Shewanella donghaensis sp. nov., a psychrophilic, piezosensitive bacterium producing high levels of polyunsaturated fatty acid, isolated from deep-sea sediments

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A Gram-negative, motile, rod-shaped, psychrophilic bacterium, LT17^T, was isolated from deep-sea sediments (3300 m depth) of the East Sea (Sea of Japan). Optimal growth of LT17^T requires the presence of 2.5 % (w/v) NaCl, a pH of 7.0–7.5 and a temperature of 17 °C. The isolate grows optimally under a hydrostatic pressure of 10 MPa and growth is possible between 0.1 and <30 MPa. The novel strain is positive in tests for catalase, oxidase, lipase, β -glucosidase and gelatinase activities and reduces nitrate to nitrite. The predominant cellular fatty acids are iso-C13:0, iso-C15:0, C16:0, C16:1 ω 7 and C20:5 ω 3. The DNA G+C content of strain LT17^T is 38.8 mol%. Phylogenetic analysis of 16S rRNA gene sequences places this bacterium in the class *Gammaproteobacteria*, within the genus *Shewanella*. The closest relatives of strain LT17^T are *Shewanella japonica* (97.8 % gene sequence similarity), *Shewanella pacifica* (97.5 %), *Shewanella olleyana* (96.8 %), *Shewanella frigidimarina* (96.5 %) and *Shewanella gelidimarina* (95.4 %). The DNA–DNA hybridization levels between the novel isolate and its closest known phylogenetic relatives, *S. japonica* and *S. pacifica*, are lower than 14 %. On the basis of this polyphasic evidence, strain LT17^T represents a novel species of the genus *Shewanella*, for which the name *Shewanella donghaensis* sp. nov. is proposed. The type strain is LT17^T (=KCTC 10635BP^T=JCM 12524^T).

The genus *Shewanella* was created by the reclassification of two species, *Alteromonas putrefaciens* and *Alteromonas hanedai*, that were previously assigned to the genus *Alteromonas* (MacDonell & Colwell, 1985). The genus *Shewanella* is phylogenetically affiliated to the class *Gammaproteobacteria* and it accommodates Gram-negative, aerobic or facultatively anaerobic and straight or curved rods. Species of the genus *Shewanella* have been isolated from a variety of sources including clinical samples (Nozue *et al.*, 1992; Brink *et al.*, 1995; Venkateswaran *et al.*, 1999), oilfield fluids (Semple & Westlake, 1987), aquatic or marine

environments (Nealson *et al.*, 1991; Ivanova *et al.*, 2001; Bozal *et al.*, 2002), marine sediments (Myers & Nealson, 1988) and deep-sea sediments (Nogi *et al.*, 1998). According to various reports, the genus *Shewanella* is ubiquitous in marine environments including the deep-sea. An interesting feature of species of the genus *Shewanella* is the ability to produce polyunsaturated fatty acids (PUFAs). Kato & Nogi (2001) proposed that deep-sea *Shewanella* species could be taxonomically recognized by two major subgenus branches, one group is characterized by high-pressure cold-adapted species that produce substantial amounts of eicosapentaenoic acid (EPA) and the other group is characterized by mesophilic, pressure-sensitive species that do not produce EPA or produce only scant amounts. Skerratt *et al.* (2002) and Ivanova *et al.* (2001, 2004), however, described several species, *Shewanella olleyana*, *Shewanella japonica* and *Shewanella pacifica*, that produce significant levels of PUFAs, such as EPA, at relatively high incubation temperatures (25–30 °C).

In this study, a novel psychrophilic EPA-producing bacterium of the genus *Shewanella* was isolated from the

Abbreviations: EPA, eicosapentaenoic acid; PUFAs, polyunsaturated fatty acids.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LT17^T is AY326275.

A graph showing the growth rate of strain LT17^T under varying pressure conditions and an extended phylogenetic tree based on 16S rRNA gene sequences are available as supplementary figures in IJSEM Online.

deep-sea and characterized. The bacterium was isolated from sediment samples collected from the East Sea (Sea of Japan; 42° 41' N 139° 41' E; 3300 m depth) by a manned Shinkai 6500 submersible (Yokosuka 01-06 Cruise). Sediment samples were diluted with sterilized seawater and the diluted samples were spread onto marine agar 2216 (MA; Difco) on board just after sampling. Colonies were isolated after incubation at 4 °C for 2 weeks and one of them, strain LT17^T, was selected for further study due to its ability to produce EPA and lipase under low temperature conditions.

Unless otherwise stated, morphological and physiological characterizations were performed as described previously (Bae *et al.*, 2005). Cells grown at 10 °C for 7 days on MA were used to observe morphology and Gram-staining. Growth characteristics under different environmental conditions, including temperature, pH, NaCl concentration and hydrostatic pressure, were examined using marine broth 2216 (MB; Difco) as a basal medium using the same methods as described by Seo *et al.* (2005a).

Strain LT17^T was Gram-negative, rod-shaped, motile and approximately 1.0–1.5 µm in length and 0.5–0.8 µm in width. Colonies formed on MA at 17 °C after 1 day were orange coloured, circular, opaque, convex with entire margins and 1.5–2 mm in diameter. The colour changed from orange to slightly pink after 3–4 days incubation. Strain LT17^T grew optimally at 17 °C and growth was possible between 5 and 20 °C. The growth rate of strain LT17^T at 5 °C was approximately half that of the optimal growth rate. No growth occurred at 22 °C or higher (Fig. 1). The novel strain grew well within the pH range 6.5–8.5 and optimally at pH 7.0–7.5. Optimal growth for strain LT17^T occurred in the presence of 2.5 % (w/v) NaCl; no growth occurred at NaCl concentrations above 4.5 % or in the absence of NaCl. In addition, strain LT17^T grew optimally at a hydrostatic pressure of 10 MPa. However, this strain might be 'piezosensitive' as no growth occurred at 50 MPa

Fig. 1. Temperature-dependent growth rate of strain LT17^T.

(see Supplementary Fig. S1 in IJSEM Online). Results of the other phenotypic characterizations are given in the species description and in Table 1.

The 16S rRNA gene was analysed as described by Seo *et al.* (2005a). The 16S rRNA gene sequence (1539 bp) of strain LT17^T was aligned manually with representative sequences of the genus *Shewanella* and related taxa by using known 16S rRNA secondary-structure information. Phylogenetic trees were inferred by the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1993) and maximum-parsimony (Fitch, 1971) methods. Evolutionary-distance matrices (for the neighbour-joining method) were generated according to the model of Jukes & Cantor (1969). The trees were rooted by using *Colwellia psychrerythraea* (GenBank accession no. AF001375) and *Alteromonas macleodii* (GenBank accession no. X82145) as an outgroup. The PHYLIP package (Felsenstein, 1993) was used for all analyses. The resultant unrooted tree topology was evaluated by bootstrap analyses (1000 replicates;

Table 1. Physiological characteristics that differentiate strain LT17^T from related species of the genus *Shewanella*

Strains: 1, strain LT17^T; 2, *S. olleyana* ACAM 644^T (data from Skerratt *et al.*, 2002); 3, *S. pacifica* KMM 3597^T (Ivanova *et al.*, 2004); 4, *S. japonica* KMM 3299^T (Ivanova *et al.*, 2001). All species are straight, rod-shaped organisms and are positive in tests for the production of oxidase, catalase, lipase and gelatinase, the reduction of nitrate to nitrite, synthesis of EPA and growth at 10 °C after 24 h. All species are negative for the utilization of D-mannose and citrate. +, Positive reaction; –, negative reaction; ND, not determined.

Characteristic	1	2	3	4
Optimal pH for growth	7.0–7.5	7.0	ND	7.5
Optimal temperature for growth (°C)	17	20–22	20–25	20–25
Growth at 32 °C	–	+	+	+
Growth in the presence of:				
0 % NaCl	–	–	–	–
3 % NaCl	+	+	+	–
6 % NaCl	–	+	+	–
Production of:				
Aesculin	+	+	ND	ND
Amylase	–	+	+	+
Indole	–	ND	+	ND
Urease	–	+	ND	ND
Utilization of:				
N-Acetylglucosamine	–	–	–	+
Arabinose	–	–	+	ND
D-Gluconate	–	+	ND	–
D-Glucose	–	–	+	+
DL-Malate	–	+	ND	–
Maltose	–	–	+	+
DNA G + C content (mol%)	38.8	44	39.5–40.5	43–44

Felsenstein, 1985) using the neighbour-joining method. Sequence similarity analysis indicated that the closest relatives of strain LT17^T were *S. japonica* (97.8 %), *S. pacifica* (97.5 %), *S. olleyana* (96.8 %), *Shewanella frigidimarina* (96.5 %) and *Shewanella gelidimarina* (95.4 %). Sequence similarities to all other species included in the phylogenetic analyses were <95.0 % (Fig. 2).

Chemotaxonomic analyses were performed according to a previously described method (Seo *et al.*, 2005a) except that the cells used were cultivated in MB at 15 °C for 1 day. Cellular fatty acids of strain LT17^T ranged in carbon chain length from C12 to C20 and included saturated, unsaturated and iso-branched components (Table 2). The dominant cellular fatty acids were iso-C13:0 (9.8 %), iso-C15:0 (10.4 %), C16:0 (15.4 %), C16:1 ω 7 (18.5 %) and C20:5 ω 3 (16.2 %); a range similar to that reported for *S. olleyana* (Skerratt *et al.*, 2002). Of particular note among the fatty acids of strain LT17^T was C20:5 ω 3 which is usually found in both psychrophilic and piezophilic marine bacteria (DeLong *et al.*, 1997; Nogi *et al.*, 1998). According to Russell & Nichols (1999), the progenitor of the genus *Shewanella* possessed the ability to synthesize PUFAs and this ability has disappeared on several occasions during the divergence of psychrotolerant and/or mesophilic species. Kato & Nogi (2001) proposed that two major subgenus branches of the

genus *Shewanella* should be recognized taxonomically. Group I species were characterized as high-pressure, cold-adapted species that produce substantial amounts of EPA (11–16 %), while Group II species included mesophilic, pressure-sensitive species that do not produce or produce only low levels of EPA (3–5 %). However, in the present study, strain LT17^T produces relatively higher concentrations of EPA than Group II species, but does not show any typical characteristics such as piezophily or psychrophily as found in *Shewanella* Group I species. There are also several contradictory descriptions reported for species of the genus *Shewanella* such as *S. olleyana* (Skerratt *et al.*, 2002), *S. japonica* (Ivanova *et al.*, 2001) and *S. pacifica* (Ivanova *et al.*, 2004). These findings imply that the presence of EPA in the genus *Shewanella* is not just associated with psychrophily and piezophily, but is instead a more broadly distributed trait amongst *Shewanella* species. Production of EPA is an important physiological and descriptive component that allows differentiation between *Shewanella* species and it may also have an important ecological role, acting as a nutrient source for marine as well as estuarine biota that require essential fatty acids yet are unable to synthesize ω 3 fatty acids *de novo* (Skerratt *et al.*, 2002).

DNA–DNA hybridization was performed according to the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes. The highest and lowest values in each sample were excluded and the remaining three values were used for calculation of the mean value. DNA–DNA reassociation values between strain LT17^T and the type strains of the closely related species *S. japonica* and *S. pacifica* were lower than 14 %. This value is significantly lower than that accepted as the genotypic delineation of a species (Wayne *et al.*, 1987). DNA G + C content was determined by using the HPLC method as described previously by Seo *et al.* (2005b). The DNA G + C content of the strain LT17^T was 38.8 mol%, which is within the accepted range for the genus *Shewanella* (39–52 mol%).

Description of *Shewanella donghaensis* sp. nov.

Shewanella donghaensis [dong.ha.en'sis. N.L. fem. adj. *donghaensis* of Donghae, the Korean name for the East Sea (Sea of Japan) from which the strain was isolated].

Cells are rod-shaped, 1.0–1.5 μ m in length and 0.5–0.8 μ m in width, single or in chains, motile, Gram-negative and facultatively anaerobic heterotrophs. Colonies are circular, opaque, convex with entire margins and orange coloured. Growth occurs between 5 and 22 °C, with optimum growth at 17 °C. The pH range for growth is 6.5–8.5; optimum growth occurs at pH 7.0–7.5. Growth occurs between 0.5 and 4.0 % (w/v) NaCl and is optimum at 2.5 % (w/v) NaCl. The hydrostatic pressure range for growth is between 0.1 and <30 MPa, with the maximum growth rate observed at 10 MPa. Catalase- and oxidase-positive. Reduces nitrate to nitrite. Negative in tests for amylase, arginine dihydrolase, urease and β -galactosidase activities, the production of indole and acidification from glucose. Exhibits gelatinase,

Fig. 2. Neighbour-joining phylogenetic tree showing relationships between strain LT17^T and members of the genus *Shewanella* based on 16S rRNA gene sequences. Numbers at nodes are levels of bootstrap support, based on neighbour-joining analyses of 1000 resampled datasets. Solid circles indicate that the corresponding nodes (groupings) are also recovered in Fitch–Margoliash, maximum-likelihood and maximum-parsimony trees. The full tree from which this figure is taken is available as Supplementary Fig. S2 in IJSEM Online. Bar, 0.01 substitutions per nucleotide position.

Table 2. Fatty acid content (%) of strain LT17^T and related species of the genus *Shewanella*

Strains: 1, strain LT17^T; 2, *S. olleyana* ACAM 644^T (data from Skerratt *et al.*, 2002); 3, *S. pacifica* KMM 3597^T (Ivanova *et al.*, 2004); 4, *S. japonica* KMM 3299^T (Ivanova *et al.*, 2001); 5, *S. gelidimarina* (Nichols *et al.*, 1997); 6, *S. frigidimarina* (Kato & Nogi, 2001); Values are percentages of total fatty acids. tr, Trace; NA, not available; –, not detected.

Fatty acid	1	2	3	4	5	6
12:0	0.5	3.8	—	3.7	1	3
13:0	1.5	1.3	—	0.6	1	—
14:0	3.9	3.2	—	4.2	4	5
15:0	3.4	3.7	—	1.1	7	5
16:0	15.4	15.2	8.9	16.6	6	12
17:0	2.0	2.0	—	0.7	1	1
iso-13:0	9.8	8.0	9.3	7.5	16	5
iso-14:0	0.2	2.5	—	0.8	1	—
iso-15:0	10.4	17.8	33.9	19.6	8	9
anteiso-15:0	0.8	2.8	—	2.3	—	—
iso-16:0	0.4	0.5	—	0.2	—	—
iso-17:0	0.4	1.9	—	1.6	tr	—
16:1 ω 7	18.5	16.2	14.8	23.4	27	42
17:1 ω 8	1.2	3.4	—	0.7	4	6
18:1 ω 7	4.9	2.4	—	5.8	1	3
18:1 ω 9	1.6	2.2	—	1.6	tr	1
20:5 ω 3	16.2	8.8	5.3	2.0	16	3
Optimum growth temperature (°C)	17	20–22	20–25	20–25	15–17	20–22
Incubation temperature (°C)	15	24	28	28	NA	NA

aesculin hydrolysis (β -glucosidase) and lipase activities. Does not utilize D-glucose, arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, D-gluconate, caprate, DL-malate, citrate or phenylacetate as sole carbon sources. Major fatty acids are iso-C13:0, iso-C15:0, C16:0, C16:1 ω 7 and C20:5 ω 3. The G + C content of the DNA is 38.8 mol%.

The type strain, LT17^T (= KCTC 10635BP^T = JCM 12524^T), was isolated from deep-sea sediment of the East Sea (3300 m depth).

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