**Yaniella fodinae** sp. nov., isolated from a coal mine

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An orange bacterial strain, designated G5T, was isolated during the study of the bacterial diversity of a coal mine. The cell wall of strain G5T contained peptidoglycan type A4α (L-Lys–Gly–L-Glu) and the sugars xylose and mannose. The major menaquinones were MK-8 (45.0 %) and MK-9 (34.0 %) and minor amounts of MK-7 and MK-8(H2) were also found. The major fatty acids were anteiso-C15:0 (44.9 %) and iso-C15:0 (44.2 %). The main cellular polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. 16S rRNA gene sequence analysis showed that strain G5T was closely related to *Yaniella halotolerans* YIM 70085T and *Yaniella flava* YIM 70178T (both 96.7 % 16S rRNA gene sequence similarity). The genomic DNA G+C content of strain G5T was 61.6 mol%. These data and other phenotypic characteristics clearly indicated that strain G5T represents a novel species of the genus *Yaniella*, for which the name *Yaniella fodinae* sp. nov. is proposed. The type strain is G5T (=MTCC 9846T=DSM 22966T).

The genus *Yaniella* has been recently described and is placed within the family *Yaniellaceae* in the suborder *Micrococinae* (Li et al., 2008). The names *Yaniella* and *Yaniellaceae* were proposed by Li et al. (2008) as replacements for the illegitimate prokaryotic names *Yania* (Li et al., 2004) and *Yaniaceae* (Li et al., 2005), respectively. Members of the genus *Yaniella* are actinobacteria and have been characterized by a vast array of chemotaxonomic characteristics, such as the quinone system, cell-wall peptidoglycan and amino acids, cellular fatty acids and polar lipid profiles. At the time of writing, the genus contained two recognized species, *Yaniella flava* (Li et al., 2005) and *Yaniella halotolerans* (Li et al., 2004), which were isolated from different hypersaline soils in China. Here, we report a *Yaniella*-like strain that was isolated from a coal mine in India.

Strain G5T was isolated from a soil sample from a coal mine using the dilution-plating method (up to 10−5) on tryptic soy agar (TSA; HiMedia) and maintained in glycerol stocks at −70°C. The reference strains *Y. halotolerans* MTCC 9575T and *Y. flava* MTCC 9576T were obtained from the Microbial Type Culture Collection and Gene Bank, Chandigarh, India.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain G5T is FJ871122.

A supplementary figure and supplementary tables are available with the online version of this paper.

Colony and cell morphology were studied according to standard methods (Murray et al., 1994). The Gram reaction was determined using the HiMedia Gram-staining kit according to the manufacturer’s instructions. Motility of cells grown in tryptic soy broth (TSB) was determined using the hanging-drop method and phase-contrast microscopy (Zeiss). To determine anaerobiosis, TSA plates were incubated in an anaerobic jar (MART), which was evacuated and flushed using an Anoxomat unit (MART) with nitrogen/carbon dioxide/hydrogen (85:10:5), at 30°C for 5 days. Growth at different temperatures and pH (adjusted using biological buffers) was studied in TSB. The tolerance of strain G5T to NaCl, KCl and MgCl2·6H2O was studied in TSB and compared with *Y. halotolerans* MTCC 9575T and *Y. flava* MTCC 9576T, which are highly salt tolerant. All physiological and biochemical tests were performed at 30°C. Catalase, citrate utilization (using Simmons’ citrate agar), H2S production and urea hydrolysis were determined as described by Cowan & Steel (1965). Casein, gelatin and starch hydrolysis, indole production, methyl red test, Voges–Proskauer test and oxidase activity were assessed as described by Smibert & Krieg (1994). Nitrate reduction was tested as described by Lányi (1987). Additional physiological and biochemical tests for strain G5T and the reference strains were performed using the API 20 NE and API ZYM kits (bioMérieux), according to the manufacturer’s instructions. Sensitivity to various antibiotics was studied using antibiotic-susceptibility discs.
(HiMedia) on TSA. Oxidation of various substrates as sole carbon, nitrogen and energy sources was tested using GP2 MicroPlates (Biolog) in accordance with the manufacturer’s instructions, except that TSB was used instead of Biolog Universal Growth agar (Mayilraj et al., 2006). The results were recorded after 24 h with a MicroPlate Reader and Microlog version 4.2 computer software. Acid production from various carbohydrates was tested on minimal medium as described by Smith et al. (1952).

For cellular fatty acid analysis, strain G5\textsuperscript{T} and the reference strains were grown on marine agar at 30°C for 36 h. Fatty acid methyl ester analysis was performed using the Sherlock Microbial Identification System (MIDI) as described by Pandey et al. (2002). Freeze-dried cells for other chemotaxonomic analyses were prepared from cultures grown in TSB at 30°C for 4 days. Isoprenoid quinones were extracted and analysed as described by Minnikin et al. (1984). The purified menaquinones were separated by reversed-phase HPLC (Shimadzu; Kroppenstedt, 1982). Polar lipids were extracted following the method of Bligh & Dyer (1959) and analysed by two-dimensional TLC. The following spray reagents were used for detection of total lipids, phospholipids, aminolipids and glycolipids, respectively: molybdatophosphoric acid (5 %, w/v, in absolute ethanol; developed at 120°C overnight), molybdenum blue spray reagent (results recorded immediately), ninhydrin (0.2 %, w/v, in acetone; developed at 100°C for 5 min) and anisaldehyde reagent (developed at 100°C for 10 min).

Whole-cell sugars were determined as described by Staneck & Roberts (1974). The peptidoglycan structure was determined from hydrolysate of purified cell-wall extracts, according to Schleifer & Kandler (1972).

Genomic DNA extraction and amplification and sequencing of 16S rRNA gene was performed as described previously (Mayilraj et al., 2005). The G+C content of the genomic DNA was determined spectrophotometrically (Lambda 35; PerkinElmer) using the thermal denaturation method (Mandel & Marmur, 1968). The 16S rRNA gene was amplified using the universal primers 27F and 1492R. Sequencing of the amplified product was conducted by the dideoxy chain-terminator method using a Big Dye Terminator kit followed by capillary electrophoresis with an ABI 310 Genetic Analyzer (Applied Biosystems). The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). The 16S rRNA gene sequence of strain G5\textsuperscript{T} was aligned with sequences of members of closely related genera retrieved from the EzTaxon server using MEGA version 4 (Tamura et al., 2007). A phylogenetic tree was constructed using the neighbour-joining and maximum-parsimony algorithms and bootstrap analysis was performed to assess the confidence limits of the tree topology.

Cells of strain G5\textsuperscript{T} were aerobic, Gram-positive and oval to coccoid in shape. Colonies were circular, orange and 0.4–3.0 mm in diameter on TSA. The phenotypic

### Table 1. Differential characteristics of strain G5\textsuperscript{T} and type strains of species of the genus Yaniella

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth at 15°C</td>
<td>+=</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Range for growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>5.2–11.0</td>
<td>6.5–8.5</td>
<td>6.0–9.0</td>
</tr>
<tr>
<td>NaCl (% w/v)</td>
<td>0–15</td>
<td>0–15</td>
<td>0.5–25</td>
</tr>
<tr>
<td>KCl (% w/v)</td>
<td>0–25</td>
<td>0–25</td>
<td>0.5–30</td>
</tr>
<tr>
<td>MgCl\textsubscript{2}·6H\textsubscript{2}O (% w/v)</td>
<td>0–30</td>
<td>0–30</td>
<td>0.5–30</td>
</tr>
<tr>
<td>Acid production from sucrose</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Enzyme activities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Leucine arylamidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Polar lipids*</td>
<td>DPG, PG, PI, PL, GL</td>
<td>DPG, PG, PI, PL, GL</td>
<td>DPG, PG, PL, GL</td>
</tr>
<tr>
<td>Major fatty acids</td>
<td>anteiso-C\textsubscript{15}:0, iso-C\textsubscript{15}:0</td>
<td>anteiso-C\textsubscript{15}:0</td>
<td>anteiso-C\textsubscript{15}:0</td>
</tr>
<tr>
<td>Major menaquinones*</td>
<td>MK-8 and MK-9</td>
<td>MK-8</td>
<td>MK-8 and MK-9</td>
</tr>
<tr>
<td>Cell-wall sugars</td>
<td>Xylose, mannose</td>
<td>Xylose, mannose</td>
<td>Xylose, mannose, galactose</td>
</tr>
<tr>
<td>DNA G+C content (mol%)*</td>
<td>61.6</td>
<td>53.5</td>
<td>57.9</td>
</tr>
</tbody>
</table>

*Data in columns 2 and 3 are from Li et al. (2004, 2005).
The cellular fatty acid compositions of strain G5\textsuperscript{T} and the reference strains are shown in Table 2. The compositions obtained in this study for the reference strains were quantitatively different from those reported by Li et al. (2004, 2005), in which the major fatty acid in both reference strains was anteiso-C\textsubscript{15:0} and \textit{Y. halotolerans} MTCC 9575\textsuperscript{T} contained a minor amount of iso-C\textsubscript{15:0}. This could be attributed to different cultivation conditions. The present study obtained fatty acid compositions under identical conditions and observed quantitative and qualitative differences in the major fatty acids of strain G5\textsuperscript{T} and the reference strains. The major components in strain G5\textsuperscript{T} were anteiso-C\textsubscript{15:0} (44.9\%) and iso-C\textsubscript{15:0} (44.2\%). The cell-wall diamino acid was lysine and the interpeptide bridge of the peptidoglycan consisted of Gly–Glu (type A4\textsubscript{v}; Schleifer & Kandler, 1972). The menaquinone analysis indicated that MK-8 (45.0\%) and MK-9 (34.0\%) were the major menaquinones in strain G5\textsuperscript{T} and there were minor amounts of MK-7 (13.5\%) and MK-8(H\textsubscript{2}) (6.3\%). Menaquinones MK-8, MK-9 and MK-7 have been found in \textit{Y. halotolerans} (Li et al., 2004) but MK-7 has not been reported in \textit{Y. flava} (Li et al., 2005). The polar lipids in strain G5\textsuperscript{T} were diphosphatidylglycerol, phosphatidylglycerol, dimannosyldiaclylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, a mannos-containing lipid, an amino lipid and two unknown phospholipids (see Supplementary Fig. S1). The polar lipid compositions of strain G5\textsuperscript{T} and the reference strains are compared in Supplementary Table S2. The polar lipid profile of strain G5\textsuperscript{T} was quite different from those of the reference strains.

The 16S rRNA gene sequence phylogenetic analysis indicated that strain G5\textsuperscript{T} belonged to the genus \textit{Yaniella} and was closely related to \textit{Y. halotolerans} YIM 70085\textsuperscript{T} and \textit{Y. flava} YIM 70178\textsuperscript{T} (both 96.7\% 16S rRNA gene sequence similarity). Less than 96\% 16S rRNA gene sequence similarity was found with other members of the suborder \textit{Micrococccinae} with validly published names. The maximum-parsimony analysis placed all of the members of the family \textit{Yaniellaceae} into a separate clade (bootstrap value 100\%) within the suborder \textit{Micrococccinae} and this topology was also seen in the neighbour-joining tree (Fig. 1). The genomic DNA G+C content of strain G5\textsuperscript{T} was 61.6 mol\%, which was comparatively higher than those for \textit{Y. halotolerans} MTCC 9575\textsuperscript{T} and \textit{Y. flava} MTCC 9576\textsuperscript{T} (53.5 and 57.9 mol\%, respectively).

On the basis of the data presented above, strain G5\textsuperscript{T} should be placed in the genus \textit{Yaniella} as a representative of a novel species, for which we propose the name \textit{Yaniella fodinae} sp. nov.

\textbf{Description of \textit{Yaniella fodinae} sp. nov.}

\textit{Yaniella fodinae} (fo.di’nae. L. gen. n. \textit{fodinae} of a pit, of a mine).

Cells are coccoid to oval shaped (1.0–1.5 μm in diameter), non-motile and form pairs, tetrads and clusters. Colonies are circular and orange (0.4–3.0 mm in diameter) on TSA. Grows at 15–37 °C (optimum 30 °C) and pH 5.2–11.0 (optimum pH 7.5). Catalase-positive and oxidase-negative.

\begin{table}[h]
\centering
\caption{Comparison of cellular fatty acid profiles of strain G5\textsuperscript{T} and its closest phylogenetic neighbours}
\begin{tabular}{|c|c|c|c|}
\hline
Fatty acid (%) & 1 & 2 & 3 \\
\hline
C\textsubscript{14:0} & – & – & 0.3 \\
C\textsubscript{15:0} & – & 0.9 & 0.6 \\
C\textsubscript{16:0} & 0.9 & 0.2 & 1.8 \\
C\textsubscript{17:0} & – & – & 0.4 \\
iso-C\textsubscript{13:0} & 1.5 & – & – \\
iso-C\textsubscript{14:0} & 1.2 & 3.0 & 11.7 \\
iso-C\textsubscript{15:0} & 44.2 & 7.6 & 10.1 \\
iso-C\textsubscript{16:0} & 0.8 & 2.6 & 15.7 \\
iso-C\textsubscript{17:0} & 1.0 & 0.9 & 0.9 \\
anteiso-C\textsubscript{13:0} & 0.9 & 0.2 & – \\
anteiso-C\textsubscript{15:0} & 44.9 & 56.9 & 47.3 \\
anteiso-C\textsubscript{17:0} & 4.3 & 14.2 & 10.4 \\
C\textsubscript{15:1} anteiso A & – & – & 0.4 \\
C\textsubscript{16:1}9\textsubscript{v}7c alcohol & – & 4.8 & – \\
C\textsubscript{16:1}10\textsubscript{c}11c & – & 0.5 & – \\
iso C\textsubscript{17:1}9\textsubscript{c}10c & – & 0.5 & – \\
Summed feature 4* & – & 7.0 & – \\
\hline
\end{tabular}
\end{table}

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 4 consisted of iso-C\textsubscript{17:1} \textsubscript{1} and/or anteiso-iso-C\textsubscript{17:1} \textsubscript{1}.
Tolerates up to 15% NaCl, 25% KCl and 30% MgCl₂·6H₂O. Negative for starch hydrolysis, gelatin hydrolysis, citrate utilization, H₂S production, indole production, nitrate reduction and methyl red and Voges-Proskauer tests. Positive for oxidation of dextrin, Tweens 40 and 80, L-arabinose, D-fructose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, α-D-glucose, α-D-lactose, methyl β-D-glucoside, D-ribose, stachyose, D-tagatose, D-xyllose, acetic acid, α-, β- and γ-hydroxybutyric acids, p-hydroxyphenylacetic acid, α-ketovaleric acid, lactamide, L-lactic acid, L-malic acid, pyruvic acid, succinic acid, L-alaninamide, L-pyroglutamic acid, L-serine, 2,3-butanediol, inosine, thymidine, D-fructose 6-phosphate and D-glucose 6-phosphate. Acid is produced from fructose, glucose and maltose but not from arabinose, adonitol, cellobiose, glucose, dulcitol, fructose, galactose, inositol, inulin, lactose, maltose, mannose, mannitol, melibiose, rhamnose, salicin, sorbitol, raffinose, trehalose, sucrose or xylitol. Sensitive to (μg per disc) ampicillin (10), chloramphenicol (10), chlorotetracycline (30), ciprofloxacin (5), gentamicin (10), nalidixic acid (30), neomycin (30), novobiocin (30), oleanolic acid (30), rifampicin (5), streptomycin (25) and tetracycline (30) and resistant to cephaloridine (30), furazolidone (5), tobramycin (10) and trimethoprim (5). The peptidoglycan type is L-Lys–Gly–L-Glu (variation A4). The major fatty acids are anteiso-C₁₅:₀ and iso-C₁₅:₀. The predominant menaquinones are MK-8 and MK-9 and minor amounts of MK-7 and MK-8(H₂) are present. The cell-wall sugars include xylose and mannose.

The type strain is G5ᵀ (≡MTCC 9846ᵀ = DSM 22966ᵀ), isolated from a soil sample collected from a coal mine. The DNA G+C content of the type strain is 61.6 mol%.

Acknowledgements

We thank Professor J.P. Euzéby, Ecole Nationale Veterinaire, France for his suggestion for the Latin nomenclature for the novel species. We would like to thank Mr Malkit Singh, Ms Anupama Joshi and Ms Ishwinder Kaur for their excellent technical assistance. Financial assistance from CSIR and DBT, Government of India is duly acknowledged. This is IMTECH communication number 052/2009.

References


