

## *Jiangella mangrovi* sp. nov., isolated from mangrove soil

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An aerobic, Gram-stain-positive actinomycete, designated strain 3SM4-07<sup>T</sup>, was characterized using a polyphasic taxonomic approach. The strain produced branching mycelium which fragmented into short or elongated rods. The whole-cell hydrolysates contained LL-2,6-diaminopimelic acid as the diagnostic diamino acid, with glucose and ribose as the main sugars. The predominant cellular fatty acids were anteiso-C<sub>15</sub>:<sub>0</sub>, iso-C<sub>15</sub>:<sub>0</sub> and iso-C<sub>16</sub>:<sub>0</sub>. The predominant menaquinone was MK-9(H<sub>4</sub>). Phospholipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and phosphatidylinositol mannoside. Mycolic acids were absent. The DNA G + C content was 72.3 mol%. Strain 3SM4-07<sup>T</sup> formed a phylogenetic line within the genus *Jiangella* and its 16S rRNA gene sequence was related most closely to *Jiangella alkaliphila* D8-87<sup>T</sup> (99.0 % similarity), *Jiangella muralis* 15-Je-017<sup>T</sup> (98.8 %), *Jiangella alba* YIM 61503<sup>T</sup> (98.6 %) and *Jiangella gansuensis* YIM 002<sup>T</sup> (98.6 %). However, mean DNA–DNA hybridization values revealed that strain 3SM4-07<sup>T</sup> differed from the closest species previously described in this genus. Data from phenotypic, chemotaxonomic and molecular analyses between strain 3SM4-07<sup>T</sup> and recognized species of the genus *Jiangella* indicate that strain 3SM4-07<sup>T</sup> is a representative of a novel species of the genus *Jiangella*, for which the name *Jiangella mangrovi* sp. nov. is proposed. The type strain is 3SM4-07<sup>T</sup> (=BCC 60398<sup>T</sup>=NBRC 109648<sup>T</sup>).

The genus *Jiangella* accommodates Gram-stain-positive, non-spore-forming filamentous actinomycetes with LL-2,6-diaminopimelic acid (DAP) in the cell-wall peptidoglycan and MK-9(H<sub>4</sub>) as the main menaquinone but that lack mycolic acids (Tang *et al.*, 2012). The genus was first proposed by Song *et al.* (2005) with *Jiangella gansuensis* as the type species. At the time of writing, the genus encompassed four recognized species, *Jiangella alba* (Qin *et al.*, 2009), *Jiangella alkaliphila* (Lee, 2008), *J. gansuensis* (Song *et al.*, 2005) and *Jiangella muralis* (Kämpfer *et al.*, 2011), which

were isolated from the stem of *Maytenus austroyunnanensis*, cave soil, desert soil and cellar wall material of a house, respectively. Representatives of these species form a separate phyletic line within the evolutionary radiation encompassed by the family *Jiangellaceae*, and the genus *Haloactinopolyspora*.

An actinomycete strain, 3SM4-07<sup>T</sup>, was isolated from mangrove soil by the dilution plate technique during a study of microbial diversity in the mangrove forests of Laemson National Park, Ranong Province, Thailand (09° 21' 53.6" N 98° 24' 26.7" E). A 10-fold dilution of mangrove soil suspension was spread onto 10-fold-diluted marine agar medium (Matsumoto *et al.*, 2011) supplemented with nystatin (50 µg ml<sup>-1</sup>) and nalidixic acid (25 µg ml<sup>-1</sup>). After incubation of the plates at 28 °C for 1 month, the strain was transferred and purified on glucose yeast extract

Abbreviation: DAP, ll-2,6-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 3SM4-07<sup>T</sup> is AB976540.

Two supplementary figures are available with the online Supplementary Material.

(GYE) agar (Cross *et al.*, 1968). The pure culture of strain 3SM4-07<sup>T</sup> was maintained on nutrient agar (NA) for further studies and as mycelia fragments in glycerol solution (20 %, v/v) at -20 °C for long-term preservation. The type strains of species of the genus *Jiangella*, *J. alkaliphila* JCM 15620<sup>T</sup>, *J. alba* CCTCC AA 208023<sup>T</sup>, *J. muralis* JCM 17970<sup>T</sup> and *J. gansuensis* CCTCC AA 204001<sup>T</sup>, were used for comparison in morphological, physiological, chemotaxonomic and molecular studies. The cultural and growth characteristics of strain 3SM4-07<sup>T</sup> were determined on International Streptomyces Project (ISP; Shirling & Gottlieb, 1966) media 2, 3, 4, 5 and 7, and on NA. The standard names of the 267 Colour Centroids of the NBS/ISCC Colour System (Kelly & Judd, 1976) were used for colour description. Mycelium formation was observed under scanning electron microscopy (Quanta 450 FEI) after culturing the strain on NA for 14 days at 28 °C.

The phenotypic characteristics of strain 3SM4-07<sup>T</sup> were determined according to Shirling & Gottlieb (1966). The temperature range for growth was determined on ISP 2 agar using a temperature gradient incubator (Tokyo Kagaku Sangyo) with low and high temperatures of 10 and 45 °C, respectively. Optimal conditions for growth such as pH (4.0–10.0 at intervals of 1.0 pH unit using biological buffers) and NaCl concentrations (0–9 % at intervals of 1 %, w/v) were examined on ISP 2 agar after incubation for 14 days at 28 °C. Acid production from carbohydrates was studied using the method of Gordon *et al.* (1974). The decomposition of (w/v) arbutin (0.1 %), casein (skimmed milk, 5 %), cellulose (1.0 %), aesculin (0.1 %), gelatin (0.4 %), hypoxanthine (0.4 %), starch (1.5 %), Tweens 20 and 80, tyrosine (0.4 %), urea (1.8 %), xanthine (0.4 %) and xylan (0.4 %) was determined using standard procedures (Gordon & Mihm, 1957; Gordon *et al.*, 1974). Catalase and oxidase tests were performed using 3 % (v/v) hydrogen peroxide and 1 % (w/v) tetramethyl-*p*-phenylenediamine dihydrochloride solution, respectively. Enzyme activities were determined using the API ZYM test kit (bioMérieux) according to the manufacturer's instructions. Nitrate reduction, H<sub>2</sub>S production and lysozyme tolerance (0.005 %) were also studied following standard methods (Gordon *et al.*, 1974).

The freeze-dried cells used for chemotaxonomic analyses were prepared from culture grown in nutrient broth (NB) on a rotary shaker at 28 °C for 7 days. Cell hydrolysates for diamino pimelic acid analysis were prepared and then analysed by TLC according to the methods of Becker *et al.* (1965) and Hasegawa *et al.* (1983). Analyses of fatty acids, DNA G + C content and respiratory quinones were carried out by the Identification Service of the DSMZ, Braunschweig, Germany. Fatty acids were extracted as described by Miller (1982) and Kuykendall *et al.* (1988), then analysed by GC (Kämpfer & Kroppenstedt, 1996) and identified with reference to the TSBA6 database. The DNA G + C content was analysed by HPLC according to

an adaptation of the method of Tamaoka & Komagata (1984), and the method of Mesbah *et al.* (1989) was used for calculation. Menaquinones were extracted and purified as described by Tindall (1990a, b), then analysed by HPLC. Sugar compositions in whole-cell hydrolysates were determined using the method of Stanek & Roberts (1974). Analysis of phospholipids was carried out by two-dimensional TLC according to standard procedures (Minnikin *et al.*, 1984). Mycolic acids were examined using the TLC method of Tomiyasu (1982).

Genomic DNA extraction was performed as described by Kieser *et al.* (2000). PCR amplification of the 16S rRNA gene was carried out using primers 1F (5'-TCACGGAGAGTTTGATCCTG-3') and 1530R (5'-AAGGAGATCCAGCCGCA-3') (Kataoka *et al.*, 1997) under the conditions as described by Mingma *et al.* (2014). Sequencing of the 16S rRNA gene was performed using the service of Macrogen (Korea) with primers 1F, 1530R, Mg4F (5'-AATTCCTGGTGTAGCGGT-3') and 782R (5'-ACCAGGTATCTAATCCTGT-3').

The nearly complete 16S rRNA gene sequence (1477 nt) of strain 3SM4-07<sup>T</sup> was compared with sequences of other type strains in the EzTaxon-e server (<http://www.ezbiocloud.net/eztaxon>; Kim *et al.*, 2012). Phylogenetic analysis was performed using the maximum-parsimony (Fitch, 1971), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms in MEGA version 5.2 (Tamura *et al.*, 2011). The Jukes & Cantor (1969) model was used for generating an evolutionary distance matrix of the neighbour-joining data. The stability of the tree formation was evaluated by bootstrap analysis. Levels of DNA–DNA relatedness were measured on nylon membranes according to the method described by Wang *et al.* (2011).

Strain 3SM4-07<sup>T</sup> exhibited good growth on ISP 2 and 3 and NA but poor growth on ISP 4, 5 and 7. The colour of aerial mycelium was white on ISP 3 and NA, but yellowish white on ISP 2, 4, 5 and 7. Substrate mycelium was yellowish white on all tested media. Soluble pigment was not produced on any of the media tested. Morphological observations by light microscopy and scanning electron microscopy of a 14-day-old culture grown on NA revealed the fragmented or elongated rods of substrate and aerial mycelia (Fig. S1, available in the online Supplementary Material). Growth was observed between 20 and 37 °C, with an optimum at 28–32 °C, and also growth in the range pH 6–8, with an optimum at pH 7–8. Strain 3SM4-07<sup>T</sup> was positive for catalase and oxidase. Melanin pigment was not produced. The strain could tolerate up to 6 % NaCl and also tolerate 0.005 % (w/v) lysozyme. Comparative phenotypic characteristics between strain 3SM4-07<sup>T</sup> and representative species of the genus *Jiangella* are given in Table 1. Acid production from L-arabinose, cellobiose, D-galactose, lactose and D-xylose was found in strain 3SM4-07<sup>T</sup> but was weakly positive or absent in the other type strains. Strain 3SM4-07<sup>T</sup> did not produce acid from D-mannitol or D-ribose in contrast

**Table 1.** Differential characteristics between strain 3SM4-07<sup>T</sup> and the type strains of related species of the genus *Jiangella*

Strains: 1, 3SM4-07<sup>T</sup>; 2, *J. alkaliphila* JCM 15620<sup>T</sup>; 3, *J. muralis* JCM 17970<sup>T</sup>; 4, *J. alba* CCTCC AA 208023<sup>T</sup>; 5, *J. gansuensis* CCTCC AA 204001<sup>T</sup>. +, Positive; w, weakly positive; (, negative. All data were derived from the present study unless otherwise indicated.

Characteristic	1	2	3	4	5
Substrate mycelium colour on:					
ISP medium 2	Yellowish white	Vivid yellow	Yellowish white	Yellowish white	Vivid yellow
NA	Yellowish white	Yellowish white	Yellowish white	Yellowish white	Vivid yellow
Acid production from:					
Adonitol	w	–	w	–	–
L-Arabinose	+	w	w	–	–
Cellobiose	+	w	w	w	–
D-Galactose	+	–	–	–	–
D-Glucose	+	w	+	w	w
D-Lactose	+	–	–	–	–
D-Mannitol	–	+	–	–	–
L-Rhamnose	+	+	w	–	+
D-Ribose	–	+	+	+	–
D-Xylose	+	–	w	w	–
Sucrose	w	w	+	w	–
Degradation of:					
Casein	+	+	w	+	+
Starch	+	+	+	+	w
Tween 20	–	–	–	+	+
Growth with 7% NaCl	–	–	+	+	–
Nitrate reduction	–	–	–	+	–
H <sub>2</sub> S production	+	+	–	–	–
Isolation source	Mangrove soil	Cave soil*	Cellar wall*	Plant stem*	Desert soil*

\*Data from Song *et al.* (2005), Lee (2008), Qin *et al.* (2009) and Kämpfer *et al.* (2011).

to *J. alkaliphila*. Tween 20 was degraded by *J. alba* and *J. gansuensis*, but not by strain 3SM4-07<sup>T</sup>. Moreover, H<sub>2</sub>S was produced by strain 3SM4-07<sup>T</sup> and *J. alkaliphila* but not by the other type strains.

DAP was found in strain 3SM4-07<sup>T</sup>, indicating wall chemotype I *sensu* Lechevalier & Lechevalier (1970). MK-9(H<sub>4</sub>) was the main menaquinone, and small amounts of MK-9(H<sub>2</sub>) were also detected. Fatty acids present were anteiso-C<sub>15:0</sub> (28.4%), iso-C<sub>15:0</sub> (13.2%), iso-C<sub>16:0</sub> (9.5%), anteiso-C<sub>17:0</sub> (7.8%), C<sub>17:0</sub> (6.7%), C<sub>17:1ω8c</sub> (5.7%), iso-C<sub>14:0</sub> (5.2%), iso-C<sub>17:0</sub> (5.0%), iso-C<sub>15:0</sub> 3-OH (4.0%), C<sub>15:0</sub> 2-OH (2.3%), iso-C<sub>16:1</sub> (2.2%), C<sub>18:0</sub> (1.1%), anteiso-C<sub>15:1</sub> (1.0%) and C<sub>18:1ω9c</sub> (0.9%). The sugar composition of cell-wall hydrolysates showed large amounts of glucose and ribose. Traces of mannose and rhamnose were also detected. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidyl-inositol, phosphatidylinositol mannoside and an unidentified phospholipid were present in strain 3SM4-07<sup>T</sup> (Fig. S2). Mycolic acids were not found. The DNA G + C content of strain 3SM4-07<sup>T</sup> was 72.3 mol%.

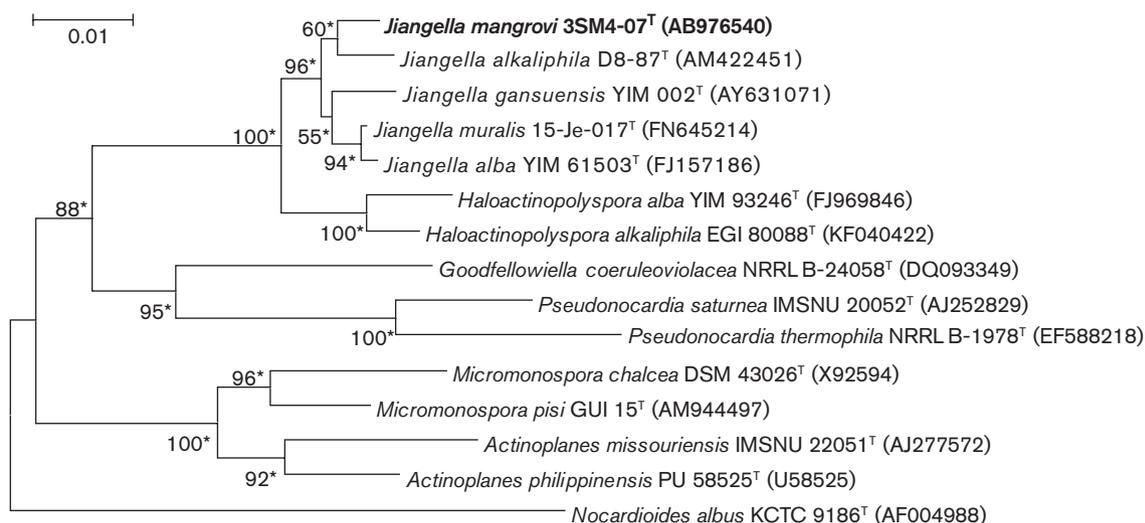
The almost-complete 16S rRNA gene sequence (1477 nt) of strain 3SM4-07<sup>T</sup> was determined, and phylogenetic analyses indicated that it should be assigned to the genus *Jiangella*. Its closest relatives were *J. alkaliphila* D8-87<sup>T</sup> (99.0%

16S rRNA gene sequence similarity), *J. muralis* 15-Je-017<sup>T</sup> (98.8%), *J. alba* YIM 61503<sup>T</sup> (98.6%) and *J. gansuensis* YIM 002<sup>T</sup> (98.6%), as shown in Fig. 1. Mean DNA–DNA hybridization values of 49.2 ± 0.3, 44.8 ± 1.5, 33 ± 4 and 31.5 ± 2.6% were observed between strain 3SM4-07<sup>T</sup> and the type strains of *J. alba*, *J. muralis*, *J. gansuensis* and *J. alkaliphila*, respectively. These values were well below the 70% cut-off value recommended for the assignment of bacterial strains to the same genomic species (Wayne *et al.*, 1987). On the basis of the phenotypic and genotypic characteristics presented above, it is evident that strain 3SM4-07<sup>T</sup> can be differentiated from previously described reference strains of species of the genus *Jiangella*. Strain 3SM4-07<sup>T</sup> should be classified as a representative of a novel species in the genus *Jiangella*, under the name *Jiangella mangrovi* sp. nov.

### Description of *Jiangella mangrovi* sp. nov

*Jiangella mangrovi* (man.gro'vi. N.L. n. *mangrovum* mangrove; N.L. gen. n. *mangrovi* of a mangrove).

Aerobic, Gram-stain-positive actinomycete that forms mycelia that fragment into short or elongated rods. Substrate mycelium is yellowish white on ISP media 2, 3, 4, 5 and 7, and on NA. Shows good growth on ISP 2, ISP 3 and NA



**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing relationships between strain 3SM4-07<sup>T</sup> and its closest phylogenetic relatives. Asterisks indicate that the corresponding branches were also recovered in both maximum-likelihood (Felsenstein, 1981), and maximum-parsimony (Fitch, 1971) phylogenetic trees. Bootstrap percentages at the nodes were based on 1000 resamplings. The sequence of *Nocardioides albus* KCTC 9186<sup>T</sup> was used as an outgroup. Bar, 0.01 nt substitutions per nucleotide position.

with an optimum temperature of 28–32 °C and optimum pH of 7–8. Catalase, oxidase and H<sub>2</sub>S production are positive, but nitrate reduction and melanin production are negative. Acid is produced from L-arabinose, cellobiose, D-galactose, D-glucose, lactose, L-rhamnose and D-xylose; acid production from adonitol, D-fructose, maltose and sucrose is weak. No acid is produced from myo-inositol, D-mannitol, melibiose, raffinose, D-ribose, D-sorbitol and xylitol. Esterase (C4), leucine arylamidase, valine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase, N-acetyl- $\beta$ -glucosaminidase and  $\alpha$ -mannosidase are detected with the API ZYM enzyme assay but no activities of alkaline phosphatase, esterase lipase (C8), lipase (C14), cystine arylamidase,  $\alpha$ -chymotrypsin, acid phosphatase,  $\beta$ -glucuronidase or  $\alpha$ -fucosidase. Hydrolysis abilities for aesculin, arbutin, casein, gelatin, Tween 80 and starch are detected, but not for cellulose, hypoxanthine, Tween 20, tyrosine, urea, xanthine or xylan. The maximum NaCl concentration for growth is 6 % and is also able to tolerate 0.005 % lysozyme. Whole-cell hydrolysates contain DAP as the cell-wall peptidoglycan. Large amounts of glucose and ribose are found as whole-cell sugars. Mycolic acids are absent. The predominant menaquinone is MK-9(H<sub>4</sub>). Phospholipids consist of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylinositol mannoside and an unidentified phospholipid. The major cellular fatty acids are anteiso-C<sub>15:0</sub>, iso-C<sub>15:0</sub> and iso-C<sub>16:0</sub>.

The type strain is 3SM4-07<sup>T</sup> (=BCC 60398<sup>T</sup>=NBRC 109648<sup>T</sup>), which was isolated from soil in the mangrove

forests of Laemson National Park, Ranong Province, Thailand. The DNA G+C content of the type strain is 72.3 mol%.

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