Jiangella gansuensis gen. nov., sp. nov., a novel actinomycete from a desert soil in north-west China

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A novel actinomycete strain, designated YIM 002^T, was isolated from a desert soil sample in Gansu Province, north-west China. This actinomycete isolate formed well-differentiated aerial and substrate mycelia. In the early stages of growth, the substrate mycelia fragmented into short or elongated rods. Chemotaxonomically, it contained LL-2,6-diaminopimelic acid in the cell wall. The cell-wall sugars contained ribose and glucose. Phospholipids present were phosphatidylinositol mannosides, phosphatidylinositol and diphosphatidylglycerol. MK-9(H_4) was the predominant menaquinone. The major fatty acids were anteiso $C_{15:0}$ (35·92 %), anteiso $C_{17:0}$ (15·84 %), iso $C_{15:0}$ (10·40 %), iso $C_{16:0}$ (7·07 %) and $C_{17:1}\omega8c$ (9·37 %). The G+C content of the DNA was 70 mol%. Phylogenetic analysis and signature nucleotide data based on 16S rRNA gene sequences showed that strain YIM 002^T is distinct from all recognized genera of the family *Nocardioidaceae* in the suborder *Propionibacterineae*. On the basis of the phenotypic and genotypic characteristics, it is proposed that isolate YIM 002^T be classified as a novel species in a new genus, *Jiangella gansuensis* gen. nov., sp. nov. The type strain is YIM 002^T (=DSM 44835^T=CCTCC AA 204001^T=KCTC 19044^T).

The family *Nocardioidaceae* was first proposed by Nesterenko *et al.* (1985) to accommodate two genera, *Nocardioides* Prauser 1976 and *Pimelobacter* Suzuki and Komagata 1983. Collins *et al.* (1989) transferred the *Pimelobacter* species to the genera *Terrabacter* and *Nocardioides*. Currently, the family *Nocardioidaceae* comprises five genera: *Nocardioides* (Prauser, 1976), *Aeromicrobium* (Miller *et al.*, 1991), *Kribbella* (Park *et al.*, 1999; Sohn *et al.*, 2003), *Marmoricola* (Urzì *et al.*, 2000) and *Actinopolymorpha* (Wang *et al.*, 2001). During a project to screen bioactive actinomycete strains from soils, one actinomycete strain, designated YIM 002^T, was isolated from a desert soil sample collected in Sunan county, Gansu Province, north-west China. It contained LL-2,6-diaminopimelic acid (LL-A_2pm) as the diagnostic amino acid in the peptidoglycan and formed well-differentiated aerial and substrate mycelia; the substrate mycelia had a tendency to fragment in the early stages of growth. In order to determine the taxonomic and phylogenetic position of this organism, we examined its morphological, physiological and biochemical characteristics and analysed its chemotaxonomic compositions and 16S rRNA gene sequence. The results indicated that strain YIM 002^T should be placed in a novel species of a new genus, for which the name *Jiangella gansuensis* gen. nov., sp. nov. is proposed.

Strain YIM 002^T was isolated from the soil sample by using the dilution plating method. The medium used for selective isolation was glycerol–asparagine agar (ISP5 medium; Shirling & Gottlieb, 1966), which was incubated at 28 °C for about 2 weeks. Following purification, the organism was maintained on yeast extract–malt extract agar (ISP2 medium; Shirling & Gottlieb, 1966) at 28 °C.

Strain YIM 002^T was grown on ISP2, ISP3, ISP4, ISP5, nutrient agar and Czapek's agar plates at 28 °C. Colour

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Abbreviation: LL-A_2pm, LL-2,6-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 002^T is AY631071.

Scanning electron micrographs of substrate and aerial mycelia of strain YIM 002^T are available as supplementary material in IJSEM Online.

determination was performed with colour chips from the ISCC-NBS Colour Charts Standard Samples no. 2106 (Kelly, 1964). Morphological characteristics were examined by light microscopy with a model BH-2 microscope (Olympus) and scanning electron microscopy with a JEOL model JSM5600LV. Morphological features were observed on ISP2 medium at 28 °C. Growth was tested over a range of temperatures (4–45 °C) and pH values (6·0–12·0). Strain YIM 002^T grew well on ISP2 agar but only slowly on the other media tested. Substrate mycelium of YIM 002^T fragmented into short or elongated rods; aerial mycelium developed well on ISP2 agar plates (see Supplementary Fig. A in IJSEM Online). The colour of the colonies was white on ISP3 medium and yellow–white on ISP2, ISP4 and ISP5 media, nutrient agar and Czapek's agar plates. No diffusible pigment was produced on any of the media tested. The optimal temperature and pH for growth were 28 °C and 7·0–8·0, respectively.

Physiological and biochemical characteristics of strain YIM 002^T are given in the genus and species descriptions below. All tests were performed at 28 °C and properties were recorded after 7, 14, 20 and 30 days, except for the nitrate reduction test, which was recorded after 1, 3 and 5 days. Carbon source utilization and sugar fermentation tests were carried out according to the method of Kämpfer *et al.* (1991).

Procedures for identification of cell-wall amino acids and sugars followed those described by Stanek & Roberts (1974). Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures of Minnikin *et al.* (1984). Menaquinones were isolated using the methods of Minnikin *et al.* (1984) and separated by HPLC (Kroppenstedt, 1982). Cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MIDI, Inc.). Amino acids in the peptidoglycan layer of strain YIM 002^T were LL-A_2pm, alanine, glycine and glutamic acid, indicating a type I wall chemotype according to the classification of Lechevalier & Lechevalier (1970). Cell-wall sugars were glucose and ribose. The predominant menaquinone was MK-9(H_4). Phospholipids of strain YIM 002^T present were phosphatidylinositol mannosides, phosphatidylinositol and diphosphatidylglycerol. The cellular fatty acid composition of strain YIM 002^T is given in the genus and species descriptions.

Chromosomal DNA from strain YIM 002^T was prepared following the method of Marmur (1961). The G+C content of the DNA was determined using the thermal denaturation method of Marmur & Doty (1962); a value of 70 mol% was measured.

Amplification of the 16S rRNA gene sequence was performed as described by Cui *et al.* (2001). Database searching was carried out using the BLAST program. The 16S rRNA gene sequence of strain YIM 002^T and previously published sequences of reference actinomycetes were aligned using the CLUSTAL X program (Thompson *et al.*, 1997). A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980, 1983). The reliability of the phylogenetic tree was evaluated using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The almost complete 16S rRNA gene sequence (1497 nt) of strain YIM 002^T was obtained (*Escherichia coli* numbering 29–1542), and BLAST search comparisons were made against the GenBank/EMBL/DDBJ databases. A sequence of 1466 nt was compared after elimination of all sites for which nucleotides were not determined in any sequences. The phylogenetic tree shown in Fig. 1 includes sequences of representative members of the family *Nocardioidaceae*.

Phylogenetic analysis of the 16S rRNA gene sequence revealed that the isolate fell within the cluster of the family *Nocardioidaceae* and represented a line of descent distinct from recognized actinomycetes of this family (Fig. 1). Strain YIM 002^T formed a monophyletic clade with *Actinopolymorpha singaporensis* at a low nucleotide sequence similarity (92·3 %). The relationship was confirmed in all three tree-making analyses [least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) (data not shown) as well as neighbour-joining (Fig. 1)] and by the high bootstrap value (>80 %), based on the neighbour-joining algorithm. The next closest neighbours were *Kribbella* species, sharing only 91·6–92·3 % 16S rRNA gene sequence similarities. Strain YIM 002^T had no more than 92 % sequence similarity to all other taxa within the family *Nocardioidaceae*.

Additionally, there are significant differences in morphology and chemotaxonomic characteristics between strain YIM 002^T and related genera of the family *Nocardioidaceae*

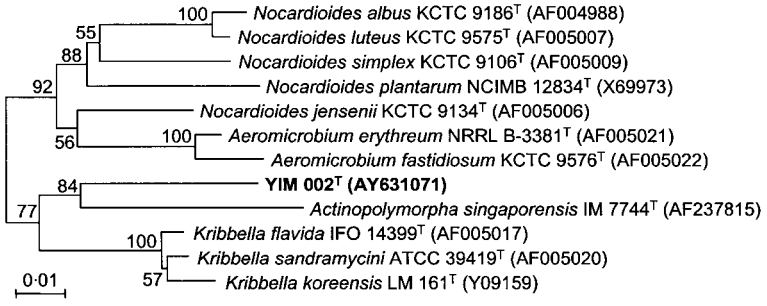

Fig. 1. A neighbour-joining phylogenetic tree of representatives from all recognized genera within the family *Nocardioidaceae*. Numbers at nodes are bootstrap percentages based on 1000 resamplings. *Streptosporangium roseum* DSM 43021^T (X89947) was used as outgroup (not shown). Bar, 1 % sequence divergence.

Table 1. Differential characteristics of *Jiangella gansuensis* gen. nov., sp. nov. YIM 002^T and related taxa in the family *Nocardioidaceae*

Data for reference taxa were taken from Prauser (1976), O'Donnell *et al.* (1982), Collins *et al.* (1989), Miller *et al.* (1991), Urzì *et al.* (2000); Park *et al.* (1999), Wang *et al.* (2001) and Sohn *et al.* (2003). ND, Not determined.

Characteristic	*Jiangella* gen. nov.	*Actinopolymorpha*	*Kribbella*	*Nocardioides*	*Aeromicrobium*	*Marmoricola*
Cell morphology	Hyphae, rods	Pleomorphism to hyphae	Hyphae	Hyphae, rods, cocci	Rods	Cocci
Major menaquinone	MK-9(H_4)	MK-9(H_6)	MK-9(H_4)	MK-8(H_4)	MK-9(H_4)	MK-8(H_4)
Phospholipids*	PI, PIM, DPG	PI, PIM, DPG, PG	DPG, PC, PG, PI	PG, DPG, PL, PG-OH	PE, PG	PI, DPG, PG
Major fatty acids	anteiso $C_{15:0}$, anteiso $C_{17:0}$, iso $C_{15:0}$	ND	anteiso $C_{15:0}$, iso $C_{16:0}$	10-Me-$C_{18:0}$, iso $C_{16:0}$, $C_{18:1}$	10-Me-$C_{18:0}$, $C_{16:0}$, $C_{18:1}$	anteiso $C_{15:0}$, iso $C_{16:0}$
G+C content (mol%)	70	69·5	68–71	66·5–71·7	71–73	72

*DPG, Diphosphatidylglycerol; GL, unknown glycolipid(s); PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PG-OH, phosphatidylglycerol containing 2-hydroxy fatty acids; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PL, unknown phospholipid(s).

(Table 1). We also compared the nucleotide signatures of strain YIM 002^T against those specific to the family *Nocardioidaceae* (Stackebrandt *et al.*, 1997). The results indicated the presence of most of the specific nucleotide signatures in the sequence of strain YIM 002^T, except for variations at some positions (see under the genus description below).

On the basis of phenotypic and genotypic data, strain YIM 002^T is distinguishable from all recognized genera of the family *Nocardioidaceae*. We consider that the polyphasic evidence presented here is sufficient to propose the classification of strain YIM 002^T within a new genus, *Jiangella* gen. nov., as *Jiangella gansuensis* sp. nov.

Description of *Jiangella* gen. nov.

Jiangella [Ji.ang.el′la. N.L. fem. dim. n. *Jiangella* after Cheng-Lin Jiang (1942–), a Chinese microbiologist, in recognition of his work on actinomycete taxonomy].

Strictly aerobic and Gram-positive. Substrate mycelium fragments into short or elongated rods in the early stages of growth. Aerial mycelium differentiates well and no spores are formed. Grow very well on ISP2 medium. Cell wall contains LL-A_2pm as the diamino acid in the peptidoglycan. MK-9(H_4) is the predominant menaquinone; cell-wall sugars are glucose and ribose. Phosphatidylinositol mannosides, phosphatidylinositol and diphosphatidylglycerol are present. Major cellular fatty acids are anteiso $C_{15:0}$, anteiso $C_{17:0}$, iso $C_{15:0}$, iso $C_{16:0}$ and $C_{17:1}\omega8c$. DNA G+C content is 70 mol%. Phylogenetically, the genus is placed in the family *Nocardioidaceae*. All family-specific nucleotide signatures (Stackebrandt *et al.*, 1997) are present except for a C : G pair at nucleotide positions 370 : 391 instead of G : C, a C : G pair at positions 602 : 636 instead of G : U and a U : U pair at positions 658 : 748 instead of U : A. The type species is *Jiangella gansuensis*.

Description of *Jiangella gansuensis* sp. nov.

Jiangella gansuensis (gan.su.en′sis. N.L. fem. adj. *gansuensis* pertaining to Gansu, a province of north-west China from where the type strain was isolated).

Morphological, chemotaxonomic and general characteristics are as given above for the genus. Colonies are white on ISP3 and yellow–white on ISP2, ISP4 and ISP5 media and nutrient and Czapek's agar plates. No diffusible pigment is produced on these agar plates. Optimal temperature and pH for growth are 28 °C and 7·0–8·0, respectively. Utilizes glucose, fructose, xylose, rhamnose, mannitol, sucrose, inositol, galactose, mannose, mannitol, arabinose, xylitol, melibiose, maltose, lactose, raffinose, cellobiose, dextrin and glycerol as sole carbon sources, but not ribose. Positive for gelatin hydrolysis, urease, milk coagulation and peptonization, but negative for cellulose hydrolysis, production of H_2S, starch hydrolysis and nitrate reduction. Fatty acids present are anteiso $C_{15:0}$ (35·92 %), anteiso $C_{17:0}$ (15·84 %), iso $C_{15:0}$ (10·40 %), $C_{17:1}\omega8c$ (9·37 %), iso $C_{16:0}$ (7·07 %), $C_{17:0}$ (3·39 %), iso $C_{17:0}$ (3·09 %), iso G-$C_{16:1}$ (2·90 %), anteiso A $C_{17:1}$ (2·86 %), $C_{15:0}$ 2-OH (2·28 %), iso $C_{14:0}$ 2-OH (2·23 %), anteiso A $C_{15:1}$ (1·31 %), $C_{18:1}\omega9c$ (1·24 %), iso $C_{17:1}\omega9c$ (1·05 %) and $C_{15:0}$ (1·05 %). DNA G+C content is 70 mol%.

The type strain, YIM 002^T (=DSM 44835^T=CCTCC AA 204001^T=KCTC 19044^T), was isolated from a desert soil sample from Gansu Province, China.

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