Haloactinopolyspora alba gen. nov., sp. nov., a halophilic filamentous actinomycete isolated from a salt lake, with proposal of *Jiangellaceae* fam. nov. and *Jiangellineae* subord. nov.

Shu-Kun Tang,[1] Xiao-Yang Zhi,[1] Yun Wang,[2] Rong Shi,[1] Kai Lou,[2] Li-Hua Xu[1] and Wen-Jun Li[1]

Correspondence
Wen-Jun Li
liact@hotmail.com

[1]The Key Laboratory for Microbial Resources of Ministry of Education, and Laboratory for Conservation and Utilization of Bio-resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China

[2]Xinjiang Institute of Microbiology, Xinjiang Academy of Agricultural Science, Urumqi, Xinjiang, 830091, PR China

A halophilic, filamentous actinomycete strain, designated YIM 93246^T, was isolated from a salt lake in Xinjiang province, north-west China, and subjected to polyphasic taxonomic characterization. The isolate grew in the presence of 7–23 % (w/v) NaCl, but not in the absence of NaCl. Strain YIM 93246^T had particular morphological properties, forming aerial mycelium that had long spore chains and pseudosporangium-like, rhiziform spore aggregates at maturity. LL-DAP was the cell-wall diamino acid and glucosamine, mannose, glucose, arabinose and galactose were the cell-wall sugars. The major fatty acids were iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. MK-9 (H_4) was the predominant menaquinone and the genomic DNA G+C content was 70.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YIM 93246^T clustered with the genus *Jiangella*. The sequence similarities between strain YIM 93246^T and *Jiangella alba*, *Jiangella gansuensis* and *Jiangella alkaliphila* were 96.9, 96.9 and 96.6 %, respectively. Based on morphological, physiological and chemotaxonomic differences, and phylogenetic analysis, a novel genus and species, *Haloactinopolyspora alba* gen. nov., sp. nov., is proposed. The type strain of the species is YIM 93246^T (=DSM 45211^T=KCTC 19409^T). Additionally, phylogenetic analysis placed the genus *Jiangella* together with strain YIM 93246^T within the order *Actinomycetales* as an independent lineage, clearly distinguished from other described suborders of the class *Actinobacteria*. Hence, based on phylogenetic characteristics, the genus *Jiangella* together with the newly proposed genus *Haloactinopolyspora* are proposed to be classified as *Jiangellaceae* fam. nov. and *Jiangellineae* subord. nov.

The genus *Jiangella* was proposed by Song *et al.* (2005) and was classified in the family *Nocardioidaceae*. It currently comprises three species with validly published names: *Jiangella gansuensis* (Song *et al.*, 2005), *Jiangella alkaliphila* (Lee, 2008) and *Jiangella alba* (Qin *et al.*, 2009). They were isolated from desert soil, a cave and a medicinal plant, respectively. Chemotaxonomically, members of the genus *Jiangella* contain LL-2,6-diaminopimelic acid (LL-DAP) in the cell-wall peptidoglycan, MK-9(H_4) as the predominant menaquinone, anteiso-$C_{15:0}$ and iso-$C_{16:0}$ or anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ as the major fatty acids, and a DNA G+C content of 70–71.9 %.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 93246^T is FJ969846.

Supplementary data are available with the online version of this paper.

Strain YIM 93246^T was isolated from a soil sample collected from Qijiaojing Lake, a salt lake in Xinjiang Province, north-west China (43°26′48″N 91°29′13″E), after 3 weeks incubation at 37 °C on cellulose-casein multi-salt (CCMS) medium described by Tang *et al.* (2008). Strain YIM 93246^T was maintained on GTY medium (Tang *et al.*, 2010) containing 15 % (w/v) NaCl at 4 °C, and as glycerol suspensions (20 %, v/v) at −80 °C. Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (about 150 r.p.m.) using GTY broth [15 % (w/v) NaCl, pH 7.0] at 37 °C for 2 weeks.

Cultural characteristics were determined after 4 weeks incubation by methods used in the International Streptomyces Project (ISP) (Shirling & Gottlieb, 1966). All media were supplemented with 15 % (w/v) NaCl for

growth. The colours of substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from ISCC-NBS colour charts (Kelly, 1964). The growth was good on GTY agar and potato agar, moderate on Czapek's agar, inorganic salts/starch agar (ISP 4) and oatmeal agar (ISP 3), weak on nutrient agar and glycerol/asparagine agar (ISP 5), but no growth was observed on yeast extract/malt extract agar (ISP 2). The colour of the aerial mycelium was white and that of the substrate mycelium was white–yellow. No soluble pigments were produced. Morphological characteristics of strain YIM 93246^T were observed by light microscopy (Model BH 2; Olympus) and scanning electron microscopy (JSM5600LV; JEOL) after incubation on GTY agar containing 15 % (w/v) NaCl at 37 °C for 4 weeks. The substrate mycelium was well-developed and fragmented into rod-like elements, while the aerial mycelium had long spore chains and formed pseudosporangium-like, rhiziform spore aggregates at maturity (Fig. 1).

The growth temperature was tested at 5–55 °C on GTY medium containing 15 % (w/v) NaCl, at intervals of 5 °C. For NaCl tolerance experiments, GTY medium was used as the basal medium and salt concentrations ranging from 0 to 30 % (w/v), at intervals of 1 %, were tested. The pH growth range was investigated between 4.0 and 10.0, at intervals of 1 pH unit, using the buffer system: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH_2PO_4/0.1 M NaOH; pH 9.0–10.0, 0.1 M $NaHCO_3$/0.1 M Na_2CO_3. Media and procedures used for determination of physiological features and carbon source utilization were those described by Williams *et al.* (1989). Enzyme activities were determined by using the API ZYM system (bioMérieux), according to the manufacturer's instructions. Anaerobic growth was determined using the GasPak Anaerobic System (BBL), according to the manufacturer's instructions. Strain YIM 93246^T could grow at 15–45 °C, pH 4.0–9.0 and 7–23 % NaCl, but no growth occurred in the absence of NaCl, showing that strain YIM 93246^T is a moderately halophilic actinomycete. The detailed physiological and biochemical characteristics of the strain are given in the species description.

The isomer of diaminopimelic acid was analysed according to the procedures developed by Hasegawa *et al.* (1983). Amino acids in cell-wall hydrolysates were analysed by precolumn derivatization with *o*-phthalaldehyde (OPA) by HPLC (Tang *et al.*, 2009b). Cell-wall sugars were detected by precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone (PMP) by HPLC (Tang *et al.*, 2009a). Polar lipids were extracted and examined by two-dimensional TLC and identified using previously described procedures (Minnikin *et al.*, 1984). Total lipid material and specific groups were analysed using Dittmer–Lester reagent (phosphate), ninhydrin (free amino groups), Dragendorff reagent (quaternary nitrogen) and anisaldehyde/sulfuric acid (glycolipids). Menaquinones were isolated according to Minnikin *et al.* (1984) and separated by APPI (+)-LC-MS (Tang *et al.*, 2008). For fatty acid analysis, cells of strain YIM 93246^T were cultured on tryptic soy agar (BD) containing 15 % NaCl at 37 °C for 7 days. Cellular fatty acid analysis was performed as described by Sasser (1990) using the Microbial Identification System (MIDI). For the determination of G+C content, the genomic DNA of strain YIM 93246^T was prepared according to the method of Marmur (1961). The G+C content of the DNA was determined by reverse-phase HPLC of nucleosides according to Mesbah *et al.* (1989). Strain YIM 93246^T contained LL-DAP (24.9 %), alanine (36.3 %), glycine (20.1 %) and glutamic acid (18.7 %) as the cell-wall amino acids. Glucosamine (35.1 %), glucose (18.7 %), galactose (12.5 %), mannose (11.5 %) and arabinose (11.5 %) were the major cell-wall sugars. A minor amount of rhamnose (5.9 %) and one unknown sugar (5.3 %) were also detected. The polar lipids were diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, one unknown

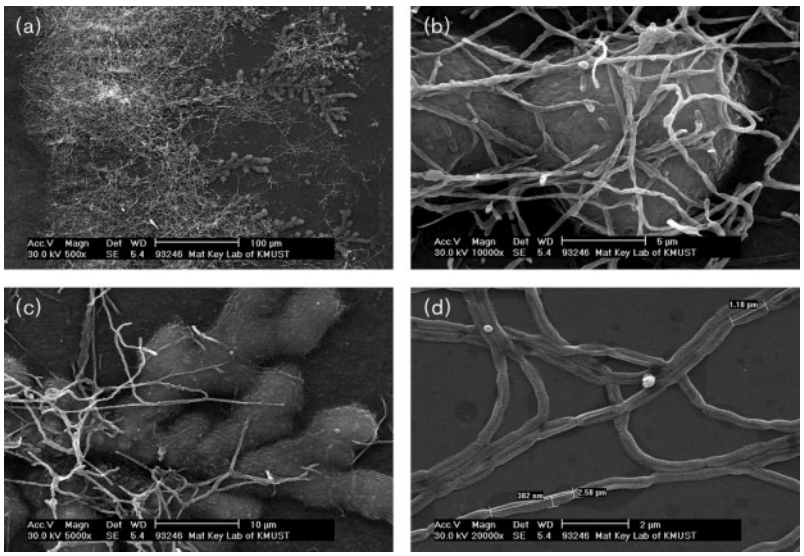

Fig. 1. Scanning electron micrographs of spore chains of *Haloactinopolyspora alba* YIM 93246^T grown on GTY medium containing 10 % (w/v) NaCl for 28 days at 37 °C. (a) Aerial mycelium and pseudosporangium-like, rhiziform spore aggregates; (b, c) long spore chains and pseudosporangium-like spore aggregates; (d) fragmented substrate hyphae. Bars: (a) 100 μm; (b) 5 μm; (c) 10 μm; (d) 2 μm.

phosphoglycolipid, one unknown phospholipid and one unknown glycolipid (Fig. S1, available with the online version of this paper). The predominant menaquinone was MK-9 (H_4) (92 %), with a minor amount of MK-8 (H_4) (8.0 %) detected. Strain YIM 93246^T had a cellular fatty acid profile that contained major amounts of branched fatty acids and minor amounts of saturated and unsaturated fatty acids. The major fatty acids were iso-$C_{16:0}$ (29.4 %), anteiso-$C_{15:0}$ (28.4 %), anteiso-$C_{17:0}$ (13.7 %); minor fatty acids were $C_{14:0}$ (0.5 %), $C_{15:0}$ (1.0 %), $C_{16:0}$ (2.7 %), $C_{17:0}$ (2.0 %), $C_{18:0}$ (1.1 %), $C_{17:1}\omega8c$ (1.7 %), $C_{18:1}\omega9c$ (0.8 %), iso-$C_{16:1}$G (4.4 %), anteiso-$C_{17:1}$A (0.9 %), iso-$C_{14:0}$ (5.8 %), iso-$C_{15:0}$ (2.7 %), iso-$C_{17:0}$ (2.0 %), $C_{15:0}$ 2-OH (1.0 %), iso-$C_{16:0}$ 3-OH (0.5 %) and iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$ (1.5 %). The G+C content of the DNA was 70.5 mol%.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li *et al.* (2007). Multiple alignments with sequences of the suborder *Propionibacterineae* and the phylum *Actinobacteria*, respectively, and calculations of levels of sequence similarity were carried out using EzTaxon server 2.1 (Chun *et al.*, 2007). Phylogenetic analyses were performed using three tree-making algorithms: the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980) using MEGA version 4.0 (Tamura *et al.*, 2007). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1,000 replicates.

An almost complete 16S rRNA gene sequence (1432 bp) was determined for strain YIM 93246^T. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the organism clustered with the genus *Jiangella* (Fig. 2). The sequence similarities between strain YIM 93246^T and *J. alba*, *J. gansuensis* and *J. alkaliphila* were 96.9, 96.9 and 96.6 %, respectively. In the phylogenetic tree based on the neighbour-joining algorithm, strain YIM 93246^T and members of the genus *Jiangella* clustered in a distinct clade supported by a high bootstrap value (100 %). This relationship was supported by all tree-making methods used in this study. Although strain YIM 93246^T was similar to the genus *Jiangella* with MK-9(H_4) as the predominant menaquinone and LL-DAP as the cell-wall diamino acid, it was different in some physiological properties (Table 1). Strain YIM 93246^T is a halophilic actinomycete: growth occurred in the presence of up to 23 % (w/v) NaCl and no growth was observed in the absence of NaCl. Members of the genus *Jiangella* are non-halophilic actinomycetes which grow well in the absence of NaCl and do not grow in the presence of 15 % (w/v) NaCl. In addition, strain YIM 93246^T exhibited some differences from the genus *Jiangella* in chemotaxonomic properties. For instance, strain YIM 93246^T contained glucosamine (35.1 %), glucose (18.7 %), galactose (12.5 %), mannose (11.5 %) and arabinose (11.5 %) as the major cell-wall sugars (no ribose), iso-$C_{16:0}$ (29.4 %), anteiso-$C_{15:0}$ (28.4 %) and anteiso-$C_{17:0}$ (13.7 %) as the major fatty acids, and diphosphatidylglycerol, an unknown phosphoglycolipid, an unknown phospholipid, phosphatidylinositol, phosphatidylinositol mannosides and an unknown glycolipid as the polar lipids (no phosphatidylglycerol or phosphatidylcholine). Members of the genus *Jiangella* contain glucose and ribose as the cell-wall sugars (rhamnose, mannose and galactose may be present, but glucosamine and arabinose are not present), anteiso-$C_{15:0}$ and iso-$C_{16:0}$ or anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$ as the major fatty acids, and phosphatidylglycerol as the predominant polar lipid (phosphatidylcholine may also be present) (see Table 1, Figs S1 and S2, available with the online version of this paper). In particular, strain YIM 93246^T is distinct from members of the genus *Jiangella* in morphological properties (Fig. 1): strain YIM 93246^T has long spore chains and formed pseudosporangium-like, rhiziform spore aggregates at maturity, whereas members of the genus *Jiangella* do not have long spore chains and do not form pseudosporangium-like spore aggregates. Therefore, on the basis of phenotypic, chemotaxonomic and phylogenetic differentiation of the new isolate from its closest neighbour, the genus *Jiangella*, we propose that strain YIM 93246^T represents a novel species in a novel genus, *Haloactinopolyspora alba* gen. nov., sp. nov.

Although the genus *Jiangella* was proposed by Song *et al.* (2005) and assigned to the family *Nocardioidaceae* within the suborder *Propionibacterineae*, phylogenetic analysis based on 16S rRNA gene sequences of the suborder *Propionibacterineae* revealed that strain YIM 93246^T clustered with members of the genus *Jiangella*, forming a monophyletic branch at the periphery of the evolutionary radiation occupied by the suborder *Propionibacterineae* (Fig. 2). This clearly showed that strain YIM 93246^T and the genus *Jiangella* did not belong to either the family *Nocardioidaceae* or the suborder *Propionibacterineae*. Then, phylogenetic analysis based on 16S rRNA gene sequences of the phylum *Actinobacteria* revealed that strain YIM 93246^T clustered with members of the genus *Jiangella*, forming a deep branch, clearly distinguished from other described suborders of the class *Actinobacteria* (Fig. 3). Representatives of members of the phylum *Actinobacteria* showed 16S rRNA gene sequence similarities to the genus *Jiangella* and strain YIM 93246^T of less than 93.1 %. Although the genus *Jiangella* and strain YIM 93246^T clearly belong to the order *Actinomycetales*, they do not belong to any of the described suborders within the order *Actinomycetales*. The 16S rRNA gene sequences of all suborders of the order *Actinomycetales* (Zhi *et al.*, 2009) and the genus *Jiangella* and strain YIM 93246^T were scanned for signature nucleotides. We found both strain YIM 93246^T and the genus *Jiangella* had many unique 16S rRNA gene signature nucleotides compared with the described suborders within the order *Actinomycetales*, particularly reflected in 11 different positions, namely 127 : 234 (G–C), 598 : 640 (C–G), 672 : 734 (G–C), 831 : 855 (U–A), 833 : 853 (G–C),

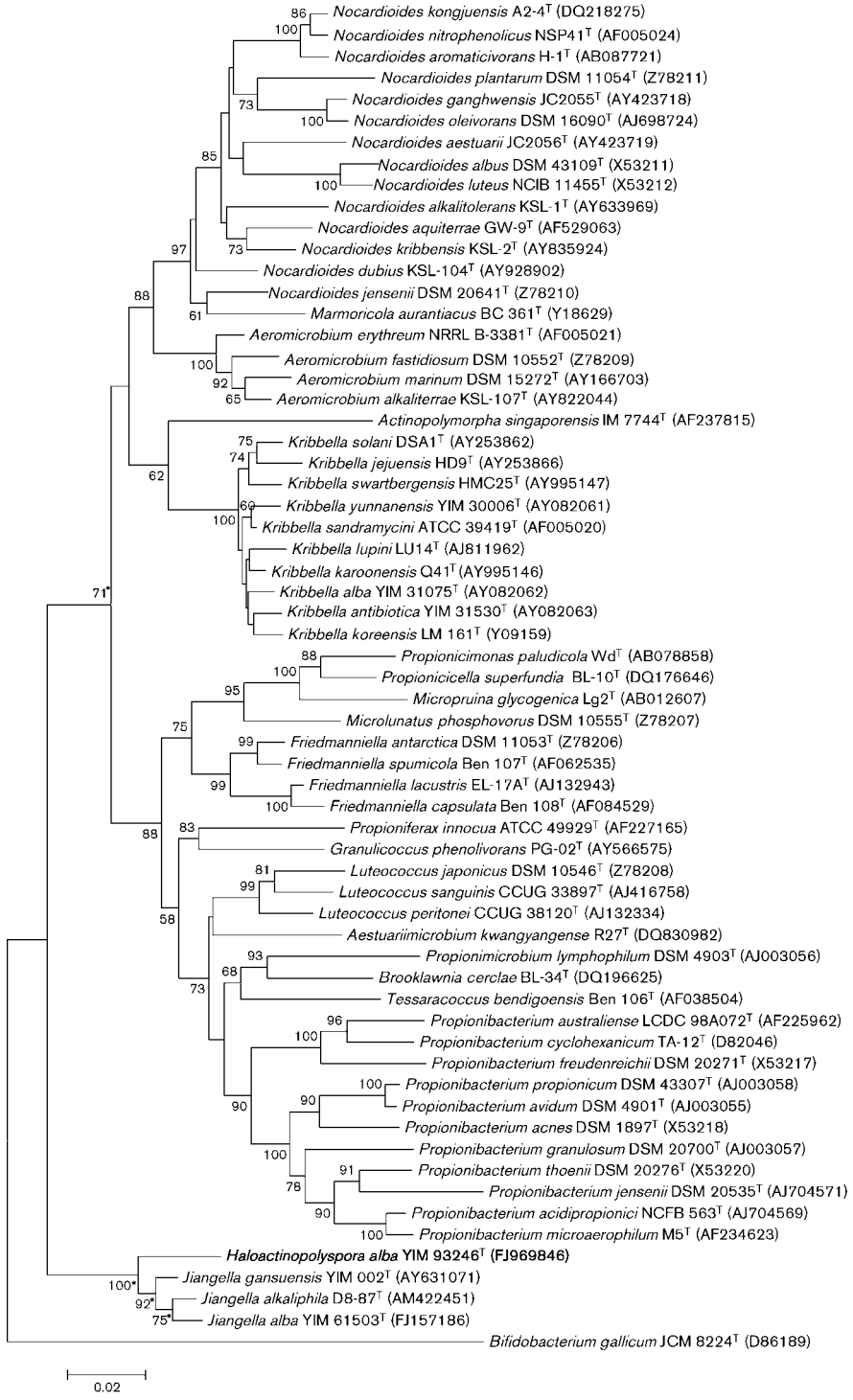

Fig. 2. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences (1471 bp length), showing the position of strain YIM 93246^T and members of the suborder *Propionibacterineae*. Asterisks indicate branches of the tree that were also found using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Numbers on branch nodes are bootstrap values (1000 resamplings; only values over 50 % are given). The sequence of *Bifidobacterium gallicum* JCM 8224^T (D86189) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

840 : 846 (A–U), 950 : 1231 (G–C), 952 : 1229 (G–C), 955 : 1225 (G–U), 986 : 1219 (U–G) and 987 : 1218 (C–G).

Higher hierarchical taxa in the class *Actinobacteria* should be mainly based on phylogenetic criteria (Stackebrandt

Table 1. Differential phenotypic characteristics of the genus *Haloactinopolyspora* and related members of the genus *Jiangella*

Strains: 1, *Haloactinopolyspora alba* YIM 93246^T (this study); 2, *J. alba* (Qin *et al.*, 2009); 3, *J. alkaliphila* (Lee, 2008); 4, *J. gansuensis* (Song *et al.*, 2005; R. M. Kroppenstedt, unpublished data). Symbols and abbreviations: +, positive; −, negative; NT, not tested; Ara, arabinose; Gal, galactose; Glc, glucose; GlcN, glucosamine; Man, mannose; Rha, rhamnose, Rib, ribose; DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PG, phosphatidylglycerol; PGL, unknown phosphoglycolipid; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, unknown phospholipid.

Characteristic	1	2	3	4
Fragmentation of aerial mycelium	−	+	+	−
Spore chain	Long	−	−	−
Spores in aggregates	+ (pseudosporangium-like)	−	−	−
Growth in:				
0 % NaCl	−	+	+	+
20 % NaCl	+	−	−	−
Nitrate reduction	−	+	−	−
Gelatin liquefaction	−	+	+	+
Decomposition of:				
Adenine	+	+	NT	NT
Casein	−	+	+	NT
Hypoxanthine	+	+	+	−
Xanthine	−	+	−	−
Urea	−	−	−	+
Utilization of:				
Cellobiose	+	−	+	+
Citrate	−	−	−	+
myo-Erythritol	−	+	−	−
D-Galactose	−	−	−	+
myo-Inositol	+	−	−	+
Lactose	+	−	+	+
D-Mannitol	−	+	+	−
Raffinose	−	+	−	−
D-Ribose	−	+	−	−
D-Sorbitol	+	−	−	+
D-Xylitol	−	−	−	+
Cell-wall sugars	GlcN, Man, Glc, Ara, Gal, Rha, one unknown sugar	Glu, Rib, Gal	Glu, Rha, Rib, Man	Rib, Glu
Polar lipids	DPG, PGL, PL, PI, PIMs, GL	DPG, PG, PI, PIM, PGL, PLs	DPG, PG, PC, PI, PIM, PL	DPG, PG, PI, PIMs, PL
Major fatty acids (>10 %)	iso-$C_{16:0}$ (29.4 %), anteiso-$C_{15:0}$ (28.4 %), anteiso-$C_{17:0}$ (13.7 %)	anteiso-$C_{15:0}$ (26.1 %), iso-$C_{16:0}$ (20.6 %)	anteiso-$C_{15:0}$ (20.4 %), iso-$C_{16:0}$ (18.0 %)	anteiso-$C_{15:0}$ (35.9 %), anteiso-$C_{17:0}$ (15.8 %)
DNA G+C (mol%)	70.5	71.9	71.5	70

et al., 1997; Zhi *et al.*, 2009). Accordingly, the genus *Jiangella* and strain YIM 93246^T are closely related phylogenetically and are clearly distinct from other suborders in the order *Actinomycetales*. Thus, a novel family *Jiangellaceae* fam. nov. and a novel suborder *Jiangellineae* subord. nov. are proposed to accommodate the genera *Jiangella* and *Haloactinopolyspora*.

Description of *Jiangellineae* subord. nov.

Jiangellineae (Ji.ang.el.li′ne.ae. N.L. fem. n. *Jiangella* type genus of the suborder; *-ineae* ending to denote a suborder; N.L. fem. pl. n. *Jiangellineae* the suborder of the genus *Jiangella*).

The pattern of 16S rRNA consists of nucleotides at positions 127 : 234 (G–C), 598 : 640 (C–G), 672 : 734 (G–C), 831 : 855 (U–A), 833 : 853 (G–C), 840 : 846 (A–U), 950 : 1231 (G–C), 952 : 1229 (G–C), 955 : 1225 (G–U), 986 : 1219 (U–G) and 987 : 1218 (C–G). The suborder contains the family *Jiangellaceae*. The nomenclatural type is the genus *Jiangella* Song *et al.* 2005.

Description of *Jiangellaceae* fam. nov.

Jiangellaceae (Ji.ang.el.la.ce′ae. N.L. fem. n. *Jiangella* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Jiangellaceae* the family of the genus *Jiangella*).

The family contains the type genus *Jiangella*, as well as the genus *Haloactinopolyspora*. The 16S rRNA nucleotide signature is as that of the suborder.

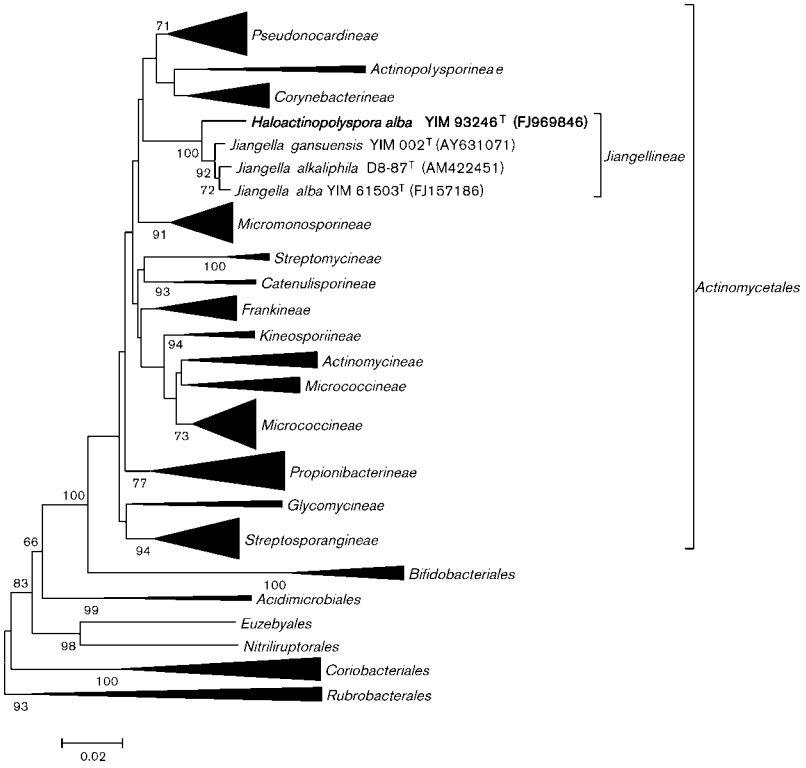

Fig. 3. Phylogenetic position of strain YIM 93246^T and members of the genus *Jiangella* within the class *Actinobacteria* based on 16S rRNA gene sequence analysis (1471 bp). Tree topology and evolutionary distances were calculated by the neighbour-joining method (Saitou & Nei, 1987). Numbers at nodes are bootstrap percentages for the clade of each group based on 1000 replications; only values above 50 % are shown. Bar, 0.02 substitutions per nucleotide position.

Description of *Haloactinopolyspora* gen. nov.

Haloactinopolyspora (Ha.lo.ac.ti.no.po.ly.spo′ra. Gr. n. *hals haloes*, salt; Gr. n. *actis actinos*, a ray; Gr. adj. *poly*, many; Gr. n. *spora*, a seed and, in biology, a spore; N.L. fem. n. *Haloactinopolyspora,* salt-loving and the many-spored ray).

Gram-positive, strictly aerobic, moderately halophilic filamentous actinomycete. The substrate mycelium fragments into rod-like elements, and the aerial mycelium has long spore chains and forms pseudosporangium-like, rhiziform spore aggregates at maturity. The whole-cell hydrolysates contain LL-DAP, alanine, glycine and glutamic acid as the cell-wall amino acids; glucosamine, glucose, galactose, mannose and arabinose are the major cell-wall sugars. The polar lipids are diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, one unknown phosphoglycolipid, one unknown phospholipid and one unknown glycolipid. The predominant menaquinone is MK-9 (H_4). The major fatty acids are iso-$C_{16:0}$, anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$. The G+C content of the DNA is about 70–71 mol%. The type species is *Haloactinopolyspora alba.*

Description of *Haloactinopolyspora alba* sp. nov.

Haloactinopolyspora alba (al′ba. L. fem. adj. *alba* white).

Displays the following properties in addition to those described for the genus. Growth occurs at 15–45 °C, pH 4.0–9.0 and 7–23 % (w/v) NaCl. Optimal growth occurs at 28–37 °C, pH 7.0–8.0 and 10–15 % (w/v) NaCl, and no growth occurs in the absence of NaCl. Aesculin, Tweens 40, 60 and 80 are degraded, but casein, starch, dextrin, chitin, Tween 20 and urea are not. Tests for milk peptonization and coagulation are positive, but gelatin liquefaction, nitrate reduction, starch hydrolysis, and H_2S and melanin production are negative. Cellobiose, dulcitol, D-fructose, inositol, lactose, maltose, D-mannose, rhamnose, sucrose, sorbitol and trehalose are utilized as sole carbon sources, while erythritol, galactose, D-glucose, glycerol, glycine, mannitol, raffinose, D-ribose, sodium propionate, trisodium citrate, xylitol and D-xylose are not. Adenine, L-arginine, L-histidine, hypoxanthine, L-lysine, L-methionine, L-proline, L-serine and L-threonine are utilized as sole nitrogen sources, whereas growth on L-alanine, D-arabinose, L-asparagine, L-phenylalanine, L-tyrosine and xanthine is not observed. In the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), α- and β-galactosidase, α- and β-glucosidase, α-mannosidase and *N*-acetyl-β-glucosaminidase are positive; acid phosphatase, α-chymotrypsin, cystine arylamidase, α-fucosidase, β-glucuronidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase are negative.

The type strain is YIM 93246^T (=DSM 45211^T=KCTC 19409^T), isolated from a salt lake in Xinjiang Province, north-west China.

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