Alteromonas addita sp. nov.

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On the basis of phenotypic, genotypic characteristics and analysis of 16S rRNA gene sequences, a novel species belonging to the genus *Alteromonas* is described. A non-pigmented, motile, Gram-negative bacterium designated R10SW13^T was isolated from sea water samples collected in Chazhma Bay (Sea of Japan, Pacific Ocean). The novel organism mainly grew between 4 °C and 37 °C, was neutrophilic and slightly halophilic, tolerating up to 10 % NaCl. Strain R10SW13^T was haemolytic and was able to degrade starch and Tween 80 and to degrade gelatin and agar weakly, but did not degrade casein. Phosphatidylethanolamine (44.3 ± 0.9 %) and phosphatidylglycerol (55.7 ± 0.9 %) were the predominant phospholipids. The major fatty acids formed were typical for the genus *Alteromonas*, including 16:0, 16:1 ω -7 and 18:1 ω -7. The G+C content of the DNA was 43.4 mol%. DNA–DNA hybridization experiments showed 38–53 % binding with the DNAs of type strains of phylogenetically related species of the genus *Alteromonas*, namely: *Alteromonas macleodii*, *Alteromonas marina*, *Alteromonas stellipolaris*, *Alteromonas litorea*, '*Alteromonas macleodii* subsp. *fijiensis*' and '*Alteromonas infernus*'. Based on these results, a novel species, *Alteromonas addita* sp. nov., is proposed, with strain R10SW13^T (=KMM 3600^T=KCTC 12195^T=LMG 22532^T) as the type strain.

Volume 1 of the first edition of *Bergey's Manual of Systematic Bacteriology* described only one genus of Gram-negative, aerobic, heterotrophic, marine bacteria with a single polar flagellum, *Alteromonas* Baumann *et al.* 1972 (Baumann *et al.*, 1984; Baumann & Baumann, 1981). During its dynamic and turbulent taxonomic history, the genus has undergone detailed investigations (Van Landschoot & De Ley, 1983; Gauthier *et al.*, 1995; Van Trappen *et al.*, 2004; Ivanova *et al.*, 2004b) and currently comprises the species *Alteromonas*

macleodii Baumann *et al.* 1972, *A. marina* Yoon *et al.* 2003, *A. stellipolaris* Van Trappen *et al.* 2004 and *A. litorea* Yoon *et al.* 2004.

In this study we report on the characterization of a novel bacterium of the genus *Alteromonas*, strain R10SW13^T, isolated from sea water samples collected in Chazhma Bay (Gulf of Peter the Great, Sea of Japan, Pacific Ocean). This work was part of a taxonomic survey of free-living microbial populations of the bay in the north-west Pacific Ocean, sediments of which were contaminated by radionuclides. Water samples were collected during October–November 2000 from two different horizons, from the first metre below the surface and from 1–2 m from the bottom (a varying depth of 9–13 m) (salinity, 32 ‰; temperature, 13.6 °C), using a standard hydrological plastic bathometer. Samples were kept at 4 °C and processed within 4–8 h. A portion of

Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Alteromonas addita* R10SW13^T is AY682202.

DNA G+C contents and DNA–DNA relatedness values for *Alteromonas* species including *A. addita* are available as supplementary material in IJSEM Online.

sea water (0.1 ml) was plated onto marine agar 2216 (Difco) or medium B which contained 0.2 % (w/v) Bacto peptone (Difco), 0.2 % (w/v) casein hydrolysate (Merck), 0.2 % (w/v) Bacto yeast extract (Difco), 0.1 % (w/v) glucose, 0.02 % (w/v) KH_2PO_4 , 0.005 % (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.5 % (w/v) Bacto agar (Difco), 50 % (v/v) natural sea water and 50 % (v/v) distilled water at pH 7.8. Plates were incubated aerobically at room temperature (approx. 22–25 °C) and growth was monitored after 5, 7 and 10 days. The isolation and purification procedure has been described elsewhere (Ivanova *et al.*, 1996). Strains were stored at –80 °C in marine broth 2216 (Difco) supplemented with 20 % (v/v) glycerol.

The following physiological and biochemical properties were examined by methods described by Smibert & Krieg (1994) unless indicated: oxidation/fermentation of glucose, denitrification (Azegami *et al.*, 1987), oxidase and catalase activity, gelatin liquefaction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, sodium requirement [0, 0.5, 1, 3, 6, 8, 10, 12 % (w/v) NaCl], indole and H_2S production and the ability to hydrolyse starch, Tween 80 and casein. Growth at different temperatures was determined in marine broth 2216 (Difco) and on plates with medium B after 24–72 h at 2, 4, 6, 29, 30, 35 and 37 °C. The Biolog GN test kit was also used to examine the primary oxidation of 95 carbon compounds as described elsewhere (Ivanova *et al.*, 1998). Antibacterial activity was assessed by the agar diffusion assay, based on the method described by Barry (1980). Haemolysis was tested on blood-agar plates (6.5 % sheep blood; Merck).

Phenotypic analysis showed that the novel organism had all the characteristics of *Alteromonas*-like bacteria: it was Gram-negative, strictly aerobic, oxidase- and catalase-positive, did not produce H_2S or indole and was negative for denitrification. The novel isolate did not show anti-bacterial activity, but possessed haemolytic activity. The morphological and physiological properties examined are also shown in Table 1 and given in the species description.

For analysis of phospholipids and fatty acids, the strains were grown at 28 °C on marine agar 2216. After 48 h growth, cells were harvested. The lipids were extracted by a modified method of Bligh & Dyer (1959). Polar lipids were separated by two-dimensional micro-thin-layer chromatography in solvent systems described by Vaskovsky & Terekhova (1979). Lipids were detected on the TLC using a 10 % solution of H_2SO_4 in methanol with subsequent heating to 180 °C and by using specific reagents for phospholipids (see Vaskovsky *et al.*, 1975), amino-containing lipids (2 % ninhydrin in acetone) and choline lipids (Dragendorff's reagent). Phospholipids were quantified by the method of Vaskovsky *et al.* (1975). The lipids were treated with 5 % HCl in methanol at 80 °C for 180 min to produce fatty acid methyl esters (FAMES) (Christie, 1982). FAMES were analysed by FID-GC (Shimadzu GC-17) with a fused silica capillary column (30 m $\times$ 0.25 mm), coated with Supelcowax 10 at 210 °C. Helium was used as the carrier gas. FAMES

Table 1. Characteristics that differentiate *Alteromonas addita* sp. nov. from phylogenetically related *Alteromonas* species

Species: 1, *A. addita* sp. nov.; 2, *A. macleodii*; 3, *A. marina*; 4, *A. stellipolaris*; 5, *A. litorea*. All species/strains are Gram-negative, motile, oxidase- and catalase-positive and negative for indole and H_2S production, grow at 3–6 % NaCl and produce lipase (Tween 80). +, Positive; –, negative; W, weak reaction; V, variable; ND, no data. Data were obtained in this study and from Yoon *et al.* (2003, 2004) and Van Trappen *et al.* (2004).

Characteristic	1	2*	3	4	5
Production of pigments	–	–	–	+	–
Growth at:					
4 °C	+	–	+	+	–
40 °C	–	+	+	–	+
Nitrate reduction to nitrite	–	V (–)	–	–	ND
Growth in NaCl at:					
10 %	+	–	+	+	+
15 %	–	–	+	–	–
Hydrolysis of:					
Gelatin	W	V (+)	+	+	+
Agar	W	–	–	–	–
Starch	+	V (+)	+	+	+
Haemolysis	+	–	ND	–	ND
Assimilation of:					
D-Mannitol	–	V (+)	–	+	–
L-Lactate	+	V (+)	–	–	–
G + C content (mol%)	43	45–46	44–45	45	46

*Data in parentheses reported by Yoon *et al.* (2003).

were identified by comparing the retention times with those of authentic standards and using equivalent chain length measurements. To ensure correct identification, FAMES were analysed by GC-MS using a model GCMS-QP5050A (Shimadzu) fitted with an MDN-5S capillary column (30 m $\times$ 0.25 mm). The column temperature was programmed for 1 min hold at 170 °C, followed by an increase to 240 °C at 2 °C min^{–1} and a hold at 240 °C for 20 min. The temperature of the injector and detector were 250 °C. Phosphatidylethanolamine (44.3 $\pm$ 0.9 %) and phosphatidylglycerol (55.7 $\pm$ 0.9 %) were the major constituents of the phospholipids. Traces of bisphosphatidic acid, lysophosphatidylethanolamine and phosphatidic acid were also detected. Neither diphosphatidyl glycerol nor glycerophospholipids were found, which is in agreement with our previous observations (Ivanova *et al.*, 2000). The fatty acids formed by the novel organism were (%): 12:0 (1.0); 13:0 (0.5); iso-14:0 (0.5); 14:0 (2.9); 14:1 (1.9); 15:0 (1.8); 15:1 ω -8 (2.7); iso-16:0 (0.4); 16:0 (15.2); 16:1 ω -7 (30.1); 17:0 (2.0); 17:1 ω -8 (4.1); iso-18:0 (7.8); 18:0 (1.0); 18:1 ω -7 (11.7); 10:0 3-OH (3.3); iso-11:0 3-OH (2.2); 11:0 3-OH (2.5); iso-12:0 3-OH (1.1); 12:0 3-OH (1.9); 14:0 3-OH (2.9); 15:0 3-OH (1.2) and 16:0 3-OH (1.4).

The small-subunit rRNA genes were sequenced as described elsewhere (Ivanova *et al.*, 2004a). The 16S rRNA gene sequence of R10SW13^T was aligned and compared to the GenBank nucleotide database using an online BLAST search. Analyses of 16S rRNA gene sequences were done using PHYLIP version 3.57c (Felsenstein, 1993). DNADIST was used to determine sequence similarities using the maximum-likelihood algorithm option. Phylogenetic trees were constructed with the neighbour-joining method by using the program NEIGHBOR. The outgroup on the *Alteromonas* trees was *Escherichia coli*. Analysis of the 16S rRNA gene sequences revealed that the novel isolate belongs to the genus *Alteromonas*, forming a coherent cluster (bootstrap value of 100 %) with all other recognized species of the genus *Alteromonas* (Fig. 1). Strain R10SW13^T shared 97 % 16S rRNA gene sequence similarity with *A. macleodii*, *A. marina*, *A. litorea*, '*Alteromonas infernus*' and 99 % similarity with *A. stellipolaris*. This level of similarity supports the phylogenetic inclusion of strain R10SW13^T in the genus *Alteromonas*.

DNA was isolated from strain R10SW13^T following the method of Marmur (1961) and the G+C content of the DNA was determined using the thermal denaturation method of Marmur & Doty (1962) as 43.4 mol%. DNA–DNA hybridization was performed spectrophotometrically as described by De Ley *et al.* (1970). Type strains were obtained from the American Type Culture Collection and the BCCM/LMG Bacteria Collection or were kindly provided by Dr J.-H. Yoon and Dr G. Barbier. DNA from strain R10SW13^T showed 38–53 % binding with the DNA of type strains of *Alteromonas* species with validly published names and some other strains, e.g. '*A. macleodii* subsp. *fijiensis*' and '*A. infernus*' (detailed results available as supplementary

material in IJSEM Online). These values are far lower than 70 %, which is the cut-off value recommended for definition of a genomic species (Wayne *et al.*, 1987), and clearly indicate that strain R10SW13^T represents a genomic species that is separate from recognized *Alteromonas* species. Further, the novel isolate can be readily distinguished from other *Alteromonas* species by the combination of phenotypic, genotypic and phylogenetic features, e.g. range of salinity and temperature for growth, presence of haemolytic activity and the ability to hydrolyse agar (Table 1). While R10SW13^T had patterns of phospholipids and cellular fatty acids that were characteristic of the genus, the large proportion of 16:1 ω -7 is an additional species-specific feature. Thus, the results obtained in this study provide evidence that R10SW13^T represents a novel species of the genus *Alteromonas* for which we propose the name *Alteromonas addita* sp. nov.

Description of *Alteromonas addita* sp. nov.

Alteromonas addita (ad.di'ta. L. fem. part. adj. *addita* added, joined to the genus).

Gram-negative, rod-shaped, single cells, about 0.7–0.9 μ m in diameter. Motile, with a single polar flagellum. Aerobic. Chemoorganotroph with respiratory metabolism. Colonies are uniformly round, 2–3 mm in diameter, regular, convex and smooth on marine agar or B medium. Does not form endospores. Does not accumulate poly- β -hydroxybutyrate as an intracellular reserve product. Requires Na⁺ ions or sea water for growth; growth occurs in media with 1–10 % NaCl. Temperature for growth ranges from 4 to 37 °C; no growth is detected at 40 °C. The pH for growth ranges from 6.0 to 10.0, with optimum at 7.5–8.0. Negative for arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase. Weakly decomposes gelatin and agar. Other physiological properties are listed in Table 1. Utilizes the following carbon sources: dextrin, glycogen, Tween-80, *N*-acetyl-D-glucosamine, adonitol, D-cellobiose, L-arabinose, D-arabitol, i-erythritol, D-fructose, L-fucose, *m*-inositol, α -D-lactose, lactulose, maltose, D-mannose, D-melibiose, methyl β -D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, acetic acid, *cis*-aconitic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketoglutaric acid, α -ketobutyric acid, α -ketovaleric acid, DL-lactic acid, malonic acid and propionic acid. Phosphatidylethanolamine (44.3 $\pm$ 0.9 %) and phosphatidylglycerol (55.7 $\pm$ 0.9 %) are the predominant phospholipids. The main cellular fatty acids are 16:1 ω -7, 16:0, 18:1 ω -7 and iso-18:0 (approx. 65 %). The G+C content of the DNA of the type strain is 43 mol%.

The type strain is strain R10SW13^T (=KMM 3600^T=KCTC 12195^T=LMG 22532^T), isolated from sea water from Chazhma Bay in the Sea of Japan, Pacific Ocean.

Fig. 1. Phylogenetic position of *Alteromonas addita* sp. nov. and related *Alteromonas* species according to 16S rRNA gene sequence analysis. The topology shown was obtained using the bioNJ algorithm and 1000 bootstrap replications with Kimura's two-parameter correction for the distances. Percentages of bootstrap are indicated only for branches that were also retrieved by maximum-parsimony and maximum-likelihood ($P < 0.01$); these branches should be considered as the only robust clusters identified by this analysis. Bar, 0.01 substitutions per nucleotide position.

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