Fodinibacter luteus gen. nov., sp. nov., an actinobacterium isolated from a salt mine

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A Gram-positive-staining, aerobic, catalase- and oxidase-positive, irregular short rod-shaped actinobacterium, designated strain YIM C003^T, was isolated from a salt mine in Yunnan, PR China. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YIM C003^T was most closely related to strains of the genera *Knoellia* (94.2–96.0% similarity), *Oryzihumus* (95.6%), *Terrabacter* (94.9–95.4%), *Janibacter* (94.9–95.4%), *Kribbia* (95.0%), *Lapillicoccus* (95.0%) and *Phycococcus* (94.2–95.0%) of the family *Intrasporangiaceae* and that it formed an independent monophyletic lineage with three strains of *Oryzihumus leptocrescens*. The DNA G+C content of strain YIM C003^T was 72.0 mol%. The diagnostic cell-wall diamino acid was *meso*-diaminopimelic acid. The predominant menaquinone was MK-8(H₄). Mycolic acids were not detected. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and two unknown phospholipids. The major cellular fatty acids were C_{18:1ω9c} and C_{16:0}. These chemotaxonomic properties, together with data from phylogenetic analysis, enabled the novel isolate to be differentiated from all other members of the family. A novel species in a new genus, *Fodinibacter luteus* gen. nov., sp. nov., is proposed, with strain YIM C003^T (=DSM 21208^T =CCTCC AA 208036^T) as the type strain of *Fodinibacter luteus*.

The family *Intrasporangiaceae* of the suborder *Micrococcineae* was proposed by Stackebrandt *et al.* (1997) on the basis of 16S rRNA gene sequence analysis. At the time of writing, the family comprised 15 recognized genera and has been divided into three groups on the basis of the diagnostic diamino acid in the cell-wall peptidoglycan: the first group has LL-diaminopimelic acid (LL-DAP) and contains six genera; the second group has L-ornithine and contains three genera; and the third group has *meso*-DAP and contains six genera [*Janibacter* (Martin *et al.*, 1997), *Tetrasphaera* (Maszenan *et al.*, 2000), *Knoellia* (Groth *et al.*, 2002), *Oryzihumus* (Kageyama *et al.*, 2005), *Phycococcus* (Lee, 2006) and *Kribbia* (Jung *et al.*, 2006)].

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Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM C003^T is EU878005.

A transmission electron micrograph of cells of strain YIM C003^T is available as supplementary material with the online version of this paper.

The present study focuses on the taxonomic description of strain YIM C003^T, which was isolated from a salt mine in Yunnan, China. On the basis of the results of a polyphasic taxonomic study, it is proposed that the strain represents a novel species in a new genus within the family *Intrasporangiaceae*.

Strain YIM C003^T was isolated from a sample collected from the wall of a salt mine by a standard dilution plating method on marine agar 2216 (MA, pH 7.2; Difco) plates. After incubating the agar plates at 28 °C for 21 days, strain YIM C003^T was purified as a single colony and subsequently stored as 20% (v/v) glycerol suspensions at –80 °C. The isolate was cultured on MA, trypticase soy agar (Difco) and nutrient agar (Difco) to determine its growth rate. Growth was slow or poor on the above media and therefore a basal medium (GTM) was designed to improve it. GTM agar medium contained 10 g glucose, 5 g tryptone, 5 g malt extract, 3 g NaCl and 15 g agar in 1 l distilled water (pH 7.2). The pure culture was then routinely maintained on GTM agar at 28 °C.

Genomic DNA extraction, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR products were performed as described previously (Cui *et al.*, 2001). An almost-complete 16S rRNA gene sequence (1444 bp) of strain YIM C003^T was obtained and compared with those available in GenBank using BLAST searches (Altschul *et al.*, 1990). The 16S rRNA gene sequence of the isolate was aligned with the corresponding sequences of members of the family *Intrasporangiaceae* (obtained from GenBank/EMBL/DBJ) by using CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic analysis was performed by using three tree-making algorithms, namely the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. The neighbour-joining tree was constructed from evolutionary distances calculated using Kimura's two-parameter method (Kimura, 1983). Tree topology was evaluated by bootstrap analysis based on 1000 resamplings.

The result of preliminary sequence comparisons with 16S rRNA gene sequences held in GenBank showed that the isolate belonged to the family *Intrasporangiaceae*. Phylogenetic analysis showed that strain YIM C003^T formed a monophyletic clade with strains of *Oryzihumus leptocrescens* within the family *Intrasporangiaceae*. This relationship was also maintained in the tree based on the maximum-likelihood algorithm, but not in the tree based on the maximum-parsimony algorithm (Fig. 1). The 16S rRNA gene sequence similarity between the novel strain and *Oryzihumus leptocrescens* KV-628^T was 95.6 %. Strain

YIM C003^T was also closely related to strains of other genera within the family *Intrasporangiaceae* from the genera *Knoellia* (94.2–96.0 % 16S rRNA gene sequence similarity), *Terrabacter* (94.9–95.4 %), *Janibacter* (94.9–95.4 %), *Phycococcus* (94.2–95.0 %), *Kribbia* (95.0 %), *Terracoccus* (95.0 %), *Lapillicoccus* (95.0 %), *Intrasporangium* (94.8 %), *Humihabitans* (94.8 %), *Tetrasphaera* (94.1–94.7 %), *Arsenicococcus* (94.5 %), *Ornithinimicrobium* (94.0 %), *Ornithinococcus* (93.9 %) and *Serinicoccus* (93.8 %).

For phenotypic characterization, strain YIM C003^T was grown routinely on GTM agar at 28 °C, unless otherwise specified. Gram staining was carried out using the standard Gram reaction combined with the KOH lysis test method (Gegersen, 1978). Cell morphology in 7-day-old cultures was examined by light microscopy with a model BH-2 microscope (Olympus) and by transmission electron microscopy. The morphology, size and colour of colonies from cultures grown aerobically for 7 days were examined. Growth at different temperatures, pH and NaCl concentrations was determined on GTM agar medium. Catalase activity was determined by assessing bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using a 1 % (w/v) solution of tetramethyl-*p*-phenylenediamine (Kovacs, 1956). Hydrolysis of hypoxanthine, starch, tyrosine, Tweens 20, 40 and 80 and xanthine was determined as described by Cowan & Steel (1965). The ability of strain YIM C003^T to grow on a range of sole carbon and energy sources at 0.5 % (w/v) was determined

Fig. 1. Neighbour-joining phylogenetic tree showing the relationship between strain YIM C003^T and representatives of the family *Intrasporangiaceae*. Filled circles indicate that the corresponding nodes were also found in the trees generated with both maximum-parsimony and maximum-likelihood algorithms; open circles indicate that the corresponding nodes were also found in the maximum-likelihood tree. Bootstrap percentages (based on 1000 replications) >50 % are shown at branching points. Bar, 0.01 substitutions per nucleotide position.

on carbon utilization medium (Pridham & Gottlieb, 1948). Acid production from 49 carbon sources was tested at 28 °C with the API 50CH kit combined with API 50CHB/E medium (bioMérieux). Enzyme activity tests were performed using the API ZYM kit (bioMérieux) according to the manufacturer's instructions. Other biochemical tests were carried out with API 20NE and API 20E kits (bioMérieux) according to the manufacturer's instructions.

Cells of strain YIM C003^T were aerobic, non-motile, non-endospore-forming and Gram-positive. Cells were irregular short rods at all growth stages and occurred singly or in clusters. On GTM medium, colonies were circular, smooth, convex and orange–yellow in colour with entire margins. The results of the physiological and biochemical tests are given in the species description.

The amino acid contents of the cell walls were determined according to the procedures described by Schleifer & Kandler (1972). The amino acid composition of complete wall hydrolysates was determined by HPLC. Whole-cell sugars were identified by the method described by Becker *et al.* (1965). Mycolic acids were extracted and analysed according to the protocol of Minnikin *et al.* (1975). Polar lipids were extracted as described by Minnikin *et al.* (1979) and identified by two-dimensional TLC sprayed with specific reagents (Collins & Jones, 1980). Menaquinones were extracted by using the method of Collins *et al.* (1977) and analysed by HPLC as described by Tamaoka *et al.* (1983). Biomass for quantitative fatty acid analysis of strain YIM C003^T was prepared by scraping cells from GTM agar plates that had been incubated for 7 days at 28 °C. Analysis of the whole-cell fatty acid pattern was carried out as described by Sasser (1990) using the Microbial Identification System (MIDI). The G+C content of the genomic DNA was determined by HPLC according to Mesbah *et al.* (1989).

The DNA G + C content of the isolate was 72.0 mol%. The cell-wall peptidoglycan contained *meso*-DAP, alanine and glutamic acid in a molar ratio of 1.0:1.7:1.0. The isolate contained peptidoglycan type A1 γ (Schleifer & Kandler, 1972). Whole-cell sugars were ribose and glucose. The polar lipids were phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and two unknown phospholipids. The menaquinone was MK-8(H₄) (100%). Mycolic acids were not detected. The cellular fatty acids (>1% of total fatty acids) were C_{18:1} ω 9c (33.5%), C_{16:0} (12.6%), iso-C_{16:0} (8.1%), C_{17:1} ω 8c (7.4%), iso-C_{14:0} (5.3%), C_{18:0} (5.1%), summed feature 3 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH, 3.76%), iso-C_{15:0} (2.9%), C_{14:0} (2.6%), C_{17:0} (2.3%), C_{18:1} ω 7c (2.1%), summed feature 5 (C_{18:2} ω 6,9c and/or anteiso-C_{18:0}, 2.1%), C_{15:0} (2.1%), anteiso-C_{15:0} (1.8%), C_{16:1} ω 9c (1.3%) and anteiso-C_{17:0} (1.2%).

The results of the phylogenetic analyses showed the phylogenetic position of strain YIM C003^T within the family *Intrasporangiaceae*. In terms of 16S rRNA gene sequence similarity, the closest phylogenetic neighbour was *Oryzihumus leptocrescens* KV-628^T (95.6% similarity).

However, strain YIM C003^T could be distinguished from *Oryzihumus leptocrescens* on the basis of the presence of phosphatidylethanolamine and phosphatidylinositol and the presence of C_{18:1} ω 9c and C_{16:0} as the major fatty acids (Table 1). Strain YIM C003^T could be readily differentiated from all other members of the family *Intrasporangiaceae* by differences in chemotaxonomic properties, particularly the diamino acid type in position 3 of the peptidoglycan and fatty acid profiles (Table 1). Strain YIM C003^T contained *meso*-DAP, which distinguished it from the genera containing LL-DAP or L-ornithine as the diagnostic diamino acid. Additionally, strain YIM C003^T contained C_{18:0} ω 9c and C_{16:0} as the major fatty acids, which distinguished it from recognized genera with *meso*-DAP as the diagnostic acid of the cell-wall peptidoglycan in the family *Intrasporangiaceae*. Although there may be differences in the proportions of some fatty acids (probably due to differences in cultivation conditions and extraction procedures) (Table 1), the fatty acid C_{16:0} was the major fatty acid in strain YIM C003^T. This fatty acid is absent (or present as a minor component) in all other genera of the family *Intrasporangiaceae*, except members of the genus *Kribbia*. However, strain YIM C003^T could be differentiated from members of the genus *Kribbia* based on the absence of 10-methyl C_{18:0} (17.9–18.5% in *Kribbia dieselivorans*; Jung *et al.*, 2006). Furthermore, the fatty acid iso-C_{15:0} was a minor component in strain YIM C003^T, whereas it is a major component in most recognized genera of the family *Intrasporangiaceae*.

On the basis of the phylogenetic data and differential chemotaxonomic properties reported here, it is suggested that strain YIM C003^T represents a novel species in a new genus within the family *Intrasporangiaceae*, for which the name *Fodinibacter luteus* gen. nov., sp. nov. is proposed.

Description of *Fodinibacter* gen. nov.

Fodinibacter (Fo.di.ni.bac'ter. L. fem. n. *fodina* mine; N.L. masc. n. *bacter* rod; N.L. masc. n. *Fodinibacter* rod bacterium isolated from a mine).

Cells stain Gram-positive. Oxidase- and catalase-positive. Cells are non-endospore-forming, non-motile and irregular short rods. Cells occur singly or in clusters. The peptidoglycan is of the A1 γ type, containing *meso*-DAP, glutamic acid and alanine. Mycolic acids are not present. The predominant menaquinone is MK-8(H₄). The polar lipid profile contains phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol, diphosphatidylglycerol and two unknown phospholipids. The predominant cellular fatty acids are C_{18:1} ω 9c and C_{16:0}. Phylogenetically, the genus belongs to the family *Intrasporangiaceae*, suborder *Micrococcineae*. The type species is *Fodinibacter luteus*.

Description of *Fodinibacter luteus* sp. nov.

Fodinibacter luteus (lu'te.us. L. masc. adj. *luteus* orange–yellow, referring to the colony colour).

Table 1. Differential characteristics of strain YIM C003^T and genera of the family *Intrasporangiaceae*

Taxa: 1, *Fodinibacter luteus* YIM C003^T; 2, *Knoellia* (data from Groth *et al.*, 2002; Weon *et al.*, 2007); 3, *Oryzihumus* (unless indicated, data from Kageyama *et al.*, 2005); 4, *Janibacter* (Martin *et al.*, 1997; Imamura *et al.*, 2000; Yoon *et al.*, 2000, 2004; Kämpfer *et al.*, 2006); 5, *Kribbia* (Jung *et al.*, 2006); 6, *Tetrasphaera* (Maszenan *et al.*, 2000; Hanada *et al.*, 2002); 7, *Phycococcus* (Lee, 2006; Yoon *et al.*, 2008); 8, *Arsenicicoccus* (Collins *et al.*, 2004); 9, *Ornithinimicrobium* (Groth *et al.*, 2001); 10, *Serinicoccus* (Yi *et al.*, 2004); 11, *Ornithinococcus* (Groth *et al.*, 1999); 12, *Intrasporangium* (Kalakoutskii *et al.*, 1967; Schumann *et al.*, 1997; Groth *et al.*, 2001); 13, *Terracoccus* (Prauser *et al.*, 1997); 14, *Terrabacter* (Collins *et al.*, 1989; Montero-Barrientos *et al.*, 2005; Lee *et al.*, 2008); 15, *Humihabitans* (Kageyama *et al.*, 2007); 16, *Lapillicoccus* (Lee & Lee, 2007). L-Orn, L-Ornithine; i-, iso-branched; ai-, anteiso-branched; Me, methyl; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cell morphology	Irregular short rods	Irregular rods and cocci	Irregular rods	Coccoid to rod-shaped	Irregular short rods or cocci	Cocci or short rods	Cocci	Cocci	Irregular rods and cocci	Cocci	Cocci	Hyphae	Cocci	Irregular rods	Hyphae	Cocci
Cell-wall di-amino acid	meso-DAP	meso-DAP	meso-DAP	meso-DAP	meso-DAP	meso-DAP	meso-DAP	LL-DAP	L-Orn	L-Orn	L-Orn	LL-DAP	LL-DAP	LL-DAP	LL-DAP	LL-DAP
Major mena-quinone	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)	MK-8(H ₄)
Polar lipids*	DPG, PE, PG, PI, 2PL	DPG, PG, PE	DPG, PG, PGL, PL†	DPG, PG, PI	ND	DPG, PG, PE, PI, DPG, PL, APL, PL	ND	DPG, PG, PI, PL, GL	DPG, PG, PI, PL, GL	DPG, PG, PI, GL	DPG, PG, PI, PS, PL	ND	DPG, PG, PI, PL	DPG, PE, PI, GL	ND	ND
Major fatty acids (>10 % of total fatty acids)‡	C _{18:1} ω9c, C _{16:0}	i-C _{15:0} , i-C _{17:0} , i-C _{16:0} , ai-C _{17:0} /i-C _{16:0} , C _{17:1} ω8c, i-C _{15:0}	i-C _{16:0} , i-C _{15:0} , i-C _{14:0}	i-C _{16:0} , C _{17:1} ω8c, C _{18:1} ω9c, C _{17:0}	10-Me, C _{18:0} , i-C _{16:0} , C _{18:1} ω9c, C _{16:0} , C _{17:0}	ai-C _{15:0} , i-C _{15:0} /i-C _{16:0} , ai-C _{17:0} , i-C _{15:0} /i-C _{16:0} , ai-C _{16:0} , C _{17:0}	i-C _{17:1} ω8c, i-C _{16:0} , i-C _{15:0} , C _{18:1} ω9c, ai-C _{15:0}	C _{16:1} ω7c, i-C _{15:0} , C _{18:1} ω9c, ai-C _{15:0}	i-C _{16:0} , i-C _{15:0} , ai-C _{15:0}	i-C _{15:0} , i-C _{16:0} , i-C _{17:1} ω9c	i-C _{15:0} , ai-C _{15:0}	i-C _{15:0} , ai-C _{15:0}	i-C _{15:0} , ai-C _{15:0} , i-C _{16:0}	i-C _{15:0} , ai-C _{15:0} /i-C _{14:0} , i-C _{16:0} /i-C _{15:0}	i-C _{15:0} , C _{16:0}	i-C _{16:0} , C _{17:1} ω8c, i-C _{15:0}
DNA G + C content (mol%)	72	68–73	72–73	69–73	69–70	68–71	70.7–74	72.2	70	72	72	68.2	73	69.8–72.6	70	74.1

*APL, Unknown aminophospholipid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid; PGL, unknown phosphoglycolipid; GL, unknown glycolipid.

†Data from this study.

‡Different groups of fatty acids, each separated by a solidus, may be present, depending on the species.

In addition to the morphological, chemotaxonomic and general characteristics described for the genus, cells are $0.3\text{--}0.6 \times 0.7\text{--}2.2\ \mu\text{m}$. Growth occurs at $15\text{--}37\ ^\circ\text{C}$ with an optimum at $28\ ^\circ\text{C}$. Optimal growth at pH 6.5–7.5. NaCl tolerance on GTM agar medium is 2.5 % (w/v). Only very weak growth is observed on GTM agar without NaCl. Anaerobic growth does not occur on GTM using the GasPak anaerobic system (BBL). Acetate, D-galactose, D-glucose, maltose, melibiose and pyruvate are utilized as sole carbon and energy sources, sucrose and trehalose are utilized weakly and L-arabinose, benzoate, cellobiose, citrate, formate, D-fructose, L-glutamate, L-malate, D-mannose, salicin, succinate and D-xylose are not utilized. Acid is produced from D-glucose. Acid is not produced from L-arabinose, cellobiose, D-fructose, D-galactose, myo-inositol, lactose, maltose, D-mannitol, melezitose, raffinose, L-rhamnose, D-ribose, sucrose, D-sorbitol or trehalose. Aesculin, gelatin and Tweens 20, 40 and 80 are hydrolysed, but hypoxanthine, starch, tyrosine and xanthine are not. With the API 20E kit (bioMérieux), arginine dihydrolase and β -galactosidase are present, lysine decarboxylase, ornithine decarboxylase and urease are absent and H_2S and indole are not produced. Nitrate is reduced to nitrite (API 20NE). In assays with the API ZYM system (bioMérieux), tests positive for alkaline phosphatase, esterase C4, esterase lipase C8, lipase C14, leucine arylamidase, acid phosphatase, cystine arylamidase, valine arylamidase, α -chymotrypsin, trypsin and β -galactosidase, but negative for N-acetyl- β -glucosaminidase, α -glucosidase, β -glucosidase, β -glucuronidase, fucosidase, naphthol-AS-BI-phosphohydrolase, α -mannosidase and α -galactosidase.

The type strain is YIM C003^T (=DSM 21208^T =CCTCC AA 208036^T), isolated from a sample collected from the wall of a salt mine in Yunnan. The DNA G+C content of the type strain is 72.0 mol%.

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