Marinomonas ostreistagni sp. nov., isolated from a pearl-oyster culture pond in Sanya, Hainan Province, China

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A Gram-negative, aerobic, halophilic, neutrophilic, rod-shaped, non-pigmented, polar-flagellated bacterium, UST010306-043T, was isolated from a pearl-oyster culture pond in Sanya, Hainan Province, China in January 2001. This marine bacterium had an optimum temperature for growth of between 33 and 37 °C. On the basis of 16S rRNA gene sequence analysis, the strain was closely related to Marinomonas aquimarina and Marinomonas communis, with 97.5–97.7 and 97.1 % sequence similarity, respectively. Levels of DNA–DNA relatedness to the type strains of these species were well below 70 %. Analyses of phylogenetic, phenotypic and chemotaxonomic characteristics showed that strain UST010306-043T was distinct from currently established Marinomonas species. A novel species with the name Marinomonas ostreistagni sp. nov. is proposed to accommodate this bacterium, with strain UST010306-043T (≡ JCM 13672T ≡ NRRL B-41433T) as the type strain.

Van Landschoot & De Ley (1983) showed that, based on rRNA gene sequences, the genus Alteromonas (Baumann et al., 1972) was composed of four distinct branches and reclassified the branch containing Alteromonas vaga and Alteromonas communis as the genus Marinomonas. At the time of writing, the genus comprises eight species with validly published names: Marinomonas vaga, Marinomonas communis (Baumann et al., 1972; Van Landschoot & De Ley, 1983), Marinomonas mediterranea (Solano & Sanchez-Amat, 1999), Marinomonas primoryensis (Romanenko et al., 2003), Marinomonas pontica (Ivanova et al., 2005), Marinomonas ushuaiensis (Prabagaran et al., 2005), Marinomonas aquimarina (Macián et al., 2005) and Marinomonas dokdonensis (Yoon et al., 2005).

In this study, we report a novel strain, UST010306-043T, which was isolated from a sea-water sample from a pearl-oyster culture pond in the HKUST-CAS Joint Laboratory in Sanya City, Hainan Province, China, on 6 January 2001. Based on polyphasic taxonomy, we propose that strain UST010306-043T represents a novel species, Marinomonas ostreistagni sp. nov.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UST010306-043T is AB242868.

A figure showing a slot-blot DNA–DNA hybridization assay with DNA of strain UST010306-043T as the probe is available as supplementary material in IJSEM Online.
was tested in artificial sea water (ASW; 0·1 % CaCl₂, 2H₂O, 0·1 % KCl, 0·5 % MgSO₄, 7H₂O, 2·5 % NaCl) (Lewin & Lounsbery, 1969), with NaCl replaced by equal molar KCl and addition of 0·4 % yeast extract. Tolerance to NaCl was tested in ASW supplemented with 0·4 % yeast extract and with NaCl added at final concentrations of 0, 1, 2, 3, 4, 5, 7·5, 10 and 15 %. Anaerobic growth was examined by using the Oxoide anaerobic system for cultures grown on MA supplemented with 0·1 % sodium nitrate or 0·1 % glucose. Cellular morphology was determined from a MB overnight culture using a Zeiss MC100 Spot microscope at a magnification of × 1000. Flagella morphology was examined by using transmission electron microscopy (JOEL 100CXII at 80 kV), using cells placed on a carbon grid and stained with 1 % (w/v) phosphotungstic acid (pH 7·0).

Gram staining was performed according to Collins et al. (1989). Tests for requirement of organic growth factors, utilization of various carbon sources and chitin, fermentation of glucose, sucrose or D-mannitol and accumulation of poly-β-hydroxybutyrate were performed according to Baumann & Baumann (1981). Alkaline phosphatase activity, H₂S generation from cysteine or thiosulfate, oxidation of glucose and hydrolysis of starch, casein, gelatin and cellulose were determined according to Smibert & Krieg (1994). Fatty acid methyl ester analysis was performed as described by Svetashev et al. (1995), except that the bacterial strains were cultured at 30 °C for 24 h. DNA hydrolysis test and sequencing of the nearly complete 16S rRNA gene were performed as described by Lau et al. (2005). Related sequences were identified by MEGABLAST search (Zhang et al., 2000), retrieved from the NCBI nucleotide database, aligned using CLUSTAL X (Thompson et al., 1997), edited with BioEdit version 5.0.9 (Hall, 1999) and analysed using MEGA version 2.1 (Kumar et al., 2001). A phylogenetic tree was generated by using the neighbour-joining method (Saitou & Nei, 1987) with the Jukes–Cantor substitution model (Jukes & Cantor, 1969) and was evaluated by bootstrap analysis (Saitou & Nei, 1987) with equal molar KCl and addition of 0·4 % yeast extract. Tolerance to NaCl was tested in ASW supplemented with 0·4 % yeast extract and with NaCl added at final concentrations of 0, 1, 2, 3, 4, 5, 7·5, 10 and 15 %. Anaerobic growth was examined by using the Oxoide anaerobic system for cultures grown on MA supplemented with 0·1 % sodium nitrate or 0·1 % glucose. Cellular morphology was determined from a MB overnight culture using a Zeiss MC100 Spot microscope at a magnification of × 1000. Flagella morphology was examined by using transmission electron microscopy (JOEL 100CXII at 80 kV), using cells placed on a carbon grid and stained with 1 % (w/v) phosphotungstic acid (pH 7·0).

For DNA–DNA hybridization experiments, genomic DNA was extracted according to Maloy (1990) with modifications: 1·5 ml overnight culture was centrifuged and resuspended in 467 μl TE buffer, containing 30 μl SDS (10 %, w/v) and 3 μl proteinase K (20 mg ml⁻¹), incubated at 37 °C for 1 h and then extracted twice with equal amounts of phenol/chloroform/isoamyl alcohol (25 : 24 : 1, by vol.). DNA was precipitated with 0·1 vol. 3 M sodium acetate (pH 5·2) and 0·6 vol. 2-propanol. The precipitate was washed with 0·5 ml 70 % ethanol, dried in a vacuum, dissolved in 100 μl TE buffer with RNase (100 μg ml⁻¹) and incubated at 37 °C for 1 h. DNA was diluted in TE buffer to 0·5–1·0 ng μl⁻¹ and run in an agarose gel (1 %) with a λ HindIII marker (100 ng; TaKaRa). Quantification of DNA was done by comparing the band intensities of isolated DNA and that of the marker using the Spot Denso function of the IS-1000 Digital Imaging System (Alpha Innotech). Slot-blot analysis of DNA was performed according to Brown (2005) with Hybond-N+ membrane (Millipore). One or 10 ng genomic DNA of each strain was loaded per well. One hundred nanogram probe DNA was labelled using the Gene Image random prime labelling module (Amersham), according to the manufacturer’s instructions. Overnight hybridization was performed at 60 °C. The membrane was washed three times with 1 × SSC/0·1 % SDS and three times with 0·5 × SSC/0·1 % SDS at 60 °C (each washing step lasted for 10 min), detected using a Gene Image CDP-Star detection module (Amersham) according to the manufacturer’s instructions and exposed to a 100NIF Xray film (Fuji) for 15 min. Levels of hybridization were determined with EagleSight software version 3·2 in the Eagle Eye II system (Stratagene). The DNA G+C content was determined by using the HPLC method (Mesbah et al., 1989), with genomic DNA extracted with a MiniBEST Bacterial Genomic DNA Extraction kit (TaKaRa). Susceptibility to antibiotics was tested by using the disc-diffusion plate method on MA. The following antibiotics were tested: ampicillin (10 μg), polymyxin B (300 U), chloramphenicol (30 μg), tetracycline (30 μg), streptomycin (10 μg), rifampicin (10 μg) and penicillin G (2 U).

### Table 1. Whole-cell fatty acid profiles of strain UST010306-043T and related members of the genus Marinomonas

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Straight-chain fatty acids:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:0</td>
<td>5–4</td>
<td>5–2</td>
<td>3–2</td>
</tr>
<tr>
<td>12:0</td>
<td>5–7</td>
<td>5–0</td>
<td>5–4</td>
</tr>
<tr>
<td>14:0</td>
<td>4–2</td>
<td>3–0</td>
<td>3–0</td>
</tr>
<tr>
<td>16:0</td>
<td>15–6</td>
<td>13–0</td>
<td>10–4</td>
</tr>
<tr>
<td>18:0</td>
<td>1–14</td>
<td>1–3</td>
<td>1–4</td>
</tr>
<tr>
<td><strong>Unsaturated fatty acids:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:1ω8c</td>
<td>0–0</td>
<td>0–0</td>
<td>0–2</td>
</tr>
<tr>
<td>18:1ω7c</td>
<td>39–5</td>
<td>40–4</td>
<td>41–8</td>
</tr>
<tr>
<td><strong>Hydroxy fatty acids:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:0 3-OH</td>
<td>7–8</td>
<td>9–2</td>
<td>10–5</td>
</tr>
<tr>
<td>12:0 3-OH</td>
<td>0–0</td>
<td>0–2</td>
<td>0–0</td>
</tr>
<tr>
<td><strong>Summed features:</strong>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19–7</td>
<td>22–4</td>
<td>24–0</td>
</tr>
<tr>
<td>2</td>
<td>1–0</td>
<td>0–2</td>
<td>0–0</td>
</tr>
<tr>
<td>Unknown ECL 11-799</td>
<td>0–0</td>
<td>0–1</td>
<td>0–0</td>
</tr>
</tbody>
</table>

*1, 16:1ω7c/15:0 iso 2-OH; 2, 19:1ω6c/19:0 cyclo/unknown ECL 18:846.
Morphological, physiological and biochemical features of UST010306-043<sup>T</sup> are given in the species description. Strain UST010306-043<sup>T</sup> shared many similarities with other Marinomonas species. Strains were all Gram-negative, isolated from marine habitats, had rod-shaped cells, were halophilic, strictly aerobic, heterotrophic, non-spore-forming and non-fermentative, utilized glucose and acetate and did not require complex organic growth factors. Strain UST010306-043<sup>T</sup> shared many similarities with M. aquimarina; in particular, their similarity in habitat (the former was found in sea water of a pearl-oyster pond and the latter was found in oyster flesh and sea water), growth up to 40 °C but not at 4 °C, oxidative response to glucose and inability to utilize maltose and D-sorbitol. The G+C content of the DNA of strain UST010306-043<sup>T</sup> was 49.8±0.5 mol%, which was close to the highest G+C content of members of the genus Marinomonas (Sanchez-Amat & Solano, 2005). The fatty acids of strain UST010306-043<sup>T</sup> and closely related species are shown in Table 1. The fatty acid profile of strain UST010306-043<sup>T</sup> was very similar to that of M. aquimarina CECT 5080<sup>T</sup>, but differed from that of M. communis CECT.
Cells are Gram-negative rods, 0.6–3.6 μm in length and 0.4–0.6 μm in diameter and motile by a single polar flagellum. Cells divide by binary fission. Heterotrophic, strictly aerobic and requires sodium ions for growth. Cell aggregates are detected when cultured in MB. Colonies are non-pigmented, circular, opaque, convex, 1–2 mm in diameter with an entire margin after 24 h incubation at 30 °C on MA. Grows in 1–10 % NaCl but not in 15 % NaCl; optimum NaCl for growth is 3–7 %. Grows at pH 4–0, but not at 4 or 42 °C; optimum temperature for growth is 33–37 °C. Grows at pH 5–0–9, with optimum growth occurring around pH 7.0. Oxidase-, catalase- and alkaline phosphatase-positive. Oxidizes glucose. Does not produce sulfide from cysteine or thiosulfate and does not degrade extracellular DNA. Does not form endospores and does not accumulate poly-β-hydroxybutyrate. Utilizes glucose, succrose, D-mannitol, aesculin, L-alanine, L-asparagine, L-serine, L-glutamic acid, acetate, citrate, pyruvate, propionate, lactate, ethanol and Tween 20, but not galactose, maltose, L-arabinose, trehalose, rhamnose, lactose, melibiose, D-sorbitol, methanol, glycerol, succinate, malate, glycine, L-leucine, L-arginine, L-lysine, L-histidine, L-methionine, L-threonine, valine, starch, gelatin, casein, agar, DNA, Tween 40, Tween 60, Tween 80 or cellulose. Does not ferment glucose, succrose or D-mannitol. Sensitive to ampicillin (10 μg), polymyxin B (300 U), chloramphenicol (30 μg), tetracycline (30 μg), streptomycin (10 μg) and rifampicin (10 μg), but resistant to penicillin G (2 U).

Phylogenetic analysis showed that strain UST010306-043T formed a distinct taxon within the genus Marinomonas and was closely related with M. aquimarina CECT 5080T with a bootstrap value of 90 % (Fig. 1). The 16S rRNA gene sequence of strain UST010306-043T shared 94.1–97.7 % similarity with other members of the genus Marinomonas, being most closely related to M. aquimarina (97.5–97.7 %) and M. communis (97.1 %). Its similarity to other species of Marinomonas was less than 97 %, indicating that they were different species (Stackebrandt & Goebel, 1994). As the 16S rRNA gene similarity of strain UST010306-043T with M. aquimarina and M. communis was at the borderline for discrimination of species by sequence analysis (Stackebrandt & Goebel, 1994), DNA–DNA hybridization, which has significantly higher resolution power than sequence analysis (Amann et al., 1992), was performed. The results of DNA–DNA hybridization (available as Supplementary Fig. S1 in IJSEM Online) showed that the levels of relatedness of strain UST010306-043T with M. aquimarina CECT 5080T and M. communis CECT 5003T were below 23 %. This was well below the 70 % threshold value for species discrimination (Wayne et al., 1987), suggesting that strain UST010306-043T represents a species that is different from M. aquimarina or M. communis. On the basis of all characteristics described above, a novel species with the name Marinomonas ostreistagni sp. nov. is proposed.

**Description of Marinomonas ostreistagni sp. nov.**

*Marinomonas ostreistagni* (os.tre’i.stag’ni. L. fem. n. ostra oyster; L. gen. neut. n. stagni of/from a pond; N.L. gen. neut. n. ostreistagni from/of oyster pond).

Cells are Gram-negative rods, 0.6–3.6 μm in length and 0.4–0.6 μm in diameter and motile by a single polar flagellum. Cells divide by binary fission. Heterotrophic, strictly aerobic and requires sodium ions for growth. Cell aggregates are detected when cultured in MB. Colonies are non-pigmented, circular, opaque, convex, 1–2 mm in diameter with an entire margin after 24 h incubation at 30 °C on MA. Grows in 1–10 % NaCl but not in 15 % NaCl; optimum NaCl for growth is approximately 3 %. Grows at 16–40 °C, but not at 4 or 42 °C; optimum temperature for growth is 33–37 °C. Grows at pH 5–0–9, with optimum growth occurring around pH 7.0. Oxidase-, catalase- and alkaline phosphatase-positive. Oxidizes glucose. Does not produce sulfide from cysteine or thiosulfate and does not degrade extracellular DNA. Does not form endospores and does not accumulate poly-β-hydroxybutyrate. Utilizes glucose, succrose, D-mannitol, aesculin, L-alanine, L-asparagine, L-serine, L-glutamic acid, acetate, citrate, pyruvate, propionate, lactate, ethanol and Tween 20, but not galactose, maltose, L-arabinose, trehalose, rhamnose, lactose, melibiose, D-sorbitol, methanol, glycerol, succinate, malate, glycine, L-leucine, L-arginine, L-lysine, L-histidine, L-methionine, L-threonine, valine, starch, gelatin, casein, agar, DNA, Tween 40, Tween 60, Tween 80 or cellulose. Does not ferment glucose, succrose or D-mannitol. Sensitive to ampicillin (10 μg), polymyxin B (300 U), chloramphenicol (30 μg), tetracycline (30 μg), streptomycin (10 μg) and rifampicin (10 μg), but resistant to penicillin G (2 U). Predominant fatty acids are 18:1ω7c, 16:0 and 16:1ω7c/15:0 iso 2-OH (Table 1; Ivanova et al., 2000, 2005; Yoon et al., 2003; Prabagar et al., 2005). Characteristics that differentiated strain UST010306-043T from other species of *Marinomonas* are shown in Table 2.

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**References**


Marinomonas ostreistagni sp. nov.


