Mariniluteicoccus flavus gen. nov., sp. nov., a new member of the family Propionibacteriaceae, isolated from a deep-sea sediment

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A Gram-staining-positive, aerobic, non-motile, irregular coccus, designated strain YIM M13146T, was isolated from a sediment sample collected from the South China Sea at a depth of 2439 m, and its taxonomic position was determined by a polyphasic approach. Optimal growth of the strain was observed at 30 °C (range 5–40 °C), pH 7.0 (pH 6.0–9.0) and 0–1 % NaCl (0–6 %, w/v) on/in tryptic soy agar/broth. Strain YIM M13146T had the major cellular fatty acid anteiso-C15:0, the predominant respiratory menaquinone MK-9(H4), peptidoglycan type A3 (LL-DAP–Gly) containing alanine, glycine, glutamic acid and LL-diaminopimelic acid (LL-DAP) and the polar lipids phosphatidylcholine, diphosphatidylglycerol, one unknown phospholipid and several glycolipids. The G+C content of the DNA was 67.2 mol%. Phenotypic and chemotaxonomic characteristics together with 16S rRNA gene sequence analyses showed that strain YIM M13146T was distinct from its close phylogenetic relatives in the genus Propionifexar and Granulicoccus of the family Propionibacteriaceae. Hence, a new genus and species, Mariniluteicoccus flavus gen. nov., sp. nov., is proposed. The type strain of Mariniluteicoccus flavus is YIM M13146T (=DSM 25892T = CCTCC AB 2012055T).

The ocean covers more than 70 % of the Earth’s surface, and extreme environments at depths greater than 200 m (the deep-sea) account for about 64 % of the Earth’s surface (Bull, 2011). The South China Sea is Chinese largest tropical marginal sea, with a complex and diverse submarine sediment environment, and is rich in biological resources, but the utilization of microbial resources in the deep-sea sediment environment is still in its infancy. Up to now, researchers taking part in this project have isolated, identified and published many novel actinobacterial taxa, such as Sciscionella marina (Tian et al., 2009a), Strepto- myces nanshensis (Tian et al., 2009b), Marinactinospora thermotolerans (Tian et al., 2009c), Janibacter alkaliphilus (Li et al., 2012a), Nocardioopsis coralliicola (Li et al., 2012b), Rhodococcus nanhaiensis (Li et al., 2012c), Streptomyces nanhaiensis (Tian et al., 2012a), Streptomyces oceani (Tian et al., 2012b), Streptomyces glycovorans, Streptomyces xishensis and Streptomyces abyssalis (Xu et al., 2012), Pseudonocardia antennualis (Tian et al., 2013), Mycobacterium sediminis (Zhang et al., 2013a) and Streptomospora nanhaiensis (Zhang et al., 2013b). There are clearly still many unknown, uncultured micro-organisms in the South China Sea. In this study, we report an actinobacterium of a new genus of the family Propionibacteriaceae isolated from the South China Sea.

The family Propionibacteriaceae was described by Delwiche (1957) and its description has been emended by Stackebrandt et al. (1997) and Zhi et al. (2009) on the basis of 16S rRNA gene sequence analyses. Currently, there are 17 known genera in the family, according to the List of Prokaryotic Names with Standing in Nomenclature (http://www.bacterio.net/index.html). Strain YIM M13146T was
is isolated from a sediment sample collected from the South China Sea (18° 1.654’ N 112° 30.203’ E) at a depth of 2439 m, by the serial dilution technique using HP agar medium [5 g fucose, 1 g proline, 1 g (NH₄)₂SO₄, 2 g CaCl₂, 1 g KH₂PO₄, B vitamin mixture (0.5 mg each thiamine hydrochloride, riboflavin, niacin, pyridoxine, calcium panthenate, inositol and p-aminobenzoic acid and 0.25 mg biotin), 35 g NaCl, 12 g agar, 1000 ml distilled water; pH 7.2] at an incubation temperature of 28 °C for 1 month. The strain was cultivated and maintained on tryptic soy agar (TSA) and stored as an aqueous glycerol suspension (20%, v/v) at −80 °C.

Gram staining was carried out by using the standard Gram reaction and was confirmed by using the KOH lysis test (Cerny, 1978). Cell motility was tested by the hanging-drop technique (Skerman, 1967) using phase-contrast microscopy (Olympus). Microscopic observation of strain YIM M13146ᵀ grown on TSA for 7 days at 28 °C was made by light microscopy (BH 2; Olympus). Cells negatively stained with 2% uranyl acetate were used for the detection of the presence of flagella using a JEM-2100 transmission electron microscope (Vreeland et al. 1980). Growth was tested at 5, 10, 15, 20, 28, 30, 35, 40, 45 and 50 °C on TSA by incubating the cultures for 14 days. The ability of the strain to grow at pH 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 [using the buffer system described by Xu et al. (2005)] and 0, 1, 2, 3, 4, 6, 7, 8, 9 and 10 % (w/v) NaCl (pH 7) was examined at 28 °C for 14 days with tryptic soy broth (TSB) as the basal medium. Anaerobic cultivation was performed on TSA using a Whitley A35 anaerobic workstation (Don Whitley Scientific). Carbon source utilization was tested using the Biolog GEN III MicroPlate according to the manufacturer’s instructions. Catalase activity was detected by the production of bubbles after the addition of a drop of 3% (v/v) H₂O₂. Other physiological and biochemical tests were performed by using the API ZYM and API 20E strips (bioMérieux) according to the manufacturer’s instructions.

Biomass for analyses of cellular fatty acids was obtained from cultures grown on TSA (Difco) for 3 days, and that for the other tests was grown for 7 days. Cellular fatty acid analysis was performed by using the Microbial Identification System (Sherlock version 6.1; MIDI database TSBA6). The amino acid composition in peptidoglycan hydrolysates was analysed by TLC (Hasegawa et al. 1983) and HPLC (Tang et al. 2009). Menaquinones were extracted by using the methods of Collins et al. (1977) and separated by HPLC (Tamaoka et al. 1983). Polar lipids were extracted and analysed by two-dimensional TLC according to Embley & Wait (1994). The genomic DNA G + C content was determined by using the HPLC method of Mesbah et al. (1989).

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were carried out as described by Li et al. (2007). The 16S rRNA gene sequence obtained in this study was compared with sequences from EzBioCloud using BLAST (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). Multiple alignments with sequences of the most closely related bacteria retrieved from the GenBank/EMBL/DDBJ database were carried out using the CLUSTAL_X 1.8 program (Thompson et al., 1997). Phylogenetic trees were reconstructed by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms by using the software package MEGA version 5.05 (Tamura et al., 2011). The stability of relationships was assessed by performing bootstrap analyses with 1000 resamplings (Felsenstein, 1985).

Strain YIM M13146ᵀ grew well on TSA and International Streptomyces Project (ISP) medium 2. Round, yellow colonies with wet, smooth surfaces were formed stably on TSA and ISP 2 within the first week, and the surfaces became dry and wrinkled during the second week. No diffusible pigment was observed in either medium. Investigations of 7-day-old cultures of strain YIM M13146ᵀ revealed that cells of the strain were Gram-stain-positive, irregular cocci, 0.5–1.0 μm in diameter, and non-motile, with no flagella, and occurred singly or in pairs or triads (Fig. 1). The isolate could grow at pH 6.0–9.0, 0–6% (w/v) NaCl and 5–40 °C; optimum growth occurred at 30 °C, 0–1% (w/v) NaCl and pH 7.0. Detailed physiological characteristics are shown in Table 1 and the species description.

The profile of cellular fatty acids (>1%) of strain YIM M13146ᵀ showed the presence of anteiso-C₁₃:₀ (3.26%), iso-C₁₄:₀ (2.11%), C₁₄:₀ (1.16%), iso-C₁₅:₀ (5.08%), anteiso-C₁₅:₀ (63.1%), C₁₄:₀ 2-OH (1.4%), iso-C₁₆:₁ H (2.91%), iso-C₁₆:₀ (3.72%), C₁₆:₀ (2.7%), anteiso-C₁₇:₀ 10–9c (2.42%), anteiso-C₁₇:₀ (2.51%), summed feature 3 (C₁₅:₀ 10–7c and/or C₁₆:₀ 10–6c 2.92%) and summed feature 9 (iso-C₁₇:₀ 10–9c and/or 10–methyl C₁₆:₀ 1.37%). The polar lipids of strain YIM M13146ᵀ were phosphatidylcholine, diphasatidylglycerol, an unknown phosphoglycolipid and several glycolipids, with phosphatidylglycerol and two unknown polar lipids as minor components (Fig. S1, available in the online Supplementary Material). TLC analysis indicated that strain YIM M13146ᵀ possessed
LL-diaminopimelic acid (LL-DAP) in the cell wall. Further HPLC analysis of the peptidoglycan showed the presence of alanine, glycine and glutamic acid (2:1:1). These results confirmed that the peptidoglycan type of the isolate was A3\(\gamma\) (LL-DAP–Gly). The predominant menaquinone of strain YIM M13146\textsuperscript{T} was MK-9(H\textsubscript{4}) and the DNA G+C content was 67.2 mol%.

An almost-complete 16S rRNA gene sequence was obtained for isolate YIM M13146\textsuperscript{T} (1536 bp). The sequence showed 90.0–95.2\% similarity to members of family Propionibacteriaceae. Comparative 16S rRNA gene sequence analysis showed that the strain was located stably in the family Propionibacteriaceae together with the genera Granulicoccus (95.2\% similarity to the type strain of the type species; 63/1315 mismatches) (Maszenan \textit{et al.}, 2007) and Propioniferax (94.1\% similarity to the type strain of the type species; 86/1451 mismatches) (Yokota \textit{et al.}, 1994) in all three tree-making methods (Figs 2, S2 and S3). The results of 16S rRNA gene sequence comparisons demonstrated that strain YIM M13146\textsuperscript{T} was most closely related to the genera Granulicoccus and Propioniferax of the family Propionibacteriaceae.

Strain YIM M13146\textsuperscript{T}, which was located phylogenetically in the family Propionibacteriaceae, exhibited obvious differences from its closely related neighbours Granulicoccus phenolivorans PG 02\textsuperscript{T} and Propioniferax innocua ATCC 49929\textsuperscript{T} (Yokota \textit{et al.}, 1994). ND, No data available. All strains showed positive reactions for gelatin hydrolysis and negative reactions for production of H\textsubscript{2}S and indole and showed peptidoglycan type A3\(\gamma\) (LL-DAP–Gly) and optimum growth at pH 7.

### Table 1. Phenotypic and biochemical characteristics of strain YIM M13146\textsuperscript{T} and its closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Character</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>O\textsubscript{2} requirement</td>
<td>Aerobe</td>
<td>Faculative anaerobe</td>
<td>Faculative anaerobe</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>Cocci, single and in pairs or triads</td>
<td>Cocci, single and in pairs</td>
<td>Pleomorphic rods</td>
</tr>
<tr>
<td>Cell size ((\mu)m)</td>
<td>0.5–1.0</td>
<td>0.3–1.4</td>
<td>0.5–1.2</td>
</tr>
<tr>
<td>Isolation source</td>
<td>Marine sediment</td>
<td>Phenol-degrading granules</td>
<td>Human epidermal surface</td>
</tr>
<tr>
<td>Growth temperature ((^\circ)C)</td>
<td>30</td>
<td>30</td>
<td>37</td>
</tr>
<tr>
<td>Optimum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>5–40</td>
<td>15–37</td>
<td>10–40</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>6.0–9.0</td>
<td>5.0–8.5</td>
<td>ND</td>
</tr>
<tr>
<td>Presence of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidase</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Major menaquinone(s)</td>
<td>MK-9(H\textsubscript{4})</td>
<td>MK-9(H\textsubscript{4}), MK-8(H\textsubscript{4})</td>
<td>MK-9(H\textsubscript{4})</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Major polar lipids*</td>
<td>DPG, PC, PGL, GL</td>
<td>PG, DPG, GL, GL</td>
<td>PE, PG, GL</td>
</tr>
<tr>
<td>Major fatty acid(s) (&gt;10%)†</td>
<td>ai-C\textsubscript{15:0}</td>
<td>i-C\textsubscript{15:0}, i-C\textsubscript{15:0} DMA</td>
<td>ai-C\textsubscript{15:0}, i-C\textsubscript{15:0}</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>67.2</td>
<td>69</td>
<td>62</td>
</tr>
</tbody>
</table>

\*DPG, Disphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidyglycerol; GL, unknown glycolipid(s); PGL, unknown phosphoglycolipid.
†ai, Anteiso-branched; i, iso-branched; i-C\textsubscript{15:0} DMA, 1,1-dimethoxy isopentadecane.

Description of \textit{Mariniluteicoccus} gen. nov.

\textit{Mariniluteicoccus} \[\textit{M}a.r\text{i}\.\text{ri}\.\text{n}\.\text{l}\.\text{u}\.\text{t}\.\text{e}\.\text{i}\.\text{c}\.\text{c}\.\text{u}\.\text{s}\] L. adj. \textit{marinus} of the sea; L. adj. \textit{luteus} yellow; N.L. masc. n. \textit{coccus} (from Gr. masc. n. \textit{kokkos} a grain, a seed) a coccus; N.L. masc. n. \textit{Mariniluteicoccus} yellow coccus living in the sea.
Cells are Gram-staining-positive, aerobic, non-motile, irregular cocci. The genus is a member of the family Propionibacteriaceae. The major cellular fatty acid is anteiso-C15:0, the predominant menaquinone is MK-9(H4), the peptidoglycan type is A3c containing alanine, glycine, glutamic acid and LL-diaminopimelic acid in a molar ratio of 2:1:1:2 and the polar lipids are phosphatidylcholine, diphosphatidylglycerol, an unknown phosphoglycolipid and several unknown glycolipids. The G+C content of the DNA of the type strain of the type species is 67.2 mol%. The type species is Mariniluteicoccus flavus.

Description of Mariniluteicoccus flavus sp. nov.

Mariniluteicoccus flavus (flavus. L. masc. adj. flavus yellow, reflecting the colour of colonies of the type strain).

The characteristics are the same as those described for the genus with the following additions. Cells are 0.5–1.0 μm in diameter. Optimal growth occurs at 30 °C (range 5–40 °C), pH 7.0 (range pH 6.0–9.0) and 0–1 % NaCl (range 0–6 %, w/v) on/in TSA/TSB. Production of catalase is negative. Positive reactions from the API ZYM system are observed for alkaline phosphatase, esterase (C4), esterase lipase (C8),

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain YIM M13146T and members of the family Propionibacteriaceae. Bootstrap values (expressed as percentages of 1000 replications) ≥50 % are shown at branch points. Asterisks denote nodes that were also recovered using the maximum-likelihood and maximum-parsimony methods. Bar, 0.01 substitutions per nucleotide position.
leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase and β-glucosidase; negative reactions for lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Positive reactions from the API 20E system for β-galactosidase, the Voges–Proskauer test, gelatin hydrolysis and nitrate reductase, while negative reactions are observed for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H₂S production, tryptophan deaminase, urease, indole production, oxidase and fermentation under anaerobic conditions. Positive reactions from the GEN III system for utilization of dextrin, maltose, cellobiose, gentiobiose, sucrose, turanose, methyl β-D-glucoside, D-salicin, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, N-acetyl neuraminic acid, α-D-glucose, D-mannose, D-fructose, D-galactose, D- and L-fucose, L-rhamnose, inosine, D-arabitol, glycerol, D-glucose 6-phosphate, D-fructose 6-phosphate, D-serine, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, L-prolylglutamic acid, L-serine, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, glucuronamide, quinic acid, D-saccharic acid, D-lactic acid methyl ester, L-lactic acid, α-ketoglutaric acid, acetoacetic acid and acetic acid and tolerance of pH 6, 1 and 4 % NaCl, 1 % sodium lactate, trehalomycin, nalidixic acid and lithium chloride. Negative reactions from the GEN III system for utilization of trehalose, stachyose, raffinose, lactose, melibiose, N-acetyl-D-galactosamine, 3-methyl glucose, D-sorbitol, D-mannitol, myo-inositol, D-aspartic acid, gelatin, pectin, mucic acid, p-hydroxyphenylacetic acid, methyl pyruvate, citric acid, D- and L-malic acid, bromosuccinic acid, Tween 40, γ-aminobutyric acid, α-hydroxybutyric acid, β-hydroxy-DL-butyric acid, α-ketobutyric acid, propionic acid and formic acid and tolerance of pH 5, 8 % NaCl, fusidic acid, D-serine, rifamycin SV, minocycline, lincomycin, guanidine hydrochloride, niaproof 4, vancomycin, tetrazolium violet, tetrazolium blue, potassium tellurite, aztreonam, sodium butyrate and sodium bromate.

The type strain YIM M13146T (= DSM 25892T = CCTCC AB 2012055) was isolated from a sediment sample collected from the South China Sea (18° 1.654’ N 112° 30.203’ E) at a depth of 2439 m.

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References


