Persicitalea jodogahamensis gen. nov., sp. nov., a marine bacterium of the family ‘Flexibacteraceae’, isolated from seawater in Japan

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An obligately aerobic, Gram-negative, non-motile, pale-pink-pigmented, rod-shaped strain, designated Shu-9-SY12-35CT, was isolated from seawater in Jodogahama, Iwate, Japan, and was subjected to a polyphasic taxonomic examination. Phylogenetic analyses based on the 16S rRNA gene sequence revealed that the novel isolate was affiliated with the family ‘Flexibacteraceae’ of the phylum Bacteroidetes and that it showed highest sequence similarity (86.4 %) with Dyadobacter hamtensis HHS 11T. The novel isolate is phenotypically and physiologically different from strains described previously. The G+C content of the DNA was 56.3 mol%, MK-7 was the major menaquinone and iso-C15 : 0, C16 : 107c and iso-C17 : 0 3-OH were the major fatty acids. On the basis of polyphasic taxonomic studies, it was concluded that strain Shu-9-SY12-35CT represents a new genus and species of the family ‘Flexibacteraceae’, for which the name Persicitalea jodogahamensis gen. nov., sp. nov. is proposed. The type strain of Persicitalea jodogahamensis is Shu-9-SY12-35CT (=MBIC07417T=IAM 15412T=KCTC 12866T).

A globally distributed, environmentally abundant group of micro-organisms, the phylum Bacteroidetes (Ludwig & Klenk, 2001; Garrity & Holt, 2001) is known to play an important role in the biogeochemical cycling and degradation of organic compounds such as chitin, cellulose (Cottrell & Kirchman, 2000) and agar (Nedashkovskaya et al., 2003). Members of the phylum Bacteroidetes constitute a significant proportion of marine microbial communities (Glockner et al., 1999) and are associated with the decay of planktonic blooms (Brettar et al., 2004). According to the second edition of Bergey’s Manual of Systematic Bacteriology, the largest family belonging to the class ‘Sphingobacteria’, ‘Flexibacteraceae’, consists of 10 published genera (Ludwig & Klenk, 2001). Recently, a number of new genera such as Arcicella, Aquifexum, Dyadobacter and Larkinella (Chelius & Triplett, 2000; Brettar et al., 2004; Nikitin et al., 2004; Vancanneyt et al., 2006) have been described as members of this family.

Strain Shu-9-SY12-35CT was isolated from seawater at Jodogahama Beach, Iwate, Japan, in 2003, after incubation at 25 °C on SY agar, containing [1T−1] Daigo’s artificial seawater SP (Nihon Seiyaku), pH 7.5 [136 μg KH2PO4, 53.5 μg NH4Cl, 19.8 mg MgCl2, 10 mg sodium succinate, 10 mg sodium pyruvate, 10 mg glycerol, 10 mg yeast extract and 19.8 mg sodium l-ascorbate. In the present study, we attempted to elucidate the phylogenetic position of strain Shu-9-SY12-35CT using a polyphasic taxonomic approach, including 16S rRNA gene sequence analysis. In parallel, we performed physiological, biochemical and chemotaxonomic analyses to characterize this novel isolate. Based on these data, we suggest that the isolate represents a novel genus of the family ‘Flexibacteraceae’ in the phylum Bacteroidetes.

The temperature and pH ranges for growth were determined by incubation in 1/5-strength marine agar 2216 (1/5 MA; Difco). Growth in peptone water (0.2 % peptone, 0.1 % NaCl, pH 7.2) was determined after 10 days of incubation. Gram-staining was performed as described by Murray et al. (1994). Cell morphology was observed using light microscopy (BX60; Olympus). Growth under anaerobic conditions was determined after 2 weeks of incubation in an AnaeroPack (Mitsubishi Gas Chemical Co., Inc.) on 1/5 MA. Catalase activity was determined by bubble formation in a 3 % H2O2 solution and oxidase activity was determined with cytochrome oxidase paper (Nissui Pharmaceutical). API 20E and API 50CH strips (bioMérieux) were used to determine the physiological and biochemical characteristics of the strain; strips were read after incubation for 48 h at 25 °C. Flexirubin-like pigments were tested for by measuring the absorbance spectrum of ethanol and alkaline-ethanol extracts of lysed cells (Weeks, 1981). Determination of the respiratory quinone system and cellular fatty acid composition (MIDI System) were carried out as described previously (Xie & Yokota, 2003).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Shu-9-SY12-35CT is AB272165.
DNA was prepared following the method described by Marmur (1961) from cells grown on 1/5 MA, and the DNA base composition was determined by the HPLC method of Mesbah et al. (1989). A fragment of approximately 1500 bp of the 16S rRNA gene was amplified from the extracted DNA using universal bacterial primers 27F and 1492R specific to the 16S rRNA gene (Weisburg et al., 1991). To ascertain the phylogenetic position of the new isolate, the 16S rRNA gene sequence of strain Shu-9-SY12-35CT was compared with sequences obtained from GenBank. Multiple alignments of the sequences were performed using CLUSTAL_X (version 1.83) (Thompson et al., 1997). Alignment gaps and ambiguous bases were not taken into consideration in the comparison of 1328 bases of the 16S rRNA gene. Phylogenetic relationships were analysed using the same software. Distances were calculated using the Kimura two-parameter model (Kimura, 1980). Clustering based on the neighbour-joining method (Saitou & Nei, 1987) was determined using bootstrap values based on 1000 replicates (Felsenstein, 1985). Similarity values were calculated using MEGA3 (Kumar et al., 2004).

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain Shu-9-SY12-35CT belongs to the family ‘Flexibacteraceae’ and shows the highest sequence similarity (86.4 %) to Dyadobacter hamtensis HHS 11T (Chaturvedi et al., 2005), followed by Dyadobacter ginsengisoli Gsoil 043T (86.1 %; Liu et al., 2006), Dyadobacter fermentans NS114T (85.9 %; Chelius & Triplett, 2000) and Dyadobacter crusticola CP183-8T (85.7 %; Reddy & Garcia-Pichel, 2005). All other species of the family ‘Flexibacteraceae’ with validly published names were related more distantly, showing 16S rRNA gene sequence similarity of less than 80 %. Thus, on the basis of phylogenetic data presented, we believe that strain Shu-9-SY12-35CT should be identified as representing a novel genus and species of the family ‘Flexibacteraceae’ within the phylum Bacteroidetes, for which we propose the name Persicitalea jodogahamensis gen. nov., sp. nov.

Strain Shu-9-SY12-35CT, an obligately aerobic bacterium, was isolated from seawater and was found to be able to tolerate 7 % NaCl, while its closest described relatives within the ‘Flexibacteraceae’ can only tolerate 1–1.5 % NaCl (except D. hamtensis JCM 12919T; Table 1). Furthermore, strain Shu-9-SY12-35CT could be distinguished by the following characteristics: flexirubin reaction, growth on peptone water, growth at 37 °C and its ability to hydrolyse agar. Differential characteristics were also obtained from the API 50CH results, including acid production from L-arabinose, fructose and lactose (Table 1).

Based on the results of phylogenetic analysis and the physiological and biochemical properties of strain Shu-9-SY12-35CT, we define it as the type strain of a novel species in a new genus belonging to the ‘Flexibacteraceae’ within the phylum Bacteroidetes, for which we propose the name Persicitalea jodogahamensis gen. nov., sp. nov.
Table 1. Characteristics that differentiate strain Shu-9-SY12-35C<sup>T</sup> from related taxa

Strains: 1, Shu-9-SY12-35C<sup>T</sup>; 2, D. crusticola DSM 16708<sup>T</sup>; 3, D. fermentans ATCC 700827<sup>T</sup>; 4, D. ginsengioli KCTC 12589<sup>T</sup>; 5, D. hamtensis JCM 12919<sup>T</sup>; 6, Runella slytherin ATCC 29530<sup>T</sup>; 7, Runella zeae ATCC BAA-293<sup>T</sup>. Data are from this and earlier studies (Larkin & Williams, 1978; Chelius & Triplett, 2000; Chelius et al., 2002; Chaturvedi et al., 2005; Reddy & Garcia-Pichel, 2005; Liu et al., 2006; Vancanneyt et al., 2006). +, Positive; w, weakly positive; −, negative; ND, no data.

<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>
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<tr>
<td>Isolation source</td>
<td>Seawater</td>
<td>Soil</td>
<td>Plants</td>
<td>Soil</td>
<td>Glacial water</td>
<td>Freshwater</td>
<td>Plants</td>
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<tr>
<td>Colony colour</td>
<td>Pale pink</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Light yellow</td>
<td>Pale pink</td>
<td>Salmon</td>
</tr>
<tr>
<td>Cell morphology</td>
<td>Straight rods</td>
<td>Curved or straight rods</td>
<td>Rods, long filaments</td>
<td>Rods</td>
<td>Long rods</td>
<td>Curved rods</td>
<td>Straight or bent rods</td>
</tr>
<tr>
<td>Cell length (µm)</td>
<td>3–6</td>
<td>0.6–1</td>
<td>0.75–2</td>
<td>3–6</td>
<td>ND</td>
<td>2.5–4</td>
<td>ND</td>
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<td>Flexirubin reaction</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>−</td>
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<td>Growth on peptone water</td>
<td>−</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Growth at 37 °C</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>−</td>
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<td>Highest NaCl tolerance (% w/v)</td>
<td>7</td>
<td>1</td>
<td>1.5</td>
<td>1</td>
<td>11.6</td>
<td>1</td>
<td>1.5</td>
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<td>Hydrolysis of agar</td>
<td>W</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>Acid production from:</td>
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<tr>
<td>l-Arabinose</td>
<td>W</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
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<td>Fructose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>−</td>
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<td>ND</td>
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<td>Lactose</td>
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<td>−</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
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<td>DNA G+C content (mol%)</td>
<td>56.3</td>
<td>48</td>
<td>48</td>
<td>48</td>
<td>49</td>
<td>49.6</td>
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Description of Persicitalea gen. nov.

Persicitalea (Per.si.ci.ta’le.a. L. n. persicum a peach; L. suff. -icus - a - um suffix used with the sense of belonging to; L. fem. n. talea a slender staff, a rod; N.L. fem. n. Persicitalea peach-coloured rod, because the colonies are peach-coloured).

Cells are straight-rod-shaped, Gram-negative and obligately aerobic. Cells lack flagella and are non-motile. No formation of endospores. Catalase- and oxidase-positive. Nitrate and nitrite reduction are negative. Flexirubin-type pigment is absent. The major respiratory quinone is MK-7. The G+C content of the genomic DNA of the type strain of the type species is 56.3 mol%. Predominant cellular fatty acids are iso-C<sub>15:0</sub>, C<sub>16:1</sub>ω7c and iso-C<sub>17:0</sub> 3-OH. The type species is Persicitalea jodogahamensis.

Description of Persicitalea jodogahamensis sp. nov.

Persicitalea jodogahamensis (jo.do.ga.ha.men’sis. N.L. fem. adj. jodogahamensis pertaining to Jodogahama, a beach located on Iwate in Japan, where the type strain was isolated).

The main characteristics are the same as those given for the genus. In addition, cells are 0.3–0.5 µm wide and 3–6 µm long. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on 1/5 MA are circular, convex and pale-pink-pigmented. The optimum temperature for growth is 25–30 °C; no growth occurs at 4 or 45 °C. The pH range for growth is 5–10, with an optimum around neutral pH. NaCl is not required for growth but can be tolerated in a solution of up to 7 % (w/v). Agar is hydrolysed but urea, starch and gelatin are not. Voges–Proskauer and o-nitrophenyl β-D-galactosidase (ONPG) tests are positive, but reactions for citrate utilization, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, hydrogen sulfide and indole production are negative. Acid is produced from D-arabinose, L-arabinose, ribose, D-xylene, L-xylene, methyl β-D-xylonopyranoside, galactose, glucose, fructose, mannose, rhamnose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, aesculin ferric citrate, salicin, cellulose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, inulin, rhamnose, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose and L-fucose, but not from glycerol, erythritol, adonitol, sorbose, dulcitol, inositol, mannitol, sorbitol, starch, glycogen, xylitol, D-arabitol, L-arabitol, glucosone, 2-ketogluconate or 5-ketogluconate. Major fatty acid components (> 3.0 %) include iso-C<sub>15:0</sub> (15.7 %), C<sub>16:1</sub>ω7c (44.9 %), C<sub>16:1</sub>ω5c (7.1 %), C<sub>16:0</sub> (4 %) and iso-C<sub>17:0</sub> 3-OH (11 %).

The type strain is Shu-9-SY12-35C<sup>T</sup> (= MBIC07417<sup>T</sup> = IAM 15412<sup>T</sup> = KCTC 12866<sup>T</sup>), which was isolated from seawater at Jodogahama, Iwate, Japan.

Acknowledgements

The authors would like to thank Atsuko Katsuta, Chiaki Komukai, Ayako Matsuzaki, Tomomi Haga and Yukiko Itazawa for their technical assistance. This work was supported by the New Energy and Industrial Technology Development Organization (NEDO).

References


