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

Zhi-Gang Wang,† Yong-Xia Wang,† Ji-Hui Liu, Yi-Guang Chen, Xiao-Xia Zhang, Meng-Liang Wen, Li-Hua Xu, Qian Peng and Xiao-Long Cui

1Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan 650091, PR China
2College of Bio-resources and Environmental Science, Jishou University, Jishou, Hunan 416000, PR China
3Agricultural Cultural Collection of China, Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100080, PR China

A Gram-positive-staining, aerobic, catalase- and oxidase-positive, irregular short rod-shaped actinobacterium, designated strain YIM C003T, was isolated from a salt mine in Yunnan, PR China. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YIM C003T 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 *Phycicoccus* (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 C003T 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 C18:1v9c and C16:0. 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 C003T (=DSM 21208T =CCTCC AA 208036T) 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), *Phycicoccus* (Lee, 2006) and *Kribbia* (Jung et al., 2006)].

†These authors contributed equally to this work.

**Abbreviation:** DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM C003T is EU878005.

A transmission electron micrograph of cells of strain YIM C003T is available as supplementary material with the online version of this paper.

The present study focuses on the taxonomic description of strain YIM C003T, 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 C003T 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 C003T 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, 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<sup>T</sup> 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/DDBJ) 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<sup>T</sup> 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-parsimony algorithm, but not in the tree based on the maximum-likelihood algorithm (Fig. 1). The 16S rRNA gene sequence similarity between the novel strain and Oryzihumus leptocrescens KV-628<sup>T</sup> was 95.6 %. Strain YIM C003<sup>T</sup> 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 %), Physicoccus (94.2–95.0 %), Kribbia (95.0 %), Terracoccus (95.0 %), Lapilllicoccus (95.0 %), Intrasporangium (94.8 %), Humihabitans (94.8 %), Tetrasphaera (94.1–94.7 %), Arsenicoccus (94.5 %), Ornithinimicrobium (94.0 %), Ornithinicoccus (93.9 %) and Serinicoccus (93.8 %).

For phenotypic characterization, strain YIM C003<sup>T</sup> 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 (Gregersen, 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) H<sub>2</sub>O<sub>2</sub> 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<sup>T</sup> to grow on a range of sole carbon and energy sources at 0.5 % (w/v) was determined.
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 C003T 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 C003T 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 A1γ (Schleifer & Kandler, 1972). Whole-cell sugars were ribose and glucose. The polar lipids were phosphatidylethanolamine, phosphatidyl-linositol, phosphatidyglycerol, diphasatidylglycerol 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 C18:1ω9c (33.5%), C16:0 (12.6%), iso-C16:0 (8.1%), C17:0ω9c (7.4%), iso-C14:0 (5.3%), C18:0 (5.1%), summed feature 3 (C16:0ω7c and/or iso-C15:0 2-OH, 3.76%), iso-C15:0 (2.9%), C14:0 (2.6%), C17:0 (2.3%), C18:1ω7c (2.1%), summed feature 5 (C18:2ω6c9c and/or anteiso-C18:0 2.1%), C15:0 (2.1%), anteiso-C15:0 (1.8%), C16:1ω9c (1.3%) and anteiso-C17:0 (1.2%).

The results of the phylogenetic analyses showed the phylogenetic position of strain YIM C003T within the family Intrasporangiaceae. In terms of 16S rRNA gene sequence similarity, the closest phylogenetic neighbour was Oryzihumus leptocrescens KV-62B T (95.6% similarity). However, strain YIM C003T could be distinguished from Oryzihumus leptocrescens on the basis of the presence of phosphatidylethanolamine and phosphatidylinositol and the presence of C18:1ω9c and C16:0 as the major fatty acids (Table 1). Strain YIM C003T 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 C003T contained meso-DAP, which distinguished it from the genera containing LL-DAP or L-ornithine as the diagnostic diamino acid. Additionally, strain YIM C003T contained C18:1ω9c and C16:0 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 C16:0 was the major fatty acid in strain YIM C003T. 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 C003T could be differentiated from members of the genus Kribbia based on the absence of 10-methyl C18:0 (17.9–18.5% in Kribbia dieselivorans; Jung et al., 2006). Furthermore, the fatty acid iso-C15:0 was a minor component in strain YIM C003T, 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 C003T 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. fodi.na mine; N.L. masc. n. bac’ter rod; N.L. masc. n. Fodinibac’ter 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 A1γ 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, phosphatidylglycerol, phosphatidylglycerol, diphasatidylglycerol and two unknown phospholipids. The predominant cellular fatty acids are C18:1ω9c and C16:0. 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’t.e.us. L. masc. adj. lute.us orange-yellow, referring to the colony colour).
Table 1. Differential characteristics of strain YIM C003\textsuperscript{T} and genera of the family Intrasporangiaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Irregular rods and cocci</td>
<td>Cocci or short rods</td>
<td>Irregular rods</td>
<td>Irregular rods or cocci</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Cocci or short rods</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Hyphae</td>
<td>Cocci</td>
<td>Hyphae</td>
<td>Cocci</td>
<td></td>
</tr>
<tr>
<td>Cell-wall diamino acid</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td>meso-DAP</td>
<td></td>
</tr>
<tr>
<td>Major menaquinone</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td>MK-8(H\textsubscript{4})</td>
<td></td>
</tr>
<tr>
<td>Major fatty acids (&gt;10% of total fatty acids)†</td>
<td>C\textsubscript{18}:1\textsubscript{ω9G}, C\textsubscript{16}:0</td>
<td>i-C\textsubscript{15}:0, i-C\textsubscript{17}:0</td>
<td>i-C\textsubscript{17}:1</td>
<td>i-C\textsubscript{16}:0, i-C\textsubscript{15}:0</td>
<td>i-C\textsubscript{15}:0, i-C\textsubscript{14}:0</td>
<td>i-C\textsubscript{16}:0, i-C\textsubscript{15}:0</td>
<td>i-C\textsubscript{16}:0, i-C\textsubscript{15}:0</td>
<td>i-C\textsubscript{17}:1, i-C\textsubscript{16}:0</td>
<td>i-C\textsubscript{17}:1, i-C\textsubscript{16}:0</td>
<td>i-C\textsubscript{17}:1, i-C\textsubscript{16}:0</td>
<td>i-C\textsubscript{17}:1, i-C\textsubscript{16}:0</td>
<td>i-C\textsubscript{17}:1, i-C\textsubscript{16}:0</td>
<td>i-C\textsubscript{17}:1, i-C\textsubscript{16}:0</td>
<td>i-C\textsubscript{17}:1, i-C\textsubscript{16}:0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>72</td>
<td>68–73</td>
<td>72–73</td>
<td>69–73</td>
<td>69–70</td>
<td>68–71</td>
<td>70–74</td>
<td>72.2</td>
<td>70</td>
<td>72</td>
<td>72</td>
<td>68.2</td>
<td>73</td>
<td>69.8–72.6</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>

*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.

Taxa: 1, Fodinibacter luteus YIM C003\textsuperscript{T}; 2, Knollenia (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., 2007); 6, Tetrasphaera (Maszenan et al., 2000; Hanada et al., 2002); 7, Physicoccus (Lee, 2006; Yoon et al., 2008); 8, Arsenicicoccus (Collins et al., 2004); 9, Ornithinicmicrobium (Groth et al., 2001); 10, Serinicoccus (Yi et al., 2004); 11, Ornithinicoccus (Groth et al., 1999); 12, Intrasporangium (Kalakoutskii et al., 1967; Schumann et al., 1997; Groth et al., 2001); 13, Terracoccus (Frauser 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).
In addition to the morphological, chemotaxonomic and general characteristics described for the genus, cells are 0.3–0.6 × 0.7–2.2 μm. Growth occurs at 15–37 °C with an optimum at 28 °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, cellulobiose, citrate, formate, D-fructose, L-glutamate, L-malate, D-mannose, salicin, succinate and D-xylene are not utilized. Acid is produced from D-glucose. Acid is not produced from L-arabinose, cellulobiose, 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 H₂S 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-Bl-phosphohydrolase, α-mannosidase and α-galactosidase.

The type strain is YIM C003 (DSM 21208T = CCTCC AA 208367), 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%.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (30860013, 30460004, 30660004, 30760006), the Ministry of Science and Technology of China (863 Program, no. 2007AA021306; 2006BA01A01-9), the Ministry of Environmental Protection of China (National Key Sciences and Technology Program for Water Solutions, 2008ZX07102-004), the Yunnan Provincial Sciences and Technology Department (2005FY01-1, 2006C0006M) and Yunnan University (2008BY005). We are grateful to Ms Ya-Ling Yang for her help during sampling and to Professor Dr Hans G. Truper for recommending the correct etymology for the genus and species names.

References


