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Jiangella muralis sp. nov., from an indoor environment

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A Gram-stain-positive, non-spore-forming actinobacterium, designated 15-Je-017^T, was isolated from wall material of an indoor environment. The isolate formed a rudimentary substrate mycelium that fragmented into rod-shaped cells. On the basis of 16S rRNA gene sequence analysis, strain 15-Je-017^T was shown to belong to the genus *Jiangella* and was most closely related to *Jiangella alba* YIM 61503^T (99.7 % 16S rRNA gene sequence similarity), *Jiangella alkaliphila* D8-87^T (99.0 %) and *Jiangella gansuensis* YIM 002^T (99.0 %). The predominant menaquinone was MK-9(H_4). Whole-cell hydrolysates contained LL-diaminopimelic acid as the diagnostic diamino acid in the cell wall and rhamnose and glucose as the main sugars. Mycolic acids were absent. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside and seven unknown phospholipids. The fatty acid profile contained major amounts (>5 %) of anteiso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$, anteiso-$C_{17:0}$ and $C_{17:1}\omega 8c$, which supported the affiliation of strain 15-Je-017^T to the genus *Jiangella*. DNA–DNA relatedness and physiological and biochemical tests allowed the differentiation of strain 15-Je-017^T from the type strains of the three known *Jiangella* species. Strain 15-Je-017^T represents a novel *Jiangella* species, for which we propose the name *Jiangella muralis* sp. nov., with type strain 15-Je-017^T (=DSM 45357^T =CCM 7680^T).

The genus *Jiangella* was described by Song *et al.* (2005) and harboured at that time a single species, *Jiangella gansuensis*. Further species, *Jiangella alkaliphila* and *Jiangella alba*, were described by Lee (2008) and Qin *et al.* (2009), respectively. All *Jiangella* species that have been described so far accommodate aerobic, Gram-positive actinobacteria with LL-2,6-diaminopimelic acid in the cell-wall peptidoglycan and MK-9(H_4) as the predominant menaquinone.

Strain 15-Je-017^T was isolated from a cellar wall that was colonized with moulds as described by Kämpfer *et al.* (2009). Strain 15-Je-017^T was maintained on an organic medium, M79 agar (medium 426; http://www.dsmz.de), and was preserved at −80 °C as described previously (Kämpfer *et al.*, 2009).

Colony morphology was determined on M79 agar, nutrient agar (NA; Difco), International *Streptomyces* Project (ISP) 2 and 3 (Shirling & Gottlieb, 1966) and GYM agar (medium 65; http://www.dsmz.de). Cell morphology and Gram staining were determined by phase-contrast microscopy (Axioscope; Carl Zeiss Jena) and stereomicroscopy (Stemi 2000; Carl Zeiss Jena). Cells of 5-day-old liquid cultures of strain 15-Je-017^T are small, irregular rods and stain Gram-positive. After transfer to fresh liquid medium or agar slides, rods gave rise to mycelium-like filaments (Supplementary Fig. S1, available in IJSEM Online). Colonies on NA were white and slightly beige with a matt surface and, after 2 weeks, a very short, white aerial mycelium was formed (Supplementary Fig. S2). The same observations were made on M79 and GYM agar, whereas on ISP 2 the formation of aerial mycelium was detected only after 4 weeks of incubation at 28 °C and on ISP 3 no aerial mycelium was observed.

Isolation of genomic DNA was performed as described by Schäfer *et al.* (2010). Multiple alignment of 16S rRNA gene sequences and analysis were performed using MEGA version 4 (Tamura *et al.*, 2007). A neighbour-joining tree was created using genetic distances calculated with distance options according to Kimura's two-parameter model (Kimura, 1980) (Fig. 1). Bootstrap values were calculated using 1000 replications. The 16S rRNA gene sequence of strain 15-Je-017^T was a continuous stretch of 1363 bp.

Abbreviations: ISP, International *Streptomyces* Project; pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 15-Je-017^T is FN645214.

Two supplementary figures are available with the online version of this paper.

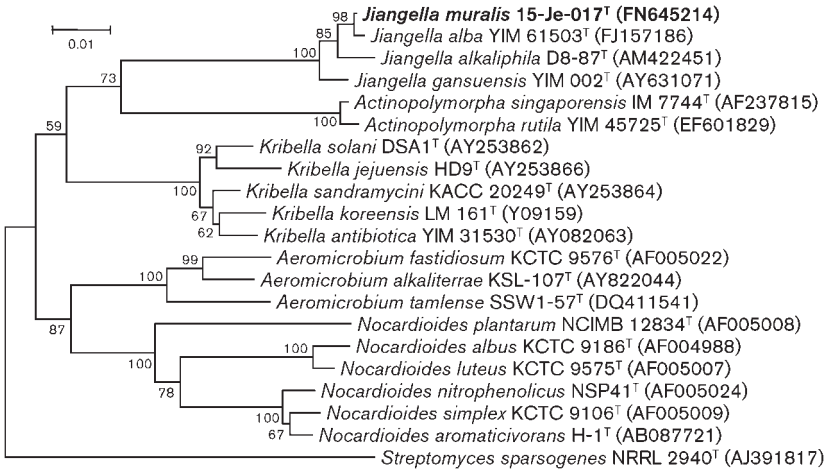

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences showing relationships between strain 15-Je-017^T and related taxa. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Bar, 0.01 substitutions per nucleotide position.

Sequence similarity calculations after the neighbour-joining analysis indicated that the closest relatives of strain 15-Je-017^T were *J. alba* YIM 61503^T (99.7 % 16S rRNA gene sequence similarity), *J. alkaliphila* D8-87^T (99.0 %) and *J. gansuensis* YIM 002^T (99.0 %).

For chemotaxonomic investigations, strain 15-Je-017^T was cultivated in liquid M79 shaken at 180 r.p.m. at 28 °C for 48 h. Cell-wall analysis was performed as described by Groth *et al.* (1996). Whole-cell hydrolysates were prepared for the detection of the isomer of diaminopimelic acid in the cell wall. The amino acids were analysed by TLC on cellulose plates as described by Schleifer & Kandler (1972). Predominant whole-cell sugars and the occurrence of mycolic acids were determined by TLC as described by Becker *et al.* (1965) and Minnikin *et al.* (1975), respectively. Menaquinones were extracted as described by Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1996). Polar lipids were extracted by the method of Minnikin *et al.* (1979) and identified by two-dimensional TLC as described by Collins & Jones (1980). Fatty acid analysis for strain 15-Je-017^T and the reference strains *J. alba* DSM 45237^T, *J. alkaliphila* DSM 45079^T and *J. gansuensis* DSM 44835^T was performed according to Kämpfer & Kroppenstedt (1996) using cells grown on tryptic soy agar at 30 °C for 48 h.

The chemotaxonomic characteristics of strain 15-Je-017^T were consistent with the affiliation to the genus *Jiangella*. Whole-cell hydrolysates contained LL-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan and the major sugars were glucose and rhamnose (wall chemotype I, *sensu* Lechevalier & Lechevalier, 1970). The predominant menaquinone was MK-9(H_4) (90 %); traces of MK-8 (5 %) and MK-7(H_2) (1 %) were also detected. Strain 15-Je-017^T showed a complex phospholipid profile (Fig. 2) that was composed of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannoside, which have been shown for the previously described *Jiangella* species, and seven unknown phospholipids. Phosphatidylcholine, found in *J. alkaliphila*, was not detected. Mycolic acids were absent. The fatty acid profile of strain 15-Je-017^T contained anteiso-$C_{15:0}$ (30.8 %), iso-$C_{16:0}$ (12.1 %), iso-$C_{15:0}$ (11.5 %), iso-$C_{14:0}$ (7.9 %), anteiso-$C_{17:0}$ (7.8 %) and $C_{17:1}\omega8c$ (7.5 %) and other fatty acids in smaller proportions. This profile was very similar to those of the reference strains obtained in this study and was congruent with the fatty acid profiles reported by Song *et al.* (2005), Qin *et al.* (2009) and Lee (2008), but some quantitative and qualitative differences were observed (Table 1).

Growth of strain 15-Je-017^T at 4, 10, 20, 25, 28, 37, 40 and 45 °C was determined on NA. Comparative physiological characterization of strain 15-Je-017^T and the type strains of species of the genus *Jiangella* was done under identical conditions using methods described previously (Kämpfer *et al.*, 1991). The results are given in Table 2 and the species

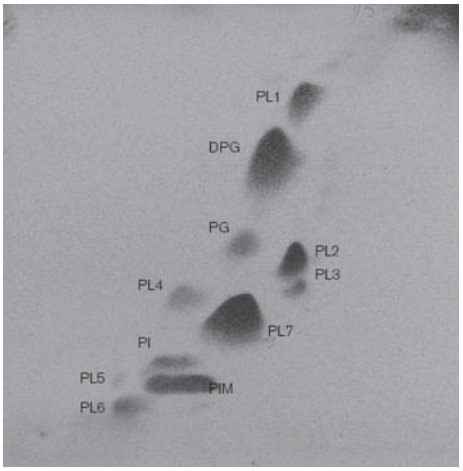

Fig. 2. Two-dimensional TLC of a polar lipid extract from strain 15-Je-017^T, stained with molybdatophosphoric acid. DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside, PL1–7, unknown phospholipids.

Table 1. Major fatty acids of type strains of species of the genus *Jiangella*

Strains: 1, *Jiangella muralis* sp. nov. 15-Je-017^T; 2, *J. alba* DSM 45237^T; 3, *J. alkaliphila* DSM 45079^T; 4, *J. gansuensis* DSM 44835^T. Data are percentages of total fatty acids and were obtained in this study.

Fatty acid	1	2	3	4
iso-$C_{14:0}$	7.9	4.1	9.1	2.0
iso-$C_{15:1}$ G	−	−	1.3	2.5
iso-$C_{14:0}$ 3-OH	−	−	1.4	−
anteiso-$C_{15:1}$ A	−	−	−	2.3
iso-$C_{15:0}$	11.5	16.1	7.7	14.1
anteiso-$C_{15:0}$	30.8	36.8	28.4	30.4
$C_{15:0}$	1.7	−	−	−
iso-$C_{16:1}$ G	1.6	1.0	4.6	3.3
iso-$C_{16:0}$	12.1	10.4	22.9	6.0
$C_{16:0}$	1.4	−	−	−
iso-$C_{15:0}$ 3-OH	3.6	1.2	−	3.7
iso-$C_{16:0}$ 3-OH	−	−	2.4	−
$C_{15:0}$ 2-OH	3.8	2.0	2.7	2.5
iso-$C_{17:1}\omega 9c$	−	0.9	−	2.9
anteiso-$C_{17:1}$ A	−	1.1	1.0	3.5
iso-$C_{17:0}$	3.1	5.8	1.9	5.6
anteiso-$C_{17:0}$	7.8	14.4	11.2	13.3
$C_{17:1}\omega 8c$	7.5	4.3	1.2	3.7
$C_{17:0}$	4.7	−	2.6	1.6
$C_{18:1}\omega 9c$	1.3	0.8	−	2.4
$C_{18:0}$	1.2	−	−	−
iso-$C_{18:0}$	−	−	1.6	−

description. Only a few tests were able to differentiate strain 15-Je-017^T from the reference strains.

DNA–DNA hybridization experiments were performed between strain 15-Je-017^T and the type strains of species of the genus *Jiangella* using the method given by Ziemke *et al.* (1998). Strain 15-Je-017^T showed moderate DNA–DNA relatedness with *J. alba* DSM 45237^T (48.1 %; 45.1 % reciprocal hybridization), *J. alkaliphila* DSM 45079^T (27.2, 22.8 %) and *J. gansuensis* DSM 44835^T (38.4, 32.1 %).

DNA–DNA relatedness and the physiological and chemotaxonomic differences between strain 15-Je-017^T and the type strains of species of the genus *Jiangella* (Tables 1 and 2) clearly warrant the description of a novel species to accommodate strain 15-Je-017^T, for which the name *Jiangella muralis* sp. nov. is proposed.

Description of *Jiangella muralis* sp. nov.

Jiangella muralis [mu.ra′lis. L. fem. adj. *muralis* pertaining or belonging to a wall(s)].

Forms mycelium-like filaments, about 1.3 μm wide. Substrate mycelium on M79 agar is white to beige; aerial mycelium is white. Gram-stain-positive and weakly oxidase-positive. Aerobic respiratory metabolism. Good growth is observed after 3 days on tryptone soy agar, M79 agar and

Table 2. Physiological characteristics of the type strains of species of the genus *Jiangella*

Strains: 1, *J. muralis* sp. nov. 15-Je-017^T; 2, *J. alba* DSM 45237^T; 3, *J. alkaliphila* DSM 45079^T; 4, *J. gansuensis* DSM 44835^T. Data were obtained in this study. All strains were positive for hydrolysis of aesculin, *p*-nitrophenyl (pNP) phenylphosphonate, 2-deoxythymidine-5′-pNP phosphate, L-alanine *p*-nitroanilide (pNA) and L-proline pNA and assimilation of *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, cellobiose, D-glucose, maltose, D-ribose, sucrose, trehalose, adonitol and pyruvate. All strains were negative for hydrolysis of *o*-nitrophenyl *β*-D-galactopyranoside and L-glutamate-*γ*-3-carboxy pNA and assimilation of gluconate, melibiose, D-sorbitol, putrescine, propionate, *cis*- and *trans*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, itaconate, mesaconate, suberate, *β*-alanine, L-leucine, L-ornithine, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. +, Positive; (+), weakly positive; −, negative.

Characteristic	1	2	3	4
Hydrolysis of:				
pNP *β*-D-glucuronide	+	+	−	+
pNP α-D-glucopyranoside	+	−	+	+
pNP *β*-D-glucopyranoside	−	+	−	+
pNP *β*-D-xylopyranoside	+	+	−	+
bis-pNP phosphate	+	−	−	+
pNP phosphorylcholine	+	+	−	+
Assimilation of:				
N-Acetyl-D-galactosamine	(+)	(+)	−	(+)
D-Fructose	(+)	+	−	+
D-Galactose	(+)	−	−	(+)
D-Mannose	+	+	−	+
L-Rhamnose	(+)	−	−	−
Salicin	+	+	−	+
D-Xylose	(+)	(+)	−	(+)
myo-Inositol	−	−	−	(+)
Maltitol	+	+	−	(+)
D-Mannitol	−	+	(+)	+
Acetate	(+)	(+)	−	(+)
Fumarate	+	+	−	−
Glutarate	−	−	−	(+)
DL-3-Hydroxybutyrate	(+)	(+)	−	(+)
DL-Lactate	+	(+)	−	+
L-Malate	+	(+)	(+)	−
Oxoglutarate	(+)	−	−	(+)
L-Alanine	(+)	−	−	−
L-Aspartate	(+)	−	−	−
L-Histidine	(+)	−	−	(+)
L-Phenylalanine	(+)	−	−	(+)
L-Proline	(+)	−	−	(+)

NA at 25–30 °C. Hydrolyses aesculin, *p*-nitrophenyl (pNP) phenylphosphonate, 2-deoxythymidine-5′-pNP phosphate, pNP *β*-D-glucuronide, pNP α-D-glucopyranoside, pNP *β*-D-xylopyranoside, bis-pNP phosphate, pNP phosphorylcholine, L-alanine *p*-nitroanilide (pNA) and L-proline pNA. Does not hydrolyse *o*-nitrophenyl *β*-galactopyranoside, pNP *β*-D-glucopyranoside or L-glutamate-*γ*-3-carboxy pNA. As

sole carbon source, utilizes D-mannose, fumarate, DL-lactate, L-malate, pyruvate, salicin and maltitol, weakly utilizes *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, cellobiose, D-fructose, D-galactose, D-glucose, maltose, L-rhamnose, D-ribose, sucrose, trehalose, D-xylose, adonitol, D-mannitol, acetate, DL-3-hydroxybutyrate, 2-oxoglutarate, L-alanine, L-aspartate, L-histidine, L-proline and L-phenylalanine, but does not utilize gluconate, melibiose, D-mannitol, *myo*-inositol, D-sorbitol, putrescine, propionate, *cis*- or *trans*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, glutarate, itaconate, mesaconate, suberate, β-alanine, L-leucine, L-ornithine, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate or phenylacetate. The quinone system is composed predominantly of menaquinone MK-9(H_4). The polar lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside and seven unknown phospholipids. Major fatty acids (<5 %) are anteiso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{15:0}$, iso-$C_{17:0}$, anteiso-$C_{17:0}$ and $C_{17:1}\omega 8c$; other fatty acids are present in smaller proportions.

The type strain, 15-Je-017^T (=DSM 45357^T =CCM 7680^T), was isolated in Jena, Germany, from a mouldy cellar wall of a house.

Acknowledgements

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