**Luteimonas abyssi** sp. nov., isolated from deep-sea sediment

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Three Gram-stain-negative, strictly aerobic, rod-shaped with single polar flagellum, yellow-pigmented bacteria, designated strains XH031\(^T\), XH038-3 and XH80-1, were isolated from deep-sea sediment of the South Pacific Gyre (41° 51’ S 153° 6’ W) during the Integrated Ocean Drilling Program (IODP) Expedition 329. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolates belonged to the genus *Luteimonas* and showed the highest 16S rRNA gene sequence similarity with *Luteimonas aestuarii* B9\(^T\) (96.95 %), *Luteimonas huabeiensis* HB9\(^T\) (96.93 %) and *Xanthomonas cucurbitae* LMG 690\(^T\) (96.92 %). The DNA G+ C contents of the three isolates were 70.2–73.9 mol%. The major fatty acids were iso-C\(_{15:0}\), iso-C\(_{16:0}\), iso-C\(_{17:1}\) and C\(_{16:0}\) 10-methyl and/or iso-C\(_{17:1}\) 9c. The major respiratory quinone was ubiquinone-8 (Q-8). The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, diphasphatidylglycerol and one unknown phospholipid. On the basis of data from polyphasic analysis, the three isolates represent a novel species of the genus *Luteimonas*, for which the name *Luteimonas abyssi* sp. nov. is proposed. The type strain is XH031\(^T\) (=DSM 25880\(^T\) = CGMCC 1.12611\(^T\)).

The genus *Luteimonas*, in the family *Xanthomonadaceae* of the class *Gammaproteobacteria*, was first proposed by Finkmann *et al.* (2000) for aerobic, Gram-negative rods with ubiquinone-8 (Q-8) as the major ubiquinone and iso-C\(_{15:0}\) as the predominant fatty acid. At the time of writing, there are ten species of the genus *Luteimonas* with validly published names: *Luteimonas mephitis* (Finkmann *et al.*, 2000), *L. composti* (Young *et al.*, 2007), *L. aestuarii* (Roh *et al.*, 2008), *L. aquatica* (Chou *et al.*, 2008), *L. marina* (Baik *et al.*, 2008) and *L. terricola* (Zhang *et al.*, 2010), *L. lutimaris* (Park *et al.*, 2011), *L. cucumeris* (Sun *et al.*, 2012), *L. vadosa* (Romanenko *et al.*, 2013), *L. huabeiensis* (Wu *et al.*, 2013). They were isolated from various environments, such as fresh water, cucumber leaf, food waste, ammonia biofilter, hydrocarbon-contaminated soil, seawater, tidal flat sediment and seashore sediment. Three novel yellow rod-shaped bacteria, designated XH031\(^T\), XH038-3 and XH80-1 were isolated from sediment at a depth of 18.1–18.2 m below the sea floor in the South Pacific Gyre at station U1370 (41° 51’ S 153° 06’ W) during the Integrated Ocean Drilling Program (IODP) Expedition 329. The aim of the present study was to determine the exact taxonomic position of the isolates using a polyphasic approach.

**Abbreviations:** DPG, diphasphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of *Luteimonas abyssi* XH031\(^T\), XH038-3 and XH80-1 are KC986351, JXS501252 and JXS501253, respectively.

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

Sediment samples were mixed with sterile 0.85 % (w/v) saline, spread on marine agar 2216 (MA; BD Difco) plates and incubated at 4 °C for 3 months or 28 °C for up to 2 weeks. Three isolates, XH031\(^T\), XH038-3 and XH80-1, which formed yellow colonies with entire margins on MA after incubation at 28 °C for 7 days, were picked and purified by streaking three times on MA. The cultures were maintained on MA plate at 28 °C, and stocks were preserved in sterile 0.85 % (w/v) saline supplemented with 15 % (v/v) glycerol at −80 °C. *Luteimonas aestuarii* DSM 19680\(^T\), *Luteimonas mephitis* DSM 12574\(^T\), *Xanthomonas cucurbitae* DSM 18957\(^T\) and *Xanthomonas campestris* DSM 3586\(^T\) obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) Braunschweig, Germany, were used as reference strains, and these and the three isolates were cultivated on nutrient agar (NA; Difco) at 28 °C, since *X. cucurbitae* DSM 18957\(^T\) did not grow well on MA plates.

Gram staining and flagellum staining were investigated using standard methods (Beveridge *et al.*, 2007). Cell morphology was determined by transmission electron microscopy (JEM-1200EX; JEOL) after cells had been negatively stained with 1 % (w/v) phosphotungstic acid. Motility was observed by the hanging-drop method (Mackie & McCartney, 1989). To test anaerobic growth, bacterial strains were cultured on NA with resazurin as an indicator of anaerobic conditions in an anaerobic jar filled with nitrogen and a packet of AnaeroPack-Anaero (Mitsubishi Gas Chemical Co.) at 28 °C for 1 month. The temperature range for growth was determined in
nutrient broth (NB; Difco) by incubating cultures at 4, 8, 12, 16, 20, 24, 28, 32, 37 or 42 °C for 5 days, and measuring the optical densities (wavelength 590 nm) at 0, 12, 24, 48, 72, 96 and 120 h. The optimal temperature for growth was determined with the growth curve at different temperatures. Growth at 0 °C was tested on NA for 30 days. Salinity and pH ranges supporting growth were investigated in 96-well microplates by measuring the optical densities (wavelength 590 nm). The NaCl concentration was adjusted to 0–15.0 % (w/v, at intervals of 1.0 %) in NB. Growth in NB was evaluated at pH 2.0–10.0 in intervals of 1 pH unit using the following buffer systems: Na2HPO4/citric acid (pH 2.0–7.0), Tris/HCl (pH 8.0–9.0) and Na2CO3/NaHCO3 (pH 9.0–10.0). Various phenotypic characterizations of the isolates and four reference strains were tested according to the standard approaches (Tindall et al., 2007), including activities of catalase and oxidase, and hydrolysis of starch, casein, gelatin, and Tweens 20, 40 and 80 (method 2). DNase activity was examined by using DNase agar (Qingdao Hope Bio-technology) according to the manufacturer’s instructions. Activities of constitutive enzymes and other physiological properties were determined after growth on NA at 28 °C for 2 days by using API 20E, API 20NE, API 50CH and API ZYM strips (bioMérieux) and GN2 MicroPlates (Biolog), according to the manufacturers’ instructions. Susceptibility to antibiotics was investigated on NA plates by using discs containing different antibiotics (Hangzhou Microbiology Reagent). Susceptibility was determined after incubating on NA at 28 °C for 24 h, when the diameter of the inhibition zone around the disc exceeded the standard diameter given in the manufacturer’s instructions. The standard diameters (mm) of different antibiotics were as follows: streptomycin (15), nalidixic acid (19), kanamycin (18), ampicillin (17), gentamicin (15), tetracycline (19), novobiocin (17), chloramphenicol (18), neomycin (24), erythromycin (23), penicillin G (28) and lincomycin (21).

Cells of strain XHO31T were Gram-stain-negative, strictly aerobic, motile rods with single polar flagellum (0.2–0.4 μm in width, 1.0–1.6 μm in length, Fig. 1), and formed yellow-pigmented, circular (1.0–1.5 mm in diameter), convex, and slightly transparent colonies with entire margins on NA after culturing for 2–3 days at 28 °C. Growth occurred at 4–37 °C, with an optimum growth rate at 28 °C. No growth was observed at 0 °C or 42 °C. The salinity range for the isolates was 0–11 % (w/v) NaCl in NB, with an optimum growth rate at 0–3 % (w/v) NaCl. The pH range for growth was pH 6.0–8.0, with an optimum growth rate at pH 7.0. Other morphological, physiological and biochemical characteristics of the isolates and the related strains are given in the species description and Table 1. The properties of strains XHO38-3 and XHO80-1 were identical with to XHO31T, unless otherwise stated.

For cellular fatty acid analysis, the three isolates and the reference strains were grown on NA at 28 °C for 3 days when they reached mid-exponential phase. Fatty acid methyl esters were prepared and analysed according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0) and identified by the TSBA6.0 database of the Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin et al. (1984), and separated by two-dimensional TLC on silica gel 60 F254 plates (Merck) using chloroform/methanol/water (65:25:4, by vol.) for the first dimension and chloroform/methanol/acetic acid/water (80:12:15:4, by vol.) for the second dimension (Collins & Shah, 1984). The identification of individual lipid spots was performed by spraying with the appropriate detection reagents and comparing with standard phosphate lipid patterns (Komagata & Suzuki, 1987). The respiratory quinones were extracted with chloroform/methanol (2:1, v/v), separated by TLC and identified by HPLC. DNA was extracted according to the procedure of Moore et al. (1999) and the G+C content was determined according to Mesbah & Whitman (1989). The cellular fatty acid profiles of the three isolates and the reference strains are listed in Table 2. The fatty acids of the three isolates were very similar, with only minor differences in quantities. The dominant fatty acids of the isolates were similar to L. aestuarii DSM 19680T and L. mephitis DSM 12574T with a large amount of iso-C15:0 (30.2–35.7 %), iso-C16:0 (10.4–13.2 %), iso-C11:0 (7.5–9.3 %) and C16:010-methyl and/or iso-C17:109c (6.9–8.4 %) although apparent quantitative differences were observed. The isolates contained larger amounts of iso-C11:0 and smaller amounts of iso-C17:0 and iso-C11:0 than L. aestuarii DSM 19680T and L. mephitis DSM 12574T.
Table 1. Differential characteristics between Luteimonas abyssi sp. nov. and type strains of phylogenetically closely related species

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<td>+</td>
<td>+</td>
<td>–</td>
<td>W</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Cystine arylamidase</td>
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<td>–</td>
<td>W</td>
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<tr>
<td>Trypsin</td>
<td>+</td>
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<td>+</td>
<td>–</td>
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<td>W</td>
<td>+</td>
<td>W</td>
<td>W</td>
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<td>+</td>
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<tr>
<td>ß-Chymotrypsin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>W</td>
<td>+</td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>+</td>
</tr>
<tr>
<td>ß-Galactosidase</td>
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<td>–</td>
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<td>W</td>
<td>+</td>
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<td>W</td>
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<td>+</td>
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<tr>
<td>ß-Glucosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>W</td>
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<td>W</td>
<td>+</td>
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<tr>
<td>N-Acetyl-ß-glucosaminidase</td>
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<td>–</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>W</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>70.2</td>
<td>73.9</td>
<td>72.0</td>
<td>64.7a</td>
<td>67.0b</td>
<td>70.3</td>
<td>68.1</td>
<td>69.6</td>
<td>67.6</td>
<td>72.0</td>
<td>NR</td>
<td>69.9</td>
<td>66.1–66.8c</td>
<td>65.8–66.6c</td>
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</table>

*Data different from the original references: Roh et al. (2008) for L. aestuarii DSM 19680T and Finkmann et al. (2000) for L. mephitis DSM 12574T. †Data from a, Roh et al. (2008); b, Finkmann et al. (2000); c, Vauterin et al. (1995).
isolates differed from *X. cucurbitae* DSM 18957T and *X. campestris* DSM 3586T by significant amounts of iso-C15:0 and iso-C16:0 and smaller amounts of C16:1ω7c and anteiso-C15:0. The polar lipid profiles of the three isolates were identical, and comprised phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and one unknown phospholipid (PL), which were similar to those of *L. aestuarii* DSM 19680T and *L. mephitis* DSM 12574T. However, the isolates differed from *L. mephitis* DSM 12574T by containing an unknown phospholipid, and differed from *L. aestuarii* DSM 19680T by comprising one unknown phospholipid at different spots. The polar lipid profile of *X. cucurbitae* comprised PE, PG, DPG, and two unknown phospholipids (PL1 and PL2) (Fig. S1, available in IJSEM Online). In accordance with other members of the genus *Luteimonas*, the isolates contained Q-8 as the major ubiquinone. The DNA G+C content of the three isolates was 70.2, 73.9 and 72.0 mol%, respectively, which fell in the range of members of the family *Xanthomonadaceae* (40.1–75 mol%).

For 16S rRNA gene sequencing, the genomic DNA of the isolates was extracted and purified using standard methods (Ausubel et al., 1995). The 16S rRNA gene was amplified by

<table>
<thead>
<tr>
<th>Table 2. Cellular fatty acid contents (%) of <em>Luteimonas abyssi</em> sp. nov. and the reference strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strains: 1, XH031T; 2, XH038-1; 3, XH80-1; 4, <em>Luteimonas aestuarii</em> DSM 19680T; 5, <em>Luteimonas mephitis</em> DSM 12574T; 6, <em>Xanthomonas cucurbitae</em> DSM 18957T; 7, <em>Xanthomonas campestris</em> DSM 3586T. All data were taken from this study. TR, Traces (&lt;1%); --, not detected. Fatty acids amounting to &lt;1% of the total fatty acids in all strains are not shown. Values in bold are predominant fatty acids.</td>
</tr>
<tr>
<td>Fatty acid</td>
</tr>
<tr>
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<tr>
<td>Straight chain</td>
</tr>
<tr>
<td>C10:0</td>
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<tr>
<td>C12:0</td>
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<tr>
<td>C14:0</td>
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<tr>
<td>C16:0</td>
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<td>C18:0</td>
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<td>Branched</td>
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<td>iso-C11:0</td>
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<td>iso-C14:0</td>
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<tr>
<td>iso-C15:0</td>
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<tr>
<td>iso-C16:0</td>
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<tr>
<td>iso-C17:0</td>
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<tr>
<td>anteiso-C15:0</td>
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<tr>
<td>anteiso-C17:0</td>
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<tr>
<td>cyclo-C17:0</td>
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<tr>
<td>Unsaturated</td>
</tr>
<tr>
<td>C15:0ω6c</td>
</tr>
<tr>
<td>C17:0ω8c</td>
</tr>
<tr>
<td>C18:1ω5c</td>
</tr>
<tr>
<td>Hydroxy</td>
</tr>
<tr>
<td>C11:0 ω3OH</td>
</tr>
<tr>
<td>C12:0 ω3OH</td>
</tr>
<tr>
<td>C13:0 ω2OH</td>
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<tr>
<td>C18:0 ω3OH</td>
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<td>iso-C11:0 ω3OH</td>
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<td>iso-C12:0 ω3OH</td>
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<td>iso-C13:0 ω3OH</td>
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<td>Summed feature*</td>
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<tr>
<td>1</td>
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<tr>
<td>3</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
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</tbody>
</table>

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 contained iso-C15:1ωH and/or C13:0ω3OH; summed feature 3 contained C16:1ω7c and/or C16:1ω6c; summed feature 8 contained C18:1ω7c and/or C18:1ω6c; summed feature 9 contained C16:0ω10-methyl and/or iso-C17:0ω9c.

http://ijs.sgmjournals.org
PCR with two universal primers (B8F: 5’-AGAGTTTGAGCTCTGGGTCAAG3’ and B1510: 5’-GGTTACCTTGTTACGACTT3’). For cloning and sequencing of the 16S rRNA gene, the PCR product was purified by TIANgel Midi Purification kit (TIANGEN Biotech), ligated into the pUCm-T vector (TaKaRa), cloned into Escherichia coli JM109T and sequenced at BGI (Qingdao, China). Pairwise similarity values between the isolates and closely related type strains were calculated using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). 16S rRNA gene sequences of related strains were downloaded from the NCBI database and aligned using the CLUSTAL X program (Thompson et al., 1997). Phylogenetic trees were reconstructed using neighbour-joining and maximum-likelihood methods with Kimura’s two-state parameter inferences, strains XH031T, XH038-3 and XH80-1 should be classified in the genus Luteimonas (Stackebrandt & Goebel, 1994). Moreover, a number of phenotypic characteristics (Table 1), namely motility, oxidase activity, production of hydrolytic enzymes, acetoin production and susceptibility to antibiotics, as well as fatty acid compositions (Table 2), clearly differentiated the novel isolates from recognized species of the genus Luteimonas.

On the basis of phenotypic characteristics and phylogenetic inferences, strains XH031T, XH038-3 and XH80-1 should be classified in the genus Luteimonas as representatives of a novel species, for which the name Luteimonas abyssi sp. nov. is proposed. The type strain is XH031T (=DSM 25880T=CGMCC 1.12611T).

**Description of Luteimonas abyssi sp. nov.**

*Luteimonas abyssi* (a.bys’i L. gen. n. abyssi from the abyss).

Cells are Gram-stain-negative, strictly aerobic, motile rods (0.2–0.4 µm in width, 1.0–1.6 µm in length) with single polar flagellum. Colonies on NA are yellow, convex,

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**Fig. 2.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing phylogenetic positions of *Luteimonas abyssi* sp. nov., other species of the genus *Luteimonas* and representatives of some other related members of the family Xanthomonadaeae. Bootstrap values >70% (1000 replicates) are shown at branch nodes. *Pseudomonas aeruginosa* LMG 1242T (GenBank accession no. Z76651) and *Escherichia coli* ATCC 11775T (X80725) were used as outgroups. Bar, 0.02 substitutions per nucleotide position.
circular, slightly transparent with entire margins and 1.0–1.5 mm in diameter after incubation for 2–3 days at 28 °C. Growth occurs at 4–37 °C (optimum 28 °C). The salinity range for growth is 0–11 % (w/v) NaCl in NB (optimum 0–3%), and the pH range is 6.0–8.0 (optimum pH 7.0). Oxidase- and catalase-positive. DNA, starch, gelatin, casein and Tween 20, 40 and 80 are hydrolysed. In the API 20E/20NE strip, there are positive results for trisodium citrate, gelatin, aesculin hydrolysis, assimilation of D-glucose, D-mannitol, N-acetylglucosamine, maltose and malic acid; a weakly positive result for D-mannose; variable results for arginine dihydrolase and phenylacetic acid (both negative for the type strain); and negative results for all other tests in the strips. In the API ZYM strip, alkaline phosphatase, esterase (C4), esterse lipase (C8), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-Bl-phosphohydrolase and α-galactosidase activities are present; valine arylamidase is variable (positive for the type strain); while lipase (C14), cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Acid is produced from aesculin, but not from other compounds in the API 50CH systems. Dextrin, glycogen, Tween 40, Tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-arabinose, D-arabitol, celllobiose, gentiobiose, α-D-glucose, maltose, D-mannitol, trehalose, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, citric acid, formic acid, β-hydroxybutyric acid, α-ketoglutaric acid, α-ketoleric acid, propionic acid, succinic acid, bromosuccinic acid, L-alaninamide, D-alanine, L-glutamic acid, glycolyl L-aspartic acid, glycolyl-L-glutamic acid and L-proline are utilized in the Biolog GN2 MicroPlate system. Susceptible to (μg per disc) streptomycin (10), nalidixic acid (30), kanamycin (30), ampicillin (10), gentamicin (10), tetracycline (30), novobiocin (30) and chloramphenicol (30); immediately susceptible to neomycin (30); and resistant to erythromycin (15), penicillin G (10) and lincomycin (2). The dominant fatty acids are isoo-C15:0 iso-C16:0 iso-C17:0 and C16:0-10-methyl and/or iso-C17:1ω9c. The major respiratory quinone is Q-8. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphasphatidylglycerol, and one unknown phospholipid.

The type strain, XH031^T (=DSM 25880^T=GCC 1.12611^T), and two additional strains XH038-3 and XH80-1 were isolated from deep-sea sediment of the South Pacific Gyre (41°51’S 153°06’W). The DNA G+C content is 70.2–73.9 mol% (70.2% for the type strain).

Acknowledgements

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Reference


