, MH and M1). The number of isolate on each medium were different, with the highest recovery of 99 isolates on MN medium, followed by 48 on M1 medium, and 8 on MH medium. The 16S rDNA PCR-RFLP of the 155 isolates generated 11 distinct restriction patterns. A total of 24 isolates were selected as representative strains for further phylogenetic analysis and were deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/GenBank/index.html) with accession numbers KC765090 to KC765113 (Table 1). Based on 16S rDNA sequence
analysis, four isolates clustered with their type strains at 100 % similarity (Table 1 and Fig. 3). Sequence similarities with the respective type strains ranged from 99.0 % to 99.9 % for 12 isolates and from 97.0 % to 98.9 % for 6 isolates. Isolates HB12036 and HB12037 were closest to *Bacillus okuhidensis* Kh10-101 \(^T\) and *Paenibacillus xylanilyticus* XIL14 \(^T\) with similarities of 94.8 % and 95.9 %, respectively (Table 1 and Fig. 3), which suggested that they were novel species (Tindall et al. 2010).

In contrast, only four genera, *Bacillus* (57 %), *Paenibacillus* (23 %), *Halobacillus* (14%), and *Virgibacillus* (6 %) were detected by the culture-dependent method (Fig. 2b). Not all the cultured bacterial species and strains were represented in the DNA sample isolated from the original sediment. Of the cultured strains, *Bacillus*, *Paenibacillus*, and *Halobacillus*, were represented in the DNA samples, but *Virgibacillus* was not. The absence of *Virgibacillus* DNA sequences might be due to the fact that the procedure used for environmental DNA extraction was not efficient in extracting DNA from endospores of *Virgibacillus*, which likely could not grow as vegetative cells and existed only as spores. These spores were then grown on media. More genera were detected by the pyrosequencing method, which suggested that molecular methods are more effective than the traditional culture-dependent method for the discovery of microbial diversity (Li et al. 2014). In addition, the previous study reported that a large proportion of the spore-forming bacteria, which were uncultivable in the sediment, could be members of taxa that were strongly energy-limited. Starved cells could be damaged by a substrate shock after exposure to the high substrate concentrations found in many standard media (Köpke et al. 2005). However, culture-dependent methods allowed the discovery of novel bacterial species as well as extensive biological studies. The common traditional approach of spore selection followed by survival in selective conditions (heat-shock) was an efficient strategy that could give significant information on cultivable *Bacillus*-like populations living in soils and other environments. In this study, two new
Bacillus-like species (Bacillus sp. HB12036 and Paenibacillus sp. HB12037 with the 16S rRNA gene similarities of 94.8% and 95.9% with phylogenetically closest relatives, respectively) and six potential new species (with the similarities ranging from 97.7% to 98.9% with the phylogenetically closest relatives) were discovered (Table 1).

Bacillus, Paenibacillus, and Halobacillus, the three genera detected by both methods were also highly abundant. Species of Bacillus and Paenibacillus are known to promote plant growth by solubilizing and mobilizing phosphate, fixing nitrogen, producing growth stimulating phytohormones, promoting antibiosis, and inducing systemic resistance to pathogens (Gutiérrez-Mañero et al. 2001; Whipps 2001; Idris et al. 2007; Richardson et al. 2009). Bacillus also participated in sulfate metabolism and cellulose degradation by producing arylsulfatase and cellulase respectively (Thatoi et al. 2013). They acted as potential nutrient suppliers, which is beneficial for plant growth in mangroves. The spores themselves may have an important ecological role by catalyzing the oxidation of Mn$^{2+}$ (Rosson & Nealson 1982). Bacillus-like bacteria in tropical mangrove sediments generally participated in mineralizing organic detritus and recycling essential nutrients as soluble matter. The presence of Alicyclobacillus groups could be attributed to the adaption of the pH and salinity conditions found in mangrove ecosystems (Dias et al. 2010). The pH and salinity of the three sediment samples from three sites were 7.45, 6.74, 7.13 and 2.63%, 1.55%, 1.38%, respectively. Alicyclobacillus was observed by the pyrosequencing method but not by the culture-dependent method because Alicyclobacillus spp. is a thermophilic (growth temperature range: 25-60 °C) and obligately acidophilic (growth pH range: 2-6) spore-forming bacterium (Wisotzkey et al. 1992; Murray et al. 2007). The culture conditions in this study (pH 7.5) were not suitable for its growth.

Biochemical characterization, antibiotic susceptibility, and antimicrobial activity of representative strains

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Biochemical characterization of two new species discovered in this study, *Bacillus* sp. HB12036 and *Paenibacillus* sp. HB12037, was carried out. Both strains produced H$_2$S and exhibited catalase activity, but did not produce melanin or degrade gelatin. Oxidase activity, cellulose degradation, and nitrate reduction were observed in *Bacillus* sp. HB12036, but not in *Paenibacillus* sp. HB12037. *Paenibacillus* sp. HB12037 could utilize sucrose, D-raffinose, cellobiose, D-fructose, D-mannose, maltose, D-glucose, $a$-lactose, D-trehalose, L-arabitol, D-galactose, D-mannitol, and D-sorbitol, but *Bacillus* sp. HB12036 could not. Inositol and starch were not utilized by both the strains. *Bacillus* sp. HB12036 exhibited hypersensitivity to kanamycin, acheomycin, and rifampicin; medium sensitivity to garamycin, neomycin, and chloromycetin; and low-alcohol sensitivity to ampicillin, erythromycin, and nalidixic acid. *Paenibacillus* sp. HB12037 exhibited medium sensitivity to nalidixic acid and low-alcohol sensitivity to acheomycin, garamycin, kanamycin, neomycin, and rifampicin.

Among 24 representative strains, *Bacillus* sp. HB12035 and HB12040 demonstrated antimicrobial activity against *Staphylococcus aureus* ATCC25923 with 15-20 mm diameter inhibition zones, and *Bacillus* sp. HB12033 acted against *Ustilago scitaminea* Syd. with 18-25 mm diameter inhibition zones. However, neither strain acted against *Escherichia coli* CGMCC 1.747 and *Ralstonia dolaanacearum*. Heat-treated and filtered supernatants of the fermentation broths of the three *Bacillus* strains still showed antimicrobial activity, indicating that the inhibitory compounds were heat-stable and filterable. *Bacillus* is a beneficial group of bacteria, belonging to phylum Firmicutes, that possess plant growth-promoting ability and can protect plants from microbial diseases (Abo-Elnaga 2006). Therefore, in mangrove ecosystems, *Bacillus* might play a role in the long-term promotion of plant growth by...
producing endospores under stressful environmental conditions and by secreting large
quantities of enzymes, such as phytases (Zhang et al. 2015).

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References


Conserv. **29** (3): 331-349.


Oceanologia **53** (3): 807-818.

diversity of cultured deep-biosphere bacteria from equatorial Pacific Ocean and Peru

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K.,
Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D.,
Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J.,
Yatsunenko, T., Zaneveld, J. and Knight, R. (2010) QIIME allows analysis of


bacterial diversity in a Brazilian non-disturbed mangrove sediment. Antonie van
Leeuwenhoek 98 (4): 541-551.

Dias, A.C.F., Dini-Andreote, F., Taketani, R.G., Tsai, S.M., Azevedo, J.L., de Melo, I.S. and
Andreote, F.D. (2011) Archaeal communities in the sediments of three contrasting


Gutiérrez-Mañero, F.J., Ramos-Solano, B., Probanza, A., Mehouachi, J., R Tadeo, F. and
Talon, M. (2001) The plant-growth-promoting rhizobacteria Bacillus pumilus and
Bacillus licheniformis produce high amounts of physiologically active gibberellins.
Physiol. Plantarum 111 (2): 206-211.


Holguin, G., Vazquez, P. and Bashan, Y. (2001) The role of sediment microorganisms in the
productivity, conservation, and rehabilitation of mangrove ecosystems: an overview.


Leeuwenhoek 93 (3): 297-304.


Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary


characterization of an isolated phytase-producing strain of SPC09 B. cereus. Appl.

### Table 1 16S rDNA sequence analysis of representative *Bacillus*-like strains isolated from Bamenwan mangrove sediment

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<td>KC765100</td>
<td><em>Virgibacillus</em></td>
<td><em>Virgibacillus</em> proomii LMG12374&lt;sup&gt;T&lt;/sup&gt;</td>
<td>99.7</td>
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<td>HB12023</td>
<td>KC765099</td>
<td><em>Halobacillus</em></td>
<td><em>Halobacillus</em> mangrovi MS10&lt;sup&gt;T&lt;/sup&gt;</td>
<td>99.3</td>
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<td>HB12026</td>
<td>KC765101</td>
<td><em>Halobacillus</em> trueperi DSM10404&lt;sup&gt;T&lt;/sup&gt;</td>
<td>96.9</td>
<td>8</td>
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<td>HB12003</td>
<td>KC765090</td>
<td><em>Paenibacillus</em></td>
<td><em>Paenibacillus</em> cineis LMG18439&lt;sup&gt;T&lt;/sup&gt;</td>
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<td><em>Paenibacillus</em> lautus NRRL</td>
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<td><em>Paenibacillus</em> xylanilyticus XIL14&lt;sup&gt;T&lt;/sup&gt;</td>
<td>95.9</td>
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Figure captions

**Fig. 1** Bacterial composition at phylum level from sediment of Bamenwan mangrove by pyrosequencing methods.

**Fig. 2** *Bacillus*-like bacterial composition at genus level from sediment of Bamenwan mangrove by pyrosequencing (a) and culture-dependent (b) methods, respectively.

**Fig. 3** Phylogenetic analysis of 24 cultivable *Bacillus*-like representatives and their closest phylogenetic neighbours based on 16S rDNA sequences. Bootstrap values (1000 replicates) are shown as percentages at each node for values. The scale bar represents 0.01 nucleotide substitutions per position.
Alicyclobacillus 11%
Tumebacillus 4%
Bacillus 42%
Halobacillus 13%
Paenibacillus 16%
Cohnella 4%
Aneurinibacillus 2%
Pontibacillus 3%
Rummeliibacillus 5%
