Psychromonas agarivorans sp. nov., a novel agarolytic bacterium

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Two heterotrophic, agarolytic bacteria were isolated from marine environments. A 16S rRNA gene sequence analysis showed the isolates (designated strains J42-3A\textsuperscript{T} and 04OZ-AS15-7A) to be positioned in a separate lineage within the genus Psychromonas. Members of the genus Psychromonas are recognized as being psychrophilic or psychrotolerant, whereas none of the currently established Psychromonas species is known to be agarolytic. The DNA G+C contents of the isolates were about 42 mol\% and the predominant cellular fatty acids were 16 : 0 and 16 : 0. Based on the results of the phylogenetic and phenotypic analyses and DNA–DNA hybridization data, the isolates represent a novel species, for which the name Psychromonas agarivorans sp. nov. is proposed. The type strain is J42-3A\textsuperscript{T} (=NBRC 104585\textsuperscript{T}=KCTC 22285\textsuperscript{T}).

Jean et al. (2006) reported that five bacterial species within the class Alteromonadaceae (Bowman & McMeekin, 2005a) were recognized as being agarolytic: PseudoalteromonasAtlantic (Akagawa-Matsushita et al., 1992; Bowman & McMeekin, 2005b), Pseudoalteromonas agarivorans (Romanenko et al., 2003a), Glaciecola nicotiphila (Romanenko et al., 2003b), Shewanella olleyana (Skerratt et al., 2002) and Thalassomonas agarivorans (Jean et al., 2006). In this study we characterized two novel agarolytic strains (designated J42-3A\textsuperscript{T} and 04OZ-AS15-7A) that were isolated from marine sediment and seawater in Japan. Our results suggest that the novel isolates should be classified as representing a novel species of the genus Psychromonas.

Strain J42-3A\textsuperscript{T} was isolated from sediment collected at Kori, off Nomozaki, Nagasaki (32°36′04.7″N 129°46′34.2″E) in Japan. The strain was isolated using solidified 1/10 strength marine broth [900 ml filtered seawater and 100 ml marine broth (Déco)] with 1.5 % (w/v) agar and supplemented with 0.5 % Rose bengal. The other strain, 04OZ-AS15-7A, was isolated from a polyurethane foam (PUF) block supplemented with sterile seawater containing 1.5 % (w/v) agar. The PUF block was placed in the sea sand close to the beach (39°20′59.6″N 141°56′09.8″E). The recovered PUF block was cut into small pieces (1 cm\textsuperscript{3}) and homogenized with 5 ml of sterile seawater by using a glass rod. The homogenate was diluted to 1/100 with sterile seawater and the bacterium was isolated from this mixture using a medium that comprised sterile seawater with 0.1 % (w/v) 7-hydroxy-flavon and 1.5 % (w/v) agar.

Nucleotide sequences of the 16S rRNA genes amplified by using primers 27F and 1492R (Weisburg et al., 1991) were determined to identify the phylogenetic relationships of the novel isolates. Sequences very similar to those of the isolates were obtained by using BLAST (Altschul et al., 1990). A phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987) using CLUSTAL_X (version 1.83; Thompson et al., 1997) after multiple alignment; the sequence used corresponded to positions 95–175, 193–442, 454–819 and 845–1393 of that of Aeromonas allosaccharophila CECT 4199\textsuperscript{T} (GenBank accession no. S39232) obtained using the method of Ivanova et al. (2004). A bootstrap analysis was conducted from 1000 bootstrapped trials. 16S rRNA gene sequence similarity was calculated according to the method of Ivanova et al. (2004). As shown in Fig. 1, the two novel isolates were positioned within the genus Psychromonas, with a high level of 16S rRNA gene sequence similarity (>99 %). The highest levels of 16S rRNA gene sequence similarity were found with Psychromonas macrocephali JAMM 0415\textsuperscript{T}, Psychromonas japonica JAMM 0394\textsuperscript{T}, Psychromonas aquimarina JAMM 0404\textsuperscript{T}, Psychromonas kaiiiae JT7304\textsuperscript{T} and Psychromonas profunda 2825\textsuperscript{T} (each showing 96 % sequence similarity). Levels of similarity with respect to other Psychromonas species were 94–95 %. To determine the genus affiliation, nucleotides at 16S rRNA gene signature sites of the novel isolates and members of the genus Psychromonas were compared, according to the method of Ivanova et al. (2004). The nucleotide signature

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains J42-3A\textsuperscript{T} and 04OZ-AS15-7A are AB374544 and AB374545, respectively.
sites of the genus *Psychromonas* were defined in the family *Psychromonadaceae* at positions 385(T), 811(A), 842(A), 845(T) and 1336(T). The nucleotides at these positions in the novel isolates were conserved, except at position 385, which was C instead of T. The levels of 16S rRNA sequence similarity and the nucleotide signature sites observed indicated that the isolates represent a novel species of the genus *Psychromonas* (Stackebrandt & Goebel, 1994; Ivanova et al., 2004).

Genomic DNA was extracted by using the method of Saitou & Miura (1963). To determine the level of DNA–DNA relatedness, DNA–DNA hybridization was carried out at 41.5 °C and was measured fluorometrically by using the method of Ezaki et al. (1989). A high level of DNA–DNA relatedness (89.3–91.9 %) was found between the two novel isolates, indicating that they represent a single species (Wayne et al., 1987).

The DNA G+C contents were determined by using HPLC according to the method of Tamaoka & Komagata (1984). The DNA G+C contents of the novel isolates were 41–42 mol%. The respiratory quinone and cellular fatty acid compositions of cells grown for 48 h at 20 °C in marine broth were determined using previously described methods (Katsuta et al., 2005). The major respiratory quinone of the isolates was Q-8 and the major fatty acids were 16:1ω7c and 16:0; the isolates also contained 16:1ω7t (Table 1).

Phenotypic characteristics of the novel isolates were determined as follows. Cells were grown for 48 h at 20 °C on marine agar (Difco) and were observed by using transmission electron microscopy after negative staining with uranyl acetate. Gram staining was performed by using the Hucker staining method described by Smibert & Krieg (1994). Growth at various temperatures (2, 8, 15, 20, 25, 30 and 37 °C) was tested on marine agar. The pH range for growth was tested on half-strength (1/2) SP5 agar (Hosoya et al., 2006), using appropriate buffers: 10 mM MES was used to adjust the pH to 5.5–7.0, 10 mM TAPS for pH 8.0–9.0 and 100 mM NaHCO3/Na2CO3 for pH 10.0. Salt tolerance was tested on salinity-free 1/2 SP5 agar supplemented with 0–10 % NaCl (w/v). Oxidase activity was tested by using oxidase test paper (Eiken), and catalase activity was tested using a 3 % H2O2 solution. Growth under anaerobic conditions was tested on marine agar for 2 weeks with the AnaeroPack system (Mitsubishi Gas Chemical). Degradation of starch was tested on 1/2 SP5 agar containing 0.2 % (w/v) soluble starch by using the method of Smibert & Krieg (1994). Hydrolysis of Tweens 20, 40, 60 and 80 was determined on 1/2 SP5 agar adjusted to pH 8.0, supplemented with 0.1 % (v/v) Tweens, by observing the opaque halo formed. Hydrolysis of agar was tested by inoculating stab cultures prepared by using Sap2 medium (Hosoya et al., 2006) containing 1 % agar. Degradation of casein [4 % (w/v) litmus milk (Difco)] and L-tyrosine (0.5 %, w/v) was determined on 1/2 SP5 agar by observing the clear zone formed. Hydrolysis of DNA was determined on DNA agar (Nissui) supplemented with half-strength artificial seawater (ASW). Acid production from sugars was assessed using API 50 CH and API 50

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**Phylogenetic Tree**

![Phylogenetic Tree](image)

**Fig. 1.** Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequence analysis, showing strains J42-3A and 04OZ-AS15-7A and other members of the genus *Psychromonas*. Bootstrap values greater than 500 are given at branch points. Bars, 0.01 substitutions per nucleotide.
Table 1. Fatty acid profiles of strains J42-3Aᵀ and 04OZ-AS15-7A and other members of the genus Psychromonas

<table>
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<tr>
<th>Fatty acid</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>12:0</td>
<td>5.9–7.6</td>
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<td>2.7–5.2</td>
<td>2.5</td>
<td>1</td>
<td>3.6</td>
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<td></td>
<td></td>
<td>0.5–7.9</td>
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<td>14:0</td>
<td>2.6–3.2</td>
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<td>1</td>
<td>2</td>
<td>1.5</td>
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<td>1.9</td>
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<tr>
<td>15:0</td>
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<td>5</td>
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<td>16:0</td>
<td>37.1–38.2</td>
<td>24</td>
<td>43.6</td>
<td>15</td>
<td>31</td>
<td>7.0–16.2</td>
<td>18.7</td>
<td>31</td>
<td>21.0</td>
<td>28.9</td>
<td>26.7</td>
<td>25.4</td>
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<td>14:1</td>
<td>3.7–4.4(ω7c)</td>
<td>8(ω7t)</td>
<td>3.2</td>
<td>10(ω7t)</td>
<td>15</td>
<td>2.7–5.2(ω5t)</td>
<td>17</td>
<td>7.3</td>
<td>4.3</td>
<td>4.5</td>
<td>5.1</td>
<td>6.1–9.9(ω7c)</td>
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<tr>
<td>16:1</td>
<td>35.0–35.3(ω7c), 58(ω7c)</td>
<td>39.4</td>
<td>52(ω7c), 44</td>
<td>−50(ω7c), 67</td>
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<td>57.7–66.6(ω7c)</td>
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<td>3(ω7c)</td>
<td>3.1</td>
<td>2(ω7c)</td>
<td>2(ω7c)</td>
<td>7.0–16.2(ω7t)</td>
<td>3.6</td>
<td>2.7</td>
<td>1.8</td>
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<td>3.5–5.1(ω7c)</td>
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<td>3-OH-14:0</td>
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<td>12:0 alde, i-16:1 or 3-OH 14:0</td>
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CHB/CHE media (bioMérieux) supplemented with half-strength ASW. The cells used to inoculate the API test strips were suspended in half-strength ASW. Assimilation of carbon sources (API 20NE) was tested using a suspension medium (Hosoya et al., 2008). The API 50 CH tests were read after incubation for 24h at 30 °C, the API ZYM tests after incubation for 4 h at 37 °C and the API 20NE tests after incubation for 48 h at 30 °C.

The phenotypic characteristics are given in the species description and in Table 2. The novel isolates caused agar plates to be pitted and eventually the agar was liquefied, unlike all other members of the genus Psychromonas. The isolates grew at 30 °C, although no recognized Psychromonas species, except for Psychromonas heitensis, could grow at 30 °C. As shown in Table 1, the novel isolates and P. arctica contained 16:1ω7t, unlike other recognized Psychromonas species. The levels of sequence similarity and signature nucleotides of the 16S rRNA gene suggested that the isolates represent a novel species of the genus Psychromonas. On the basis of the data from this study, strains J42-3Aᵀ and 04OZ-AS15-7A represent a novel species of the genus Psychromonas, for which the name Psychromonas agarivorans sp. nov. is proposed.

Description of Psychromonas agarivorans sp. nov.

Psychromonas agarivorans (a.ga.ri.vo’rans, N.L. n. agarum agar; L. part. adj. vorans devouring, destroying; N.L. part. adj. agarivorans agar-devouring).

Cells are Gram-negative, facultatively anaerobic, rod-shaped, approximately 0.8–1.2 μm long and 0.5–0.8 μm wide. Cells are motile by means of a subpolar flagellum. Cytochrome oxidase-positive and catalase-negative. Optimal growth temperature is 20–25 °C. Growth occurs at 2 and 30 °C; no growth occurs at 37 °C. The pH range for growth is 6.0–9.0. Growth occurs at NaCl concentrations of 1–4%. Does not grow in the absence of NaCl. Pitting of agar plates occurs and eventually the agar is liquefied. Positive for degradation of DNA and aesculin. Positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-galactosidase. Does not reduce nitrate (API 20E; bioMérieux). Acid is produced from glucose by fermentation (API 20NE). Acid is produced by oxidation from galactose, glucose, cellobiose and maltose (API 50 CH). Hydrolysis of and acid production from starch is variable. Does not decompose gelatin, Tween 20, 40, 60 and 80, tyrosine or urea. Does not produce indole. Positive for arginine dihydrolase, esterase (C4), lipase (C14), cystine arylamidase, valine arylamidase, trypsin, chymotrypsin, x-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, x-mannosidase and x-fucosidase. Does not produce acid from glycerol, erthritol, DL-arabinose, ribose, DL-xylene, adonitol, methyl β-D-xylopyranoside, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl x-D-mannopyranoside, methyl x-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, glycogen, xyitol, gentiobiose, turanose, D-lyxose, D-tagatose, DL-fucose, DL-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate (API 50 CH).
carbon sources is positive for glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, maltose, gluconate, DL-malate and citrate. Assimilation is negative for n-capric acid, adipic acid and phenyl acetate (API 20NE). Predominant fatty acids are 16:0, 16:1ω7c, 16:1ω6c, 16:1ω9c and 16:0.

The type strain, J42-3A\textsuperscript{T} (=NBRC 104585\textsuperscript{T}=KCTC 22285\textsuperscript{T}), was isolated from marine sediments collected at Kori, off Nomoizaki, Nagasaki in Japan. Strain 04OZ-AS15-7A (NBRC 104632) is a reference strain. The DNA G+C contents of the type strain and strain 04OZ-AS15-7A are 41–42 mol%.

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References


*Data were obtained through personal communication with Dr Yuichi Nogi and Masayuki Miyazaki.
Psychromonas macrocephali

Shewanellaceae

fam. nov.,
Pseudoalteromonadaceae

nov.

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sp. nov.,


Psychromonas marina


Psychromonas marina


Ferrimonas marina


