Shewanella marisflavi sp. nov. and *Shewanella aquimarina* sp. nov., slightly halophilic organisms isolated from sea water of the Yellow Sea in Korea

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Two Gram-negative, motile, non-spore-forming, rod-shaped organisms, strains SW-117^T and SW-120^T, were isolated from sea water of the Yellow Sea in Korea and subjected to a polyphasic taxonomic study. Strains SW-117^T and SW-120^T simultaneously contained both menaquinones (MK) and ubiquinones (Q) as isoprenoid quinones; the predominant menaquinone was MK-7 and the predominant ubiquinones were Q-7 and Q-8. The major fatty acid detected in the two strains was iso-C_{15:0}. The DNA G + C content of strains SW-117^T and SW-120^T was 51 and 54 mol%, respectively. Phylogenetic analyses based on 16S rRNA gene sequences showed that strains SW-117^T and SW-120^T fall within the radiation of the cluster comprising *Shewanella* species. Strains SW-117^T and SW-120^T showed a 16S rRNA gene sequence similarity of 97.4% and a DNA–DNA relatedness level of 10.1%. Strains SW-117^T and SW-120^T exhibited 16S rRNA gene sequence similarity levels of 93.8–98.5% and 92.4–97.0%, respectively, to *Shewanella* species. Strain SW-117^T exhibited DNA–DNA relatedness levels of 8.3–20.3% to the type strains of six phylogenetically related *Shewanella* species. On the basis of phenotypic, phylogenetic and genetic data, strains SW-117^T and SW-120^T were classified in the genus *Shewanella* as two distinct novel species, for which the names *Shewanella marisflavi* sp. nov. (type strain, SW-117^T = KCCM 41822^T = JCM 12192^T) and *Shewanella aquimarina* sp. nov. (type strain, SW-120^T = KCCM 41821^T = JCM 12193^T) are proposed, respectively.

The genus *Shewanella* was created by MacDonell & Colwell (1985) with two species that had been assigned to the genus *Alteromonas*, *Alteromonas putrefaciens* and *Alteromonas hanedai*. Subsequently, many *Shewanella* species have been isolated from a variety of sources, including aquatic and marine environments (Nealson *et al.*, 1991; Ivanova *et al.*, 2001; Bozal *et al.*, 2002), clinical samples (Nozue *et al.*, 1992; Brink *et al.*, 1995; Venkateswaran *et al.*, 1999), sediments (Myers & Nealson, 1988), oilfield fluids (Semple & Westlake, 1987) and others. The genus *Shewanella* is phylogenetically affiliated to the γ -Proteobacteria

(Anzai *et al.*, 2000). There are at least 25 recognized *Shewanella* species at the time of writing. In this study, we describe two Gram-negative, slightly halophilic, rod-shaped organisms, strains SW-117^T and SW-120^T, which were isolated from sea water of the Yellow Sea in Korea. From 16S rRNA gene sequence comparisons, these organisms were considered to be phylogenetically related to the genus *Shewanella*. Accordingly, the aim of the present work was to establish the taxonomic positions of strains SW-117^T and SW-120^T by using a combination of polyphasic taxonomic data.

Strains SW-117^T and SW-120^T were isolated by a standard dilution plating technique on marine agar 2216 (MA; Difco) at 30 °C. *Shewanella marinintestina* JCM 11558^T, *Shewanella sairae* JCM 11563^T and *Shewanella schlegeliana* JCM 11561^T were obtained from the Japan Collection of Microorganisms (JCM), Saitama, Japan. *Shewanella affinis* KMM 3587^T and *Shewanella waksmanii* KMM 3823^T were obtained from Professor Elena P. Ivanova, Industrial Research Institute, Swinburne University of Technology, Australia. *Shewanella colwelliana* ATCC 39565^T was obtained

Published online ahead of print on 17 September 2004 as DOI 10.1099/ijs.0.63198-0.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains SW-117^T and SW-120^T and *Shewanella colwelliana* ATCC 39565^T are AY485224, AY485225 and AY653177, respectively.

Detailed phenotypic characteristics of *Shewanella marisflavi*, *Shewanella aquimarina* and related species, and an expanded neighbour-joining tree are available as supplementary material in IJSEM Online.

from the American Type Culture Collection (ATCC), Manassas, USA. Cell biomass of strains SW-117^T and SW-120^T for respiratory lipoquinone analysis and for DNA extraction was obtained from cultivation in marine broth 2216 (MB; Difco) at 30 °C. For fatty acid methyl ester (FAME) analysis, cell mass of strains SW-117^T and SW-120^T was obtained from agar plates after cultivation for 2 days at 30 °C on MA and trypticase soy agar (TSA; Difco). Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy (TEM). Flagellum type was examined by TEM using cells from exponentially growing cultures. Gram reaction was determined using a Gram Stain kit (bioMérieux) according to the manufacturer's instructions. The pH range for growth was determined in MB that was adjusted to various pH values (pH 4.5–9.0 at intervals of 0.5 pH units). Growth at

various NaCl concentrations was investigated in MB or trypticase soy broth (Difco). Growth in the absence of NaCl was investigated in trypticase soy broth without NaCl. Growth at various temperatures (4–50 °C) was measured on MA. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on anaerobically prepared MA. Catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1 % (w/v) *p*-aminodimethylaniline oxalate. Hydrolysis of casein, starch and Tween 80 was determined as described by Cowan & Steel (1965). Hydrolysis of hypoxanthine, tyrosine and xanthine was performed on MA using substrate concentrations described by Cowan & Steel (1965). Hydrolysis of gelatin and aesculin and nitrate reduction were determined as described by Lanyi (1987) with a modification

Table 1. Phenotypic characteristics of *Shewanella marisflavi* sp. nov., *Shewanella aquimarina* sp. nov. and related *Shewanella* species

Species: 1, *S. marisflavi*; 2, *S. aquimarina*; 3, *S. affinis*, data from Ivanova *et al.* (2004); 4, *S. colwelliana*, data from Weiner *et al.* (1988) and Ivanova *et al.* (2004); 5, *S. waksmanii*, data from Ivanova *et al.* (2003b). +, Positive reaction; –, negative reaction; V, variable reaction; ND, not determined; *n*, number of strains. All species are rod-shaped, Gram-negative and polarly flagellated. All species are positive for growth in 3 % NaCl, catalase, oxidase and gelatinase. All species are negative for utilization of D-fructose, glycerol, lactose and sucrose.

Characteristic	1 (<i>n</i> =1)	2 (<i>n</i> =1)	3 (<i>n</i> =4)	4 (<i>n</i> =1)	5 (<i>n</i> =1)
Optimal pH for growth	7–8	7–8	ND	7.4–7.8	7.5
Optimal temperature for growth (°C)	30–37	30–37	20–25	25	2–25
Maximum temperature for growth (°C)	42	42	34	30	30
Growth at:					
4 °C	+	–	–	–	+
35 °C	+	+	–	–	–
40 °C	+	+	–	–	–
NO ₃ to NO ₂	+	+	+	+	ND
NO ₂ to N ₂	–	–	–	ND	ND
Haemolysis	+	+	+	ND	+
H ₂ S production	+	+	+	ND	ND
Production of:					
Amylase	–	+	–	+	–
Lipase	+	+	+	ND	+
Growth at:					
0 % NaCl	+	–	–	–	–
6 % NaCl	+	+	+	–	+
8 % NaCl	+	+	V	–	–
Utilization of:					
D-Galactose	–	+	–	–	–
Maltose	+	+	ND	–	ND
Succinate	+	+	–	–	–
Fumarate	–	–	–	+	–
Citrate	–	–	+	–	–
D-Mannitol	–	–	+	–	ND
D-Sorbitol	–	–	ND	–	ND
DL-Malate	+	+	ND	–	ND
DL-Lactate	+	+	+	–	–
DNA G + C content (mol%)	51	54	45	46	43

that artificial sea water was used. The artificial sea water contained (per litre of distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Levring, 1946). Hydrolysis of birchwood xylan (Sigma) was determined on solid marine salts basal medium (Baumann & Baumann, 1981) containing 0.5 % (w/v) xylan as the sole carbon source. H₂S production was tested as described by Bruns *et al.* (2001). Haemolytic activity was recorded on MA with 5 % defibrinated sheep blood. Enzyme activity was determined using the API ZYM system (bioMérieux). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981).

Isoprenoid quinones were extracted and analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. For quantitative analysis of the cellular fatty acid compositions, a loop of cell mass was harvested and FAMES were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), except that ribonuclease T1 was used with ribonuclease A. The DNA G+C content was determined by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The 16S rRNA gene was amplified by PCR using two universal primers as described by Yoon *et al.* (1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded; reported DNA–DNA relatedness values are the mean of the remaining three values.

Morphological, cultural, physiological and biochemical characteristics of strains SW-117^T and SW-120^T are shown in Table 1 or are given in the species descriptions below. Strain SW-117^T grew at 4 °C and without NaCl, but strain SW-120^T did not. Starch was hydrolysed by strain SW-120^T, but not by strain SW-117^T. D-Galactose was utilized by strain SW-120^T, but not by strain SW-117^T. Acid from D-glucose, D-cellobiose and maltose was produced by strain SW-117^T, but not by strain SW-120^T. Strains SW-117^T and SW-120^T contained simultaneously both menaquinones (MK) and ubiquinones (Q) as isoprenoid quinones. The predominant ubiquinones detected in strain SW-117^T were Q-7 and Q-8 at a peak area ratio of about 49 and 48 %, respectively, and the predominant ubiquinones detected in strain SW-120^T were Q-7 (67 %) and Q-8 (31 %). The two strains contained MK-7 as the predominant menaquinone (about 92 and 86 %, respectively). Strains SW-117^T and SW-120^T had cellular fatty

acid profiles that contained large amounts of straight-chain, branched, unsaturated and hydroxy fatty acids (Table 2). There were differences in the proportions of some fatty acids when the two strains were grown on MA and TSA (Table 2). Strains SW-117^T and SW-120^T contained iso-C_{15:0}, iso-C_{15:0} 2-OH and/or C_{16:1}ω7c and C_{16:0} as the major fatty acids when they were grown on MA (Table 2). The proportions of iso-C_{15:0} 2-OH and/or C_{16:1}ω7c and C_{16:0} decreased when the two strains were grown on TSA (Table 2). The proportions of some fatty acids, for example iso-C_{13:0}, iso-C_{17:0} and iso-C_{13:0} 3-OH, increased when the strains were grown on TSA (Table 2). The DNA G+C contents of strains SW-117^T and SW-120^T were 51 and 54 mol%, respectively, values higher than those

Table 2. Percentage cellular fatty acid composition of strains SW-117^T and SW-120^T on marine agar (MA) and trypticase soy agar (TSA)

Fatty acid*	SW-117 ^T		SW-120 ^T	
	MA	TSA	MA	TSA
Straight-chain fatty acid				
C _{12:0}	1.6	0.6	1.9	0.6
C _{13:0}	0.5	0.4	0.9	0.4
C _{14:0}	1.5	0.31	1.3	0.2
C _{15:0}	4.1	3.1	4.4	1.6
C _{16:0}	14.5	4.1	11.6	2.3
C _{17:0}	1.9	1.8	1.7	0.8
Branched fatty acid				
iso-C _{11:0}	2.0	4.8	0.1	0.2
iso-C _{13:0}	5.9	9.9	7.4	13.7
iso-C _{14:0}	0.7	0.6	0.9	1.1
iso-C _{15:0}	22.9	30.9	27.5	34.9
iso-C _{16:0}	0.2	0.6	0.4	1.3
iso-C _{17:0}	1.6	6.0	1.9	9.9
Unsaturated fatty acid				
C _{16:1} ω9c	0.7	0.3	0.7	0.3
C _{17:1} ω6c	0.6	0.9	0.8	1.0
C _{17:1} ω8c	5.3	9.5	6.7	7.8
C _{18:1} ω7c	4.1	2.9	3.4	2.3
C _{18:1} ω9c	1.7	1.2	1.6	0.9
Hydroxy fatty acid				
C _{12:0} 3-OH	1.3	0.7	0.8	0.2
iso-C _{13:0} 3-OH	4.5	11.3	5.1	10.9
Summed features†				
1	0.5	0.7	0.6	0.3
2	0.9	0.3	0.9	0.2
3	18.6	5.7	15.9	6.0

*Fatty acids representing less than 0.5 % in all rows were omitted.

†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 1 contained one or more of C_{13:0} 3-OH and/or iso-C_{15:1}. Summed feature 2 contained one or more of iso-C_{16:1} I and/or C_{14:0} 3-OH. Summed feature 3 contained one or more of C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

of most *Shewanella* species (Table S1, supplementary table in IJSEM Online).

Almost-complete 16S rRNA gene sequences of strains SW-117^T and SW-120^T comprising, respectively, 1500 and 1499 nt (about 96% of the *Escherichia coli* 16S rRNA gene sequence) were determined in this study. Phylogenetic analysis based on 16S rRNA gene sequences showed that strains SW-117^T and SW-120^T fall within the radiation of the cluster comprising *Shewanella* species. In a phylogenetic tree based on the neighbour-joining algorithm, strains SW-117^T and SW-120^T occupied independent evolutionary lineages within the phylogenetic radiation encompassed by the genus *Shewanella*, particularly within the clade comprising *Shewanella affinis*, *S. colwelliana*, *S. waksmanii*, *S. marinintestina*, *S. sairae*, *S. schlegeliana*, *S. pealeana*, *S. gelidimarina*, *S. benthica*, *S. violacea*, *S. hanedai*, *S. woodyi*, *S. fidelis*, *S. amazonensis* and *S. algae* (Fig. 1). The relationship between this clade and the cluster comprising other *Shewanella* species was supported by a high bootstrap confidence level of 99.9% (Fig. 1). The level of 16S rRNA gene sequence similarity between strains SW-117^T and SW-120^T was 97.4%. Strains SW-117^T and SW-120^T exhibited highest 16S rRNA gene sequence similarity values to *S. affinis* KMM 3587^T and *S. colwelliana* ATCC 39565^T (98.5%, respectively) and *S. waksmanii* KMM 3823^T (97.0%) (Fig. 1). Strains SW-117^T and SW-120^T exhibited 16S rRNA gene sequence similarity levels of 93.8–97.9 and 92.4–96.9%, respectively, to the type strains of the other recognized *Shewanella* species. Levels of 16S rRNA gene sequence similarity between strains SW-117^T and SW-120^T and other species used in the phylogenetic analysis were lower than 91.9% (Fig. 1). DNA–DNA hybridization was performed to determine the genetic relatedness between strains SW-117^T and SW-120^T, between strain SW-120^T and the type strains of three *Shewanella* species, and between strain SW-117^T and

the type strains of six *Shewanella* species that showed 16S rRNA gene sequence similarity levels of more than 97% to strain SW-117^T. Strains SW-117^T and SW-120^T exhibited mean DNA–DNA relatedness of 10.1%, when their DNAs were used individually as labelled DNA probes for cross-hybridization. This value indicates that strains SW-117^T and SW-120^T are members of different genomic species. Strain SW-117^T exhibited DNA–DNA relatedness levels of 8.3–20.3% to *S. affinis* KMM 3587^T, *S. colwelliana* ATCC 39565^T, *S. waksmanii* KMM 3823^T, *S. marinintestina* JCM 11558^T, *S. sairae* JCM 11563^T and *S. schlegeliana* JCM 11561^T. Strain SW-120^T exhibited DNA–DNA relatedness levels of 6.3–13.7% to *S. affinis* KMM 3587^T, *S. colwelliana* ATCC 39565^T and *S. waksmanii* KMM 3823^T.

16S rRNA gene sequence analysis revealed that strains SW-117^T and SW-120^T have closest phylogenetic affiliations to the γ -Proteobacteria, particularly to the genus *Shewanella* (Fig. 1). The results obtained in chemotaxonomic analyses are consistent with the results of 16S rRNA gene sequence analysis and phylogenetic inference. The cellular fatty acid profiles of the two strains are similar to those of recognized *Shewanella* species, although there are some differences in the proportions of the major fatty acids (Bowman *et al.*, 1997; Venkateswaran *et al.*, 1999; Bozal *et al.*, 2002; Ivanova *et al.*, 2003a, b; Satomi *et al.*, 2003). Strains SW-117^T and SW-120^T have respiratory lipoquinone patterns similar to those of *Shewanella* species, containing Q-7 and Q-8 as the predominant ubiquinones. Menaquinone detected in strain SW-117^T and SW-120^T has also been detected in some *Shewanella* species together with ubiquinones, although their compositions are variable for different strains or species (Nogi *et al.*, 1998; Venkateswaran *et al.*, 1999; Bozal *et al.*, 2002).

Strains SW-117^T and SW-120^T have approximately 39 bp (2.6%) differences between their 16S rRNA gene sequences.

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strains SW-117^T and SW-120^T and representatives of related taxa based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branch points. Bar, 0.01 substitution per nucleotide position. The tree from which Fig. 1 was taken is available as supplementary material in IJSEM Online.

Both strains are considered to be members of different species based on DNA–DNA hybridization data, together with differences in the DNA G + C contents and in their phenotypic properties, including, among others, growth at 4 °C and in the absence of NaCl, and starch hydrolysis (Table 1). Strains SW-117^T and SW-120^T are differentiated from phylogenetically related *Shewanella* species by some physiological and biochemical characteristics, such as temperature for growth, NaCl tolerance and the ability to utilize certain substrates (Table 1 and Table S1 in IJSEM Online). Strain SW-117^T exhibited 16S rRNA gene sequence similarity levels of less than 96.3 % to the type strains of other *Shewanella* species except *S. affinis*, *S. colwelliana*, *S. waksmanii*, *S. marinintestina*, *S. sairae* and *S. schlegeliana*. Levels of DNA–DNA relatedness between strains SW-117^T and SW-120^T and the type strains of some phylogenetically related *Shewanella* species are far below the threshold value (70 %) suggested for species delineation in current bacterial systematics (Wayne *et al.*, 1987). Levels of 16S rRNA gene sequence similarity (92.4–97.0 %) between strain SW-120^T and the type strains of *Shewanella* species are low enough to categorize strain SW-120^T as representing a species distinct from recognized *Shewanella* species (Stackebrandt & Goebel, 1994). Therefore, in view of the combined phenotypic, chemotaxonomic and phylogenetic data, together with genomic distinctiveness, strains SW-117^T and SW-120^T should be placed in the genus *Shewanella* as two distinct novel species, for which the names *Shewanella marisflavi* sp. nov. and *Shewanella aquimarina* sp. nov. are proposed, respectively.

Description of *Shewanella marisflavi* sp. nov.

Shewanella marisflavi (ma.ris fla'vi. L. gen. neut. n. *maris* of the sea; L. masc. adj. *flavus* yellow; N.L. gen. n. *marisflavi* of the Yellow Sea).

Cells are straight rods, 0.8–1.1 × 2.0–3.5 µm on MA. Non-spore-forming. Motile by means of a single polar flagellum. Colonies are smooth, glistening, circular, flat to slightly raised, light brown in colour and 2.0–4.0 mm in diameter after 3 days incubation at 30 °C on MA. Growth occurs at 4 and 42 °C, but not above 43 °C. Growth is observed at pH 5.0, but not at pH 4.5. Optimal growth occurs in the presence of 2–3 % NaCl. No growth occurs in the presence of more than 9 % NaCl. Growth occurs under anaerobic conditions on MA. Casein, tyrosine and Tween 80 are hydrolysed. Aesculin, hypoxanthine, urea, xanthine and xylan (birch wool) are not hydrolysed. When assayed with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase are present and leucine arylamidase is weakly present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase are absent. Acid is produced from D-cellobiose, D-glucose, maltose and D-ribose. Acid is not produced from L-arabinose, D-fructose, D-galactose,

lactose, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, stachyose, sucrose, D-trehalose, D-xylose, adonitol, D-mannitol, *myo*-inositol or D-sorbitol. Both menaquinones and ubiquinones are present; the predominant menaquinone is MK-7 and the predominant ubiquinones are Q-7 and Q-8. The major fatty acid is iso-C_{15:0}. The DNA G + C content is 51 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain (SW-117^T = KCCM 41822^T = JCM 12192^T) was isolated from sea water of the Yellow Sea in Korea.

Description of *Shewanella aquimarina* sp. nov.

Shewanella aquimarina (a.qui.ma.ri'na. L. n. *aqua* water; L. adj. *marinus* of the sea; N.L. fem. adj. *aquimarina* pertaining to sea water).

Cells are straight rods, 0.6–0.9 × 2.0–4.0 µm on MA. Non-spore-forming. Motile by means of a single polar flagellum. Colonies are smooth, glistening, circular, flat to slightly raised, light brown in colour and 2.0–4.0 mm in diameter after 3 days incubation at 30 °C on MA. Growth occurs at 10 and 42 °C, but not at 4 °C or above 43 °C. Growth is observed at pH 5.0, but not at pH 4.5. Optimal growth occurs in the presence of 2–3 % NaCl. No growth occurs in the presence of more than 9 % NaCl. Growth occurs under anaerobic conditions on MA. Casein, tyrosine and Tween 80 are hydrolysed. Aesculin, hypoxanthine, urea, xanthine and xylan (birch wool) are not hydrolysed. When assayed with the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase are present and leucine arylamidase is weakly present, but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase are absent. Acid is produced from D-ribose. Acid is not produced from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, stachyose, sucrose, D-trehalose, D-xylose, adonitol, D-mannitol, *myo*-inositol or D-sorbitol. Both menaquinones and ubiquinones are present; the predominant menaquinone is MK-7 and the predominant ubiquinones are Q-7 and Q-8. The major fatty acid is iso-C_{15:0}. The DNA G + C content is 54 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain (SW-120^T = KCCM 41821^T = JCM 12193^T) was isolated from sea water of the Yellow Sea in Korea.

Acknowledgements

This work was supported by the 21C Frontier program of Microbial Genomics and Applications (grant MG02-0401-001-1-0-0) from the Ministry of Science and Technology (MOST) of the Republic of Korea. We are grateful to Professor Elena P. Ivanova for providing *S. affinis* KMM 3587^T and *S. waksmanii* KMM 3823^T.

References

- Anzai, Y., Kim, H., Park, J.-Y., Wakabayashi, H. & Oyaizu, H. (2000). Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol* **50**, 1563–1589.
- Baumann, L. & Baumann, P. (1981). The marine Gram-negative eubacteria; genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In *The Prokaryotes. A Handbook on Habitats, Isolation, and Identification of Bacteria*, pp. 1302–1330. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- Bowman, J. P., McCammon, S. A., Nichols, D. S., Skerratt, J. H., Rea, S. M., Nichols, P. D. & McMeekin, T. A. (1997). *Shewanella gelidimarina* sp. nov. and *Shewanella frigidimarina* sp. nov., novel Antarctic species with the ability to produce eicosapentaenoic acid (20:5 ω 3) and grow anaerobically by dissimilatory Fe(III) reduction. *Int J Syst Bacteriol* **47**, 1040–1047.
- Bozal, N., Montes, M. J., Tudela, E., Jiménez, F. & Guinea, J. (2002). *Shewanella frigidimarina* and *Shewanella livingstonensis* sp. nov. isolated from Antarctic coastal areas. *Int J Syst Evol Microbiol* **52**, 195–205.
- Brink, A. J., van Straten, A. & van Rensburg, A. J. (1995). *Shewanella* (*Pseudomonas*) *putrefaciens* bacteremia. *Clin Infect Dis* **20**, 1327–1332.
- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* **51**, 1997–2006.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Ivanova, E. P., Sawabe, T., Gorshkova, N. M., Svetashev, V. I., Mikhailov, V. V., Nicolau, D. V. & Christen, R. (2001). *Shewanella japonica* sp. nov. *Int J Syst Evol Microbiol* **51**, 1027–1033.
- Ivanova, E. P., Sawabe, T., Hayashi, K., Gorshkova, N. M., Zhukova, N. V., Nedashkovskaya, O. I., Mikhailov, V. V., Nicolau, D. V. & Christen, R. (2003a). *Shewanella fidelis* sp. nov., isolated from sediments and sea water. *Int J Syst Evol Microbiol* **53**, 577–582.
- Ivanova, E. P., Nedashkovskaya, O. I., Zhukova, N. V., Nicolau, D. V., Christen, R. & Mikhailov, V. V. (2003b). *Shewanella waksmanii* sp. nov., isolated from a sipuncula (*Phascolosoma japonicum*). *Int J Syst Evol Microbiol* **53**, 1471–1477.
- Ivanova, E. P., Nedashkovskaya, O. I., Sawabe, T., Zhukova, N. V., Frolova, G. M., Nicolau, D. V., Mikhailov, V. V. & Bowman, J. P. (2004). *Shewanella affinis* sp. nov., isolated from marine invertebrates. *Int J Syst Evol Microbiol* **54**, 1089–1093.
- Komagata, K. & Suzuki, K.-I. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–203.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **85**, 1183–1184.
- Levring, T. (1946). Some culture experiments with *Ulva* and artificial seawater. *K Fysiogr Sällsk Lund Förh* **16**, 45–56.
- MacDonell, M. T. & Colwell, R. R. (1985). Phylogeny of the *Vibrionaceae*, and recommendation for two new genera, *Listonella* and *Shewanella*. *Syst Appl Microbiol* **6**, 171–182.
- Myers, C. R. & Nealson, K. H. (1988). Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Science* **240**, 1319–1321.
- Nealson, K. H., Myers, C. R. & Wimpsee, B. (1991). Isolation and identification of manganese-reducing bacteria, and estimates of microbial Mn(IV)-reducing potential in the Black Sea. *Deep Sea Res* **38**, Suppl 2, S907–S920.
- Nogi, Y., Kato, C. & Horikoshi, K. (1998). Taxonomic studies of deep-sea barophilic *Shewanella* strains and description of *Shewanella violacea* sp. nov. *Arch Microbiol* **170**, 331–338.
- Nozue, H., Hayashi, T., Hashimoto, Y., Ezaki, T., Hamasaki, K., Ohwada, K. & Terawaki, Y. (1992). Isolation and characterization of *Shewanella alga* from human clinical specimens and emendation of the description of *S. alga* Simidu et al. 1990, 335. *Int J Syst Bacteriol* **42**, 628–634.
- Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *Technical Note 101*. Newark, DE: MIDI.
- Satomi, M., Oikawa, H. & Yano, Y. (2003). *Shewanella marinintestina* sp. nov., *Shewanella schlegeliana* sp. nov. and *Shewanella sairae* sp. nov., novel eicosapentaenoic-acid-producing marine bacteria isolated from sea-animal intestines. *Int J Syst Evol Microbiol* **53**, 491–499.
- Semple, K. M. & Westlake, D. W. S. (1987). Characterization of iron-reducing *Alteromonas putrefaciens* strains from oil field fluids. *Can J Microbiol* **33**, 366–371.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reverse-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Venkateswaran, K., Moser, D. P., Dollhopf, M. E. & 10 other authors (1999). Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. *Int J Syst Bacteriol* **49**, 705–724.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Weiner, R. M., Coyne, V. E., Brayton, P., West, P. & Raiken, S. F. (1988). *Alteromonas colwelliana* sp. nov., an isolate from oyster habitats. *Int J Syst Bacteriol* **38**, 240–244.
- Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.
- Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998). Inter- and intraspecific phylogenetic analysis of the genus *Nocardioideis* and related taxa based on 16S rDNA sequences. *Int J Syst Bacteriol* **48**, 187–194.
- Yoon, J.-H., Kim, I.-G., Shin, D.-Y., Kang, K. H. & Park, Y.-H. (2003). *Microbulbifer salipaludis* sp. nov., a moderate halophile isolated from a Korean salt marsh. *Int J Syst Evol Microbiol* **53**, 53–57.