Shewanella pacifica sp. nov., a polyunsaturated fatty acid-producing bacterium isolated from sea water

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Six marine bacterial strains, KMM 3597^T, KMM 3775, KMM 3590, KMM 3772, KMM 3605 and KMM 3601, that produce polyunsaturated fatty acids were isolated from sea water samples collected from different locations and depths in Chazhma Bay (Sea of Japan, Pacific Ocean) and characterized to clarify their taxonomic position. The DNA G+C contents of these strains were 39·5–40·3 mol%. The level of DNA hybridization between these strains was conspecific (83–96 %), indicating that they represent a single genospecies. 16S rRNA gene sequence-based phylogenetic analysis of the novel strains revealed that *Shewanella japonica* KMM 3299^T was the closest relative (99 % similarity). However, DNA–DNA hybridization experiments demonstrated only 45–50 % binding with DNA of *S. japonica*. The novel organisms grew between 4 and 33 °C, were neutrophilic and haemolytic, and were able to degrade starch, gelatin, agar and Tween 80. The predominant fatty acids were (% ± SD): i13 : 0 (9·3 ± 1·1); i15 : 0 (33·9 ± 1·5); 16 : 0 (8·9 ± 1·6); and 16 : 1ω7 (14·8 ± 1·1). The fatty acid 20 : 5ω3, formed at 28 °C, was present at up to 5·3 % total fatty acids. The major isoprenoid quinones were Q7 (21–41 %) and Q8 (50–59 %). The phylogenetic, genetic and physiological properties of the six strains placed them within a novel species, *Shewanella pacifica* sp. nov., the type strain of which is R10SW1^T (=KMM 3597^T=CIP 107849^T).

The genus *Shewanella* MacDonell and Colwell 1986 comprises an ubiquitous group of Gram-negative, aerobic and facultatively anaerobic γ-*Proteobacteria* (MacDonell & Colwell, 1985; Gauthier *et al.*, 1995; Venkateswaran *et al.*, 1999; Garrity & Holt, 2001). One of the striking features of these bacteria is the ability of some recently described species to produce polyunsaturated fatty acids (PUFAs) at relatively high incubation temperatures (25–30 °C; Ivanova *et al.*, 2001, 2003a; Skerratt *et al.*, 2002), which contradicts the notion that only barophilic or cold-adapted species are able to produce significant levels of PUFAs such as eicosapentaenoic acid (EPA, 20 : 5ω3*c*; Russell & Nichols, 1999; Nogi *et al.*, 1998).

In this study, a novel mesophilic, PUFA-producing bacterium of the genus *Shewanella* was characterized. The bacterium was isolated from sea water samples collected in Chazhma

Published online ahead of print on 19 December 2003 as DOI 10.1099/ijs.0.02993-0.

Abbreviations: EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KMM 3597^T, KMM 3590 and KMM 3506 are AF500076, AF500075 and AY366086, respectively.

Tables showing the *S. pacifica* cellular fatty acid and isoprenoid quinone compositions and comparative fatty acid compositions of *S. pacifica* and related species are available in IJSEM Online.

Bay (Sea of Japan, Pacific Ocean) and was able to form EPA at relatively high incubation temperatures. 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 have been contaminated by radionuclides. During the course of this work, 70 presumptive *Shewanella* strains of different phenotypes were isolated. The majority of the strains had a few phenotypic features (e.g. agar-digesting and haemolytic activities, PUFA production) that were similar to those of a previously described species, *Shewanella japonica* (Ivanova *et al.*, 2001), and also showed a high level of 16S rRNA gene sequence similarity (99 %) to the 16S rRNA gene of *S. japonica*. However, further detailed taxonomic investigation revealed a number of atypical phenotypic traits, such as different proportions of cellular fatty acids and low levels of genetic identity (45–50 %) with *S. japonica* KMM 3299^T. It is therefore concluded that this group of strains constitutes a novel species, for which the name *Shewanella pacifica* sp. nov. is proposed.

Water samples were collected in October/November 2000 from depths of 1 m and 9–13 m (salinity 32 ‰; temperature 13·6 °C) using a standard hydrological plastic bathometer in different locations in Chazhma Bay, Gulf of Peter the Great, Sea of Japan, Pacific Ocean. Samples were kept at 4 °C and processed within 4–8 h. A portion of sea water (0·1 ml) was plated onto marine agar 2216 (Difco) or medium B (Ivanova *et al.*, 2004). Plates were incubated aerobically at room temperature (about 22–25 °C) for 5, 7 and 10 days. Isolation and purification of the bacterial strains have been described elsewhere (Ivanova *et al.*, 1996). Strains were stored at −80 °C in marine broth 2216 (Difco) supplemented with 20 % (v/v) glycerol.

Unless otherwise indicated, phenotypic characteristics were studied using standard procedures (Baumann *et al.*, 1972; Smibert & Krieg, 1994) described previously (Ivanova *et al.*, 1996, 1998, 2003a; Sawabe *et al.*, 1998). The following physiological and biochemical properties were examined: oxidation/fermentation of glucose, denitrification, catalase and oxidase activities, gelatin liquefaction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, indole and H_2S production, and the ability to hydrolyse starch, alginate, chitin, elastin, Tween 80 and casein. The medium used to study Na^+ ion requirements is described by Ivanova *et al.* (2004). Tests for salt tolerance, dissimilatory iron reduction, haemolytic activity and antimicrobial activities were carried out as described by Ivanova *et al.* (2004). Phenotypic analysis showed that all isolates were essentially identical to each other and differed only in their ability to grow at 33 °C. Morphological and physiological properties are listed in Table 1 and given in the species description.

Analysis of fatty acid methyl esters and isoprenoid quinones was carried out as described by Ivanova *et al.* (2004). The cellular fatty acid profile was genus-specific and included

Table 1. Characteristics that differentiate *Shewanella pacifica* from phylogenetically related species

Species: 1, *Shewanella pacifica*; 2, *Shewanella japonica*; 3, *Shewanella olleyana*; 4, *Shewanella baltica*; 5, *Shewanella hanedai*; 6, *Shewanella woodyi*; 7, *Shewanella waksmanii*; 8, *Shewanella colwelliana*; 9, *Shewanella frigidimarina*; 10, *Shewanella violacea*. All strains are Gram-negative, motile, rod-shaped organisms that are oxidase- and catalase-positive, and can reduce nitrate to nitrite. V, Variable reaction depending on the strain; ND, data not available. Data from this study, Weiner *et al.* (1988), Bowman *et al.* (1997), Nogi *et al.* (1998), Venkateswaran *et al.* (1999), Skerratt *et al.* (2002) and Ivanova *et al.* (2003a).

Characteristic	1	2	3	4	5	6	7	8	9	10
DNA G+C content (mol%)	40	43	44	48	45	39	43	46	40–43	47
Growth at:										
4 °C	+	−	+	−	+	+	+	−	+	+
32 °C	+	+	+	−	−	+	+	+	−	−
0 % NaCl	−	−	−	−	−	−	−	−	+	−
6 % NaCl	+	−	+	−	−	−	+	−		−
Haemolysis	+	+	−	−	ND	ND	+	ND	ND	ND
Production of:										
Lipase	+	+	+	+	+	−	+	ND	ND	ND
Amylase	+	+	+	−	−	V	−	+	−	−
Gelatinase	+	+	−	+	+	+	+	+	+	+
Chitinase	−	−	−	+	+	−	−	−	−	ND
Utilization of:										
D-Galactose	+	−	+	ND	+	+	−	−	+	+
DL-Lactate	−	−	−	ND	ND	ND	−	−	+	ND
Succinate	+	−	−	−	ND	ND	−	−	−	ND
Citrate	−	−	−	+	−	−	−	−	−	ND

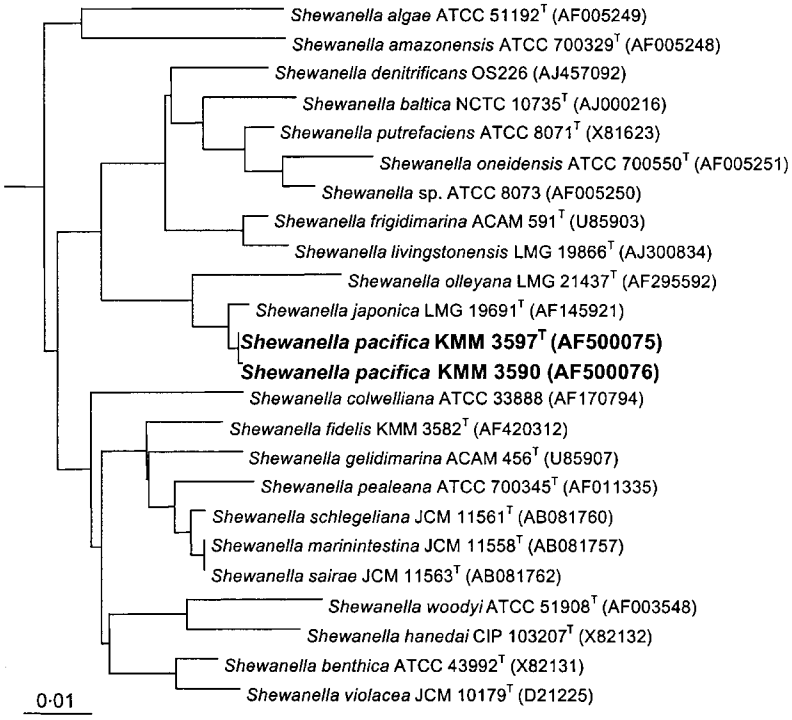

Fig. 1. Phylogenetic position of *Shewanella pacifica* according to 16S rRNA gene sequence analysis. The tree was generated from maximum-likelihood distances clustered by the neighbour-joining method. Bar, 0·01 substitutions per nucleotide position.

saturated, monoenoic, monounsaturated, straight-chain and iso-branched components (complete cellular fatty acid and isoprenoid quinone compositions are available as supplementary material in IJSEM Online). The high proportion of i15 : 0 (up to 36 %) and 20 : 5ω3 (EPA; up to 5·3 %) produced during growth at 28 °C are characteristic features of the novel strains. The mean fatty acid contents (% ± SD) were as follows: i13 : 0, 9·3 ± 1·1; i15 : 0, 33·9 ± 1·5; 16 : 0, 8·9 ± 1·6; and 16 : 1ω7, 14·8 ± 1·1.

The 16S rRNA gene sequences of KMM 3597^T and KMM 3590 were amplified and sequenced as described elsewhere (Ivanova *et al.*, 2001, 2003a, b). The 16S rRNA gene sequences of KMM 3590, KMM 3597^T and KMM 3605 were 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 using the program NEIGHBOR. The outgroup on the *Shewanella* trees was *Psychromonas antarctica* DSM 10704^T (GenBank accession no. Y14697). According to our phylogenetic analysis, strains KMM 3597^T, KMM 3590 and KMM 3605 formed a cluster with *S. japonica* and shared high (99 %) 16S rRNA gene sequence similarity (Fig. 1).

DNA was extracted from cells grown overnight on medium B following the method of Marmur (1961). DNA–DNA hybridization was performed spectrophotometrically and initial renaturation rates were recorded as described elsewhere (Marmur & Doty, 1962; De Ley *et al.*, 1970). The DNA G + C content was 39·5–40·5 (± 0·4) mol% (Table 2). DNA–DNA hybridization data revealed high levels of DNA relatedness among the six strains (83–96 %). DNA from *S. japonica* KMM 3299^T showed intrageneric relatedness with the newly isolated strains of 45–50 % (Table 2). This clearly indicated that the strains from Chazhma Bay belong to the same genospecies, but constitute a distinct *Shewanella* species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Strains of the novel species can be phenotypically, chemotaxonomically and genetically distinguished from other species, in particular from *S. japonica*, by a combination of phenotypic traits listed in Table 1 (e.g. obligate requirement for Na^+ ions, tolerance to 6 % NaCl, the ability to grow at 4 °C, different carbon source utilization patterns, lower DNA G + C content, and higher proportions of i15 : 0 and EPA).

Description of *Shewanella pacifica* sp. nov.

Shewanella pacifica (pa.ci′fi.ca. L. adj. *pacificus -a, -um* pacific; L. fem. adj. *pacifica* pacific, from, or related to, the Pacific Ocean).

Cells are rod-shaped, 1·0–2·0 × 0·6–0·8 μm, motile with a single polar flagellum and Gram-negative. Facultatively anaerobic chemoheterotroph. Does not form endospores. Anaerobic growth occurs by fermentation of D-glucose by anaerobic respiration of nitrate. Colonies on marine agar 2216 are slightly pink, circular, smooth and convex with an entire edge. Organic growth factors are not required. Has absolute requirement for Na^+ ions and grows in 0·5–6·0 % NaCl. No growth detected at 8 % NaCl. The temperature growth range is 4–33 °C; optimum growth occurs at 20–25 °C and no growth is detected at 35 °C.

Table 2. DNA relatedness (%) and G+C contents of tested strains

Organism	G+C content (mol%)	*S. japonica* KMM 3299^T	*S. pacifica* KMM 3597^T	*S. pacifica* KMM 3590	*S. pacifica* KMM 3775
S. japonica KMM 3299^T	43·4	100			
S. pacifica KMM 3597^T	39·7	46	100		
S. pacifica KMM 3590	39·8	45	93		
S. pacifica KMM 3775	39·5	50	95	96	83
S. pacifica KMM 3772	39·9		92		
S. pacifica KMM 3605	40·5	45	87	85	
S. pacifica KMM 3601	40·3				93

Oxidase- and catalase-positive. Reduces nitrate to nitrite. Arginine dihydrolase and lysine decarboxylase are not observed. Haemolytic, produces amylase, esterase (Tween 20, 40, 80), proteinase (gelatinase), alginase (depending on strain) and agarase. Chitin is not hydrolysed. H_2S is formed from thiosulfate anaerobically. Indole is not formed from L-tryptophan. Voges–Proskauer test is negative. D-Glucose is utilized as sole source of carbon. Does not utilize D-galactose, D-fructose, *N*-acetylglucosamine, succinate, D-mannose, lactose, fumarate or L-tyrosine. The following substrates (according to Biolog) are utilized: dextrin, Tween 40 and 80, L-arabinose, D-cellobiose, maltose, γ-hydroxybutyric acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, D-saccharic acid, succinic acid, L-alanine, L-alanyl-glycine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-ornithine, L-proline, L-pyroglutamic acid, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, α-D-glucose 1-phosphate and D-glucose 6-phosphate. Major cellular fatty acids are i13 : 0, i15 : 0, 16 : 0 and 16 : 1ω7 (60–70 % of total), and 20 : 5ω3 (4–5 %). The major isoprenoid quinones are Q7 (21–41 %) and Q8 (50–59 %). Isolated from the sea water of Chazhma Bay, Sea of Japan, Pacific Ocean. The DNA G+C content is 39·5–40·5 mol%.

Type strain is R10SW1^T (=KMM 3597^T=CIP 107849^T).

Acknowledgements

This study was partially supported by funds from the Defence Advanced Research Projects Agency (DARPA) grant number N66001-00-18952, the Russian Foundation for Basic Research (RFBR) 02-04-49517, grant 03-19 from Ministry for Industry, Science and Technology of Russian Federation, and grant 03-1-0-05-005 from the Far-Eastern Branch of the Russian Academy of Sciences.

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