Jiangella alkaliphila sp. nov., an actinobacterium isolated from a cave

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A mycelium-forming actinomycete, designated strain D8-87^T, was isolated from a soil collected from a cave in Jeju, Republic of Korea. Comparative 16S rRNA gene sequence analysis showed that the organism was closely related to the type and only species of the genus *Jiangella*. The level of 16S rRNA gene sequence similarity between strain D8-87^T and the type strain of *Jiangella gansuensis* was 99.2 %. The novel organism produced extensively branched aerial and substrate mycelia, which fragmented into short or elongated rod-shaped cells. Whole-cell hydrolysates of the isolate contained LL-diaminopimelic acid, glucose, mannose and arabinose. The predominant menaquinone was MK-9(H₄). The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylinositol mannoside and an unknown phospholipid. The predominant fatty acids were anteiso-C_{15:0}, iso-C_{16:0} and iso-C_{14:0}. The DNA G + C content of strain D8-87^T was 71.5 mol%. Levels of DNA–DNA relatedness between strain D8-87^T and *J. gansuensis* KCTC 19044^T were 23.4 and 34.6 %. On the basis of the phenotypic and DNA–DNA hybridization data presented, strain D8-87^T is considered to represent a novel species of the genus *Jiangella*, for which the name *Jiangella alkaliphila* is proposed. The type strain is D8-87^T (=JBRI 2008^T=KCTC 19222^T=DSM 45079^T).

The genus *Jiangella* was proposed by Song *et al.* (2005) to accommodate an actinomycete isolated from a desert soil in north-west China. At the time of writing, *Jiangella gansuensis* remains the type and only species of the genus. Chemotaxonomically, the genus is characterized by the presence of LL-2,6-diaminopimelic acid (LL-DAP) in the cell-wall peptidoglycan, MK-9(H₄) as the major menaquinone, anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{15:0}, iso-C_{16:0} and C_{17:1}ω8c as major fatty acids and a DNA G + C content of 70 mol%. 16S rRNA gene sequence analyses (Song *et al.*, 2005) indicated that the genus belongs to the family *Nocardioideae* Nesterenko *et al.* 1985 within the suborder *Propionibacterineae*.

During the course of studies on the diversity of cave bacteria, a mycelium-forming actinomycete, designated strain D8-87^T, was isolated from the soil of a natural cave in Jeju, Republic of Korea, and its taxonomic status was investigated by a polyphasic approach. Strain D8-87^T was isolated by a dilution plating method on starch-casein agar

as the selective medium. After the agar plates were incubated at 30 °C for 14 days, the purified organism was maintained as mycelial fragments or spores in 20 % (v/v) glycerol. For DNA–DNA hybridization experiments and phenotypic comparisons, *J. gansuensis* KCTC 19044^T was grown on ISP 2 medium (Shirling & Gottlieb, 1966).

The cultural characteristics of strain D8-87^T were examined by using ISP 2, ISP 3, ISP 4, trypticase soy agar (TSA; Difco) and nutrient agar (NA; Difco). Cell morphology was investigated by using field-emission scanning electron microscopy (model JSM 6700F; JEOL), with the cells grown on ISP 4 for 14 days at 28 °C. Specimens showing growth were fixed for 1 h with 1 % OsO₄ and dehydrated by using a graded series of ethanol or a mixture of ethanol and isoamyl acetate. After critical-point drying with liquid carbon dioxide, the sample was coated with gold. Strain D8-87^T grew well on ISP 2, ISP 3 and NA, but only moderately on ISP 4 and TSA. Aerial mycelium was abundantly produced on most media tested, but only poorly produced on ISP 3. The colour of aerial mycelium was white on ISP 2, NA and TSA, but light greyish-yellow–brown on ISP 4 and ISP 3. The reverse colour of the colony was brilliant orange–yellow on ISP 2, brownish black on ISP 4, deep yellowish brown on ISP 3, strong yellow on TSA and light brown on NA. No diffusible pigment was produced on any of the media tested. Both aerial and substrate mycelia fragmented into short or elongated rods (see Supplementary Fig. S1 in IJSEM Online).

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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain D8-87^T is AM422451.

A scanning electron micrograph of cells of strain D8-87^T and a neighbour-joining tree showing the phylogenetic relationships of strain D8-87^T within the radiation of the order *Actinomycetales* based on 16S rRNA gene sequences are available with the online version of this paper.

The pH for growth was tested on ISP 2 over a range of initial pH values of 4.1–12.1, pH was adjusted at intervals of 1.0 pH units with 6 M HCl or 10 M NaOH, prior to sterilization. For other physiological characterizations, all test media were adjusted to an initial pH of 9.1 prior to sterilization unless otherwise indicated. Growth was investigated over the temperature range 10–42 °C. NaCl tolerance for growth was studied on ISP 2 amended with 1–9 % (w/v) NaCl. The following tests were performed as described by Lee & Jeong (2006): Gram stain, catalase activity, nitrate reduction, H₂S production and degradation and utilization of carbohydrates. The results were recorded after incubating the plates for 14 days at 28 °C. Strain D8-87^T was mesophilic; it grew at 20–37 °C, but not at 10 or 42 °C. Strain D8-87^T showed growth at initial pH values of 6.1–10.1, with good growth at pH 8.1–10.1, but it did not grow at pH 11.1. *J. gansuensis* KCTC 19044^T also showed good growth at alkaline pH (8.1–10.1). Other physiological characteristics of strain D8-87^T are given in Table 1 and in the species description below.

Isolation of genomic DNA and amplification of the 16S rRNA gene by PCR were performed as described by Lee *et al.* (2000). The resultant PCR product was purified with the Wizard genomic DNA purification kit (Promega) and its sequence was determined by using an ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 3730xl; Applied Biosystems). The 16S rRNA gene sequence determined in the present study was aligned with corresponding sequences available from public databases by using the CLUSTAL_X program (Thompson *et al.*, 1997) and was then optimized manually. Phylogenetic analyses were performed by using the neighbour-joining method (Saitou & Nei, 1987). Evolutionary distances for the neighbour-joining method were computed according to the method of Jukes & Cantor (1969). The 16S rRNA gene sequence of strain D8-87^T (1408 nt; determined herein) was compared with corresponding sequences of representatives of the order *Actinomycetales*. In the neighbour-joining tree (see Supplementary Fig. S2 in IJSEM Online) based on 16S rRNA gene sequences, strain D8-87^T formed a tight clade with *J. gansuensis* YIM 002^T at a bootstrap value of 100 %. The relationship was also supported in trees based on the maximum-parsimony and maximum-likelihood algorithms. The level of 16S rRNA gene sequence similarity between strain D8-87^T and *J. gansuensis* YIM 002^T was 99.2 %. The genus *Jiangella* has been reported to be phylogenetically related to members of the family *Nocardioideaceae* (Song *et al.*, 2005), but, in the present study, its phylogenetic position was variable depending on the tree-making method applied. A *Jiangella* clade including the new isolate was related to members of the suborder *Micromonosporineae* when the neighbour-joining and maximum-likelihood methods were applied, whereas it was united with members of the suborders *Micromonosporineae* and *Catenulisporineae* in the maximum-parsimony tree (data not shown).

Table 1. Differential characteristics between strain D8-87^T and *Jiangella gansuensis* KCTC 19044^T

Both strains grew in the presence of up to 3 % NaCl, at temperatures of 20–37 °C and at pH 6.1–10.1, with good growth at pH 8.1–10.1. Both strains utilized D-arabinose, L-arabinose, cellobiose, dextran, D-fructose, D-glucose, inulin, D-lactose, maltose, D-mannose, L-rhamnose, sucrose, D-xylose, adonitol and glycerol as sole carbon and energy sources, but not methyl α -D-mannoside, raffinose, L-sorbose, 2,3-butanediol, D-dulcitol, *myo*-erythritol, 1,2-propanediol, benzoate, formate, malate, succinate or DL-tartrate. Both strains degraded casein, DNA and elastin, but not chitin, starch, DL-tyrosine or xanthine. Gelatin liquefaction was detected for both strains, but not nitrate reduction or H₂S production. *J. gansuensis* KCTC 19044^T showed conflicting results for utilization of raffinose, D-ribose and D-mannitol, as compared with those reported previously (Song *et al.*, 2005). +, Positive; –, negative.

Characteristic	Strain D8-87 ^T	<i>J. gansuensis</i> KCTC 19044 ^T
Utilization of:		
Acetate	–	+
Citrate	–	+
D-Galactose	–	+
<i>myo</i> -Inositol	–	+
D-Mannitol	+	–
Melezitose	–	+
Melibiose	–	+
Methyl α -D-glucoside	–	+
D-Ribose	–	+
Salicin	–	+
D-Sorbitol	–	+
Trehalose	–	+
D-Xylitol	–	+
Growth at:		
5 % NaCl	–	+
Degradation of:		
Urea	–	+
Hypoxanthine	+	–

The phylogenetic relationship of strain D8-87^T was also supported by results for most chemotaxonomic characteristics. Analyses of the diamino acid (Staneck & Roberts, 1974) and sugar composition (Saddler *et al.*, 1991) of cell-wall hydrolysates revealed that strain D8-87^T contained LL-DAP and large amounts of glucose (81 % of the total sugars), together with minor amounts of rhamnose, ribose and mannose. The major menaquinone was MK-9(H₄), as determined according to the method of Kroppenstedt (1985). The polar lipid profile comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylinositol mannoside and an unknown phospholipid, as determined by using two-dimensional TLC and staining with several compounds (Minnikin *et al.*, 1977). Phosphatidylglycerol and phosphatidylcholine were also detected in extracts of *J. gansuensis* KCTC 19044^T, in contrast to the results reported previously (Song *et al.*, 2005). Mycolic acids were not

present, as determined by the method of Minnikin *et al.* (1980). The DNA G+C content of strain D8-87^T was 71.5 mol%, as determined by HPLC (Mesbah *et al.*, 1989).

DNA–DNA hybridization experiments with strain D8-87^T and *J. gansuensis* KCTC 19044^T were carried out at the DSMZ (Germany). Genomic DNA was isolated by using a French pressure cell (Thermo Spectronic) and purified by hydroxyapatite chromatography as described by Cashion *et al.* (1977). DNA–DNA hybridization was performed as described by De Ley *et al.* (1970), with the modifications described by Huß *et al.* (1983), by using a Bio UV/VIS-spectrophotometer (Cary 100) and a temperature controller with an *in-situ* temperature probe (Varian). Experiments were performed in duplicate. Low levels of DNA–DNA relatedness (23.4 and 34.6 %, duplicate measurements with same probe) were obtained between strain D8-87^T and *J. gansuensis* KCTC 19044^T, suggesting that the novel strain represents a species distinct from *J. gansuensis* (Stackebrandt & Goebel, 1994).

Fatty acid analyses of strain D8-87^T and *J. gansuensis* KCTC 19044^T were performed by using cells grown on nutrient broth (Difco) (adjusted to pH 9.1 prior to sterilization) at 30 °C for 3 days, with shaking at 170 r.p.m. Fatty acid methyl esters were prepared and analysed by using the Sherlock Microbial Identification System (version 6.0; MIDI Inc.) according to the manufacturer's instructions. Strain D8-87^T and *J. gansuensis* KCTC 19044^T showed cellular fatty acid profiles that contained saturated, unsaturated and branched components, although that of strain D8-87^T additionally contained 3-hydroxy fatty acids as minor components. The predominant fatty acids of strain D8-87^T were anteiso-C_{15:0} (20.4 %), iso-C_{16:0} (18.0 %) and iso-C_{14:0} (8.9 %), whereas the predominant components of *J. gansuensis* KCTC 19044^T were anteiso-C_{15:0} (26.0 %), anteiso-C_{17:0} (14.0 %) and iso-C_{16:0} (9.6 %). Strain D8-87^T showed notable differences from *J. gansuensis* KCTC 19044^T in the proportions of iso-C_{14:0}, iso-C_{16:0}, anteiso-C_{17:0} and anteiso-C_{17:1}ω9c, and in the presence of iso-C_{14:0} 3-OH, iso-C_{15:0} 3-OH, iso-C_{16:0} 3-OH, C_{14:0}, C_{15:0} and iso-C_{17:1}A (Table 2). Other differential physiological properties between strain D8-87^T and *J. gansuensis* KCTC 19044^T are given in Table 1.

On the basis of the data presented, strain D8-87^T is considered to represent a novel species of the genus *Jiangella*, for which the name *Jiangella alkaliphila* sp. nov. is proposed.

Description of *Jiangella alkaliphila* sp. nov.

Jiangella alkaliphila (al.kal.i.phil'a. Arabic article *al* the; Arabic n. *qaliy* ashes of saltwort; N.L. n. *alkali* alkali; Gr. adj. *philos* loving; N.L. fem. adj. *alkaliphila* loving alkaline conditions).

Aerobic, Gram-positive, non-acid–alcohol-fast. Catalase-positive. Forms well-developed aerial and vegetative mycelia that fragment into short or elongated rods.

Table 2. Cellular fatty acid profiles of strain D8-87^T and *Jiangella gansuensis* KCTC 19044^T

Both strains were cultivated in nutrient broth (Difco) for 3 days at 30 °C. Culture broth was adjusted to pH 9.1 prior to sterilization. Components comprising <1 % of the total are not shown. —, Not found.

Fatty acid	Strain D8-87 ^T	<i>J. gansuensis</i> KCTC 19044 ^T
Saturated fatty acids:		
C _{12:0}	1.4	1.4
C _{14:0}	1.1	—
C _{15:0}	1.9	—
C _{16:0}	4.7	4.4
C _{17:0}	4.1	1.3
C _{18:0}	4.4	5.6
Unsaturated fatty acids:		
C _{17:1} ω8c	3.8	2.8
C _{18:1} ω9c	2.0	3.9
iso-C _{15:1} G	—	1.1
iso-C _{16:1} G	4.8	4.9
iso-C _{17:1} ω9c	—	1.0
anteiso-C _{15:1} A	1.7	4.3
anteiso-C _{17:1} ω9c	—	4.9
anteiso-C _{17:1} A	1.1	—
Branched fatty acids:		
iso-C _{14:0}	8.9	2.5
iso-C _{15:0}	5.3	5.3
iso-C _{16:0}	18.0	9.6
iso-C _{17:0}	1.1	1.7
anteiso-C _{15:0}	20.4	26.0
anteiso-C _{17:0}	5.2	14.0
Hydroxy fatty acids:		
iso-C _{14:0} 3-OH	1.5	—
iso-C _{15:0} 3-OH	1.7	—
iso-C _{16:0} 3-OH	2.3	—
Summed feature 4*	—	1.3

*Summed feature 4 comprised C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

Aerial mycelium is white or greyish yellow–brown. The reverse colour of colonies is variable depending on growth medium. Mesophilic. The temperature for growth is in the range 20–37 °C, with good growth at 30 °C. The initial pH range for growth is 6.1–10.1, with good growth at pH 8.1–10.1. Growth occurs in the presence of up to 3 % NaCl. Nitrate is not reduced to nitrite. H₂S is not produced. Gelatin is liquefied. Casein, DNA, elastin, aesculin and hypoxanthine are degraded, but chitin, starch, DL-tyrosine, urea and xanthine are not. D-Arabinose, L-arabinose, cellobiose, dextran, D-fructose, D-glucose, inulin, D-lactose, maltose, D-mannose, L-rhamnose, D-xylose, adonitol (weak), glycerol and D-mannitol are assimilated as sole carbon and energy sources for growth. The following carbon sources are not utilized: D-galactose, melezitose, melibiose, methyl α-D-glucoside, methyl α-D-mannoside, raffinose, D-ribose, salicin, L-sorbose, sucrose, trehalose,

2,3-butanediol, *myo*-erythritol, *myo*-inositol, 1,2-propanediol, D-sorbitol and D-xylitol. Assimilation of organic acids (acetate, benzoate, citrate, formate, malate, succinate, DL-tartrate) is not observed. Cell wall contains LL-DAP as the diagnostic diamino acid and glucose as the major sugar. The major menaquinone is MK-9(H₄). The phospholipid profile comprises diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylinositol mannoside and an unknown phospholipid. Mycolic acids are not present. The major cellular fatty acids are anteiso-C_{15:0} (20.4%), iso-C_{16:0} (18.0%) and iso-C_{14:0} (8.9%). The G+C content of the DNA is 71.5 mol%.

The type strain, D8-87^T (=JBRI 2008^T=KCTC 19222^T=DSM 45079^T), was isolated from soil from a natural cave in Jeju, Republic of Korea.

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