Marihabitans asiaticum gen. nov., sp. nov., a meso-diaminopimelic acid-containing member of the family Intrasporangiaceae

Akiko Kageyama,1 Tomomi Haga,2 Hiroaki Kasai,2 Yoshikazu Shizuri,2 Satoshi Ōmura1,3 and Yoko Takahashi1

1Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan
2Marine Biotechnology Institute, 3-75-1 Heita, Kamaishi, Iwate 026-0001, Japan
3The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan

Strain HG667T, isolated from surface seawater collected at the Kesennuma ferry port in Miyagi Prefecture, Japan, was found to be a Gram-positive, catalase-positive bacterium comprising irregular short rods and cocci. The diagnostic diamino acid in the cell-wall peptidoglycan was meso-diaminopimelic acid. The major menaquinone was MK-8(H4). Mycolic acids were not detected. The G+C content of the DNA was 70 mol%. Analysis of the 16S rRNA gene sequence revealed that the strain represents a novel lineage within the family Intrasporangiaceae, order Actinomycetales, being associated with the genus Kribbia. On the basis of morphological, biochemical and chemotaxonomic properties of the strain, together with phylogenetic data relating to the 16S rRNA gene sequence, HG667T represents a novel genus and species in the family Intrasporangiaceae, for which the name Marihabitans asiaticum gen. nov., sp. nov. is proposed. The type strain of Marihabitans asiaticum is HG667T (=MBIC07497T =DSM 18935T).

The family Intrasporangiaceae was proposed by Rainey et al. (in Stackebrandt et al., 1997) and its description was emended by Stackebrandt & Schumann (2000). The genera belonging to this family can be divided into three groups on the basis of the diagnostic diamino acid types in the cell-wall peptidoglycan: the LL-diaminopimelic acid (LL-A2pm) type, the meso-A2pm type and the L-ornithine type. The genera Janibacter (Martin et al., 1997), Knoellia (Groth et al., 2002), Kribbia (Jung et al., 2006), Oryzihumus (Kageyama et al., 2005), Physicoccus (Lee, 2006) and Tetrasphaera (Maszenan et al., 2000) belong to the meso-A2pm group. The strain described in the present study, HG667T, also contained meso-A2pm as the diagnostic diamino acid in the cell-wall peptidoglycan.

Strain HG667T was isolated from surface seawater collected in November 2003 at the Kesennuma ferry port in Miyagi Prefecture, Japan (GPS location: 38°33′52″N 141°35′35″E). A colony was picked from 1/10-strength marine agar 2216 (Difco) after incubation for 1 month at 27 °C. The cell morphology of the strain was studied with a light microscope during growth (at 21 h and at 2, 3, 4 and 5 days) in marine broth at 27 °C. Bacterial cells (3-day-old culture on MA) were also studied with a scanning electron microscope (model JSM-5600; JEOL). Motility was investigated using microscopic analysis. Colonies were suspended in artificial seawater (Lyman & Fleming, 1940) after incubation for 3 days, 1 week and 1 month at 27 °C on MA medium. NaCl tolerance was determined on 1/5-strength nutrient agar and pH and temperature ranges for growth were determined on MA. The ability of the strain to grow on various substrates serving as sole carbon sources (at 1 %, w/v) was determined using a carbon-utilization medium (Pridham & Gottlieb, 1948) (Nihon Pharmaceutical). The isolate was characterized biochemically using API ZYM (bioMérieux) in accordance with the manufacturer’s instructions.

Whole-cell hydrolysates were analysed for A2pm isomers by using TLC (Becker et al., 1965; Hasegawa et al., 1983). Purified cell walls were obtained using the method of Kawamoto et al. (1981). Purified cell wall (1 mg) was hydrolysed at 100 °C with 1 ml 6 M HCl for 16 h. The residue was dissolved in 100 μl water and then used for amino acid analysis by TLC. Sugars in the purified cell wall broth (Difco) at 27 °C for 3 or 4 days and then harvesting them by centrifugation.
were determined according to the procedures described by Stanek & Roberts (1974). The presence of mycolic acid was checked using the TLC method of Tomiyasu (1982). Menaquinones were extracted and purified using the method of Collins et al. (1977) and then analysed by HPLC (model 802-SC; Jasco) on a chromatograph equipped with a Capcell Pak C18 column (Shiseido) (Tamaoka et al., 1983). Polar lipids were extracted according to the method described by Minnikin et al. (1984). The two-dimensional TLC method (Komagata & Suzuki, 1987) was used to determine the phospholipid profile. To determine the cellular fatty acid methyl esters, cell mass of strain HG667T was harvested from TSA plates after cultivation for 3 days at 30 °C and subjected to analysis using GLC (model HP6890; Hewlett Packard). The method described in the manual (version 6) of the Sherlock Microbial Identification System (version 5.0) (MIDI) was used for sample preparation and analysis.

To determine the G+C content, chromosomal DNA was isolated as described by Saito & Miura (1963) and the content estimated using HPLC (Tamaoka & Komagata, 1984).

DNA for 16S rRNA gene sequence analysis was prepared using an InstaGene matrix (Bio–Rad). The 16S rRNA gene was amplified by using the PCR with a forward primer corresponding to positions 8–27 and a reverse primer corresponding to positions 1492–1510 (Escherichia coli numbering system; Weisburg et al., 1991) and sequenced using an automatic sequence analyser (3730 DNA analyser; Applied Biosystems) with the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Species related to the novel strain were identified by performing sequence database searches with BLAST (Altschul et al., 1990). Sequence data for related species were retrieved from GenBank and used for a detailed phylogenetic analysis. The phylogenetic analysis was performed using CLUSTAL W software (Thompson et al., 1994). Nucleotide substitution rates (K_{nucl} values) were calculated (Kimura & Ohta, 1972) and phylogenetic trees were constructed using the neighbour-joining method as described by Saitou & Nei (1987). The DNAML program in the PHYLIP 3.5c package (Felsenstein, 1985) was used for maximum-likelihood analysis, with a default transition/transversion ratio of 2.000000. Sequence similarity values were determined by means of visual comparison and manual calculation.

The cells of strain HG667T were aerobic, Gram-positive, non-spore-forming, irregular short rods or cocci (Fig. 1). Observation using light microscopy showed that the cells occur singly and also in pairs, short chains or clumps. No distinct life cycle and no changes in cell morphology were observed under the light microscope during growth (for 21 h to 5 days) in marine broth. Motility was not observed under the conditions used. The DNA G+C content of the strain was 70 mol%. The cell wall contained meso-A2pm as the diagnostic diamino acid. The cell-wall sugars were galactose, glucose and ribose. The major menaquinone (constituting about 90 %) was MK-8(H4). The phospholipids were phosphatidyglycerol, an unknown phospholipid, diphosphatidylglycerol and phosphatidylinositol mannosides. The cellular fatty acids were iso-C17:0 (38.5 %), C17:1ω9c (19.3 %), C18:1ω9c (10.5 %), iso-C15:0 (7.4 %), iso-C17:1ω9c (5.2 %), C16:0 (3.0 %), C17:0 (2.7 %), C18:1ω7c (1.7 %), iso-C16:0 (1.1 %) and anteiso-C17:0 (1.1 %).

To determine the phylogenetic position of strain HG667T, an almost-complete 16S rRNA gene sequence was determined. A database search demonstrated that the strain belonged to the family Intrasporangiaceae. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain HG667T (1435 bp) was closely related to Kribbia dieselivorans N113T (1476 bp), with 96.0 % similarity (Fig. 2). The 16S rRNA gene sequence identities of HG667T with respect to type strains of type species of other genera with meso-A2pm in the cell wall were as follows: 94.9 % with Janibacter limosus DSM 11140T (1473 bp), 94.8 % with Knoellia sinensis DSM 12331T (1479 bp), 92.8 % with Oryzihumus leptocrescens KV-628T (1429 bp), 93.4 % with Physicoccus jejensis KSW2-15T (1413 bp) and 93.0 % with Tetrasphaera japonica ACM 5116T (1445 bp).

Strain HG667T can be differentiated from other genera of the family Intrasporangiaceae on the basis of its cell-wall diamino acid, cell morphology and major fatty acid composition. Table 1 shows the phenotypic characteristics of the isolate and of meso-A2pm-containing members of the family Intrasporangiaceae. The phylogenetically closest genus is Kribbia, members of which also contain meso-A2pm. Strain HG667T can be differentiated from the genus Kribbia on the basis of major fatty acids and growth temperature. While strains of the genus Kribbia can grow over a wide range of temperatures (8–42 °C), strain HG667T grows at 18–34 °C. Strain HG667T can be differentiated from the genera Janibacter, Knoellia, Tetrasphaera and Physicoccus on the basis of cell morphology and the pattern of phospholipids. The differential

Fig. 1. Scanning electron micrograph of cells from a 3-day-old culture of strain HG667T grown on MA medium at 27 °C. Bar, 2 μm.
characteristic between strain HG667 and the genus Oryzihumus is cell morphology. Other characteristics of strain HG667 are given in the genus and species descriptions.

On the basis of the data presented here, we consider that strain HG667 merits description as a novel genus and species within the family Intrasporangiaceae, for which the name Marihabitans asiaticum gen. nov., sp. nov. is proposed.

Table 1. Differential characteristics of strain HG667 and meso-Apm-containing members of the family Intrasporangiaceae

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Cell morphology</th>
<th>Major fatty acids*</th>
<th>Polar lipids†</th>
<th>DNA G+C content (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain HG667</td>
<td>Irregular short rods and cocci</td>
<td>i-C_{17:0}, C_{17:1}, C_{18:0}, C_{18:1}</td>
<td>DPG, PG, PL, PIMs</td>
<td>70</td>
</tr>
<tr>
<td>Kribbia</td>
<td>Irregular short rods or cocci</td>
<td>10-Methyl C_{18:0}, C_{17:0}, C_{17:1}</td>
<td>ND</td>
<td>69–70</td>
</tr>
<tr>
<td>Janibacter</td>
<td>Coccoid to rod-shaped</td>
<td>i-C_{16:1}, C_{17:0}, C_{18:0}</td>
<td>DPG, PG, PI</td>
<td>69–73</td>
</tr>
<tr>
<td>Knoellia</td>
<td>Irregular rods and cocci</td>
<td>i-C_{16:0}, i-C_{17:0}, i-C_{18:0}</td>
<td>DPG, PE, PG, PL, PL§</td>
<td>68–69</td>
</tr>
<tr>
<td>Oryzihumus</td>
<td>Irregular rods</td>
<td>i-C_{16:0}, i-C_{17:0}, i-C_{18:0}</td>
<td>ND</td>
<td>72–73</td>
</tr>
<tr>
<td>Phycicoccus</td>
<td>Cocci</td>
<td>i-C_{16:0}, i-C_{17:0}, i-C_{18:0}</td>
<td>DPG, PE, PI</td>
<td>74</td>
</tr>
<tr>
<td>Tetrasphaera</td>
<td>Cocci or short rods</td>
<td>ai-C_{15:0}, i-C_{16:0}, i-C_{17:0}</td>
<td>DPG, PG, PI, PL§</td>
<td>68–71</td>
</tr>
</tbody>
</table>

*Major fatty acids are defined as constituting >10% of the total fatty acid content. ai, Anteiso; i, iso.
†DPG, Diphostidglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PL, unknown phospholipid(s); PIMs, phosphatidylinositol mannosides.
§Data for: a, T. elongata; b, T. australiensis; c, T. japonica.
§Detected in only some representatives of the genus.
casein and gelatin. Negative for urease activity, starch hydrolysis and oxidase activity. D-Galactose, D-glucose and trehalose are assimilated, but L-arabinose, D-fructose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, sucrose and D-xylene are not assimilated. Esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, acid phosphatase, \( \alpha \)-glucosidase and \( \beta \)-glucosidase are detected in the API ZYM enzyme assay; tests for alkaline phosphatase, lipase (C14), valine arylamidase, trypsin, chymotrypsin, \( \alpha \)-galactosidase, \( \beta \)-galactosidase, \( \beta \)-glucuronidase, N-acetyl-\( \beta \)-glucosaminidase, \( \varepsilon \)-mannosidase and \( \varepsilon \)-fucosidase are negative. Weakly positive for naphthol-AS-BI-phosphohydrolase. The DNA G+C content of the type strain is 70 mol%.

The type strain, HG667\(^T\) (=MBIC07497\(^T\) =DSM 18935\(^T\)), was isolated from surface seawater collected at the Kesennuma ferry port in Miyagi Prefecture, Japan.

**Acknowledgements**

This study was supported, in part, by a grant from the 21st Century COE Program of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), by a JSPS Grant-in-Aid for Science Research and by the New Energy and Industrial Technology Development Organization (NEDO).

**References**


