Shewanella marina sp. nov., isolated from seawater

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A motile, rod-shaped, pale-brown-pigmented bacterium, designated strain C4^T, was isolated from seawater collected from the South Sea (Republic of Korea). Cells were Gram-negative, facultatively anaerobic, and catalase- and oxidase-positive. The major fatty acids were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 19.4%), C_{16:0} (16.3%), C_{17:1}ω8c (9.5%) and iso-C_{15:0} (7.7%). The DNA G + C content was 40.8 mol%. A phylogenetic tree based on 16S rRNA gene sequences showed that strain C4^T formed a lineage within the genus *Shewanella* (92.7–96.1% sequence similarity to representative strains of the genus *Shewanella*) and was part of a distinct branch with the clade comprising *Shewanella haliotis* DW01^T and *Shewanella algae* ATCC 51192^T. Phenotypic characteristics enabled strain C4^T to be distinguished from *S. haliotis* and *S. algae*. On the basis of the data presented in this study, strain C4^T represents a novel species, for which the name *Shewanella marina* sp. nov. is proposed. The type strain is C4^T (=KCTC 22185^T=JCM 15074^T).

The genus *Shewanella* is a member of the class *Gammaproteobacteria* (Anzai *et al.*, 2000) and comprises a group of Gram-negative, motile, rod-shaped, oxidase-positive, non-fermentative and facultatively anaerobic aquatic and marine bacteria (Bowman, 2005; Gauthier *et al.*, 1995; MacDonell & Colwell, 1985; Venkateswaran *et al.*, 1999). At the time of writing, the genus *Shewanella* comprised 47 recognized species (<http://www.bacterio.cict.fr/s/shewanella.html>). Strains of the genus *Shewanella* have been isolated from a variety of sources, i.e. marine environments including seawater, sediment, invertebrates and fish (Bowman *et al.*, 1997; Bozal *et al.*, 2002; Brettar *et al.*, 2002; Gao *et al.*, 2006; Gram *et al.*, 1987; Gram & Huss, 1996; Hirota *et al.*, 2005; Ivanova *et al.*, 2001, 2003a, b, 2004a, b; Kim *et al.*, 2007; Lee *et al.*, 2006; Leonardo *et al.*, 1999; Makemson *et al.*, 1997; Miyazaki *et al.*, 2006; Myers & Nealson, 1988; Nealson *et al.*, 1991; Nogi *et al.*, 1998; Satomi *et al.*, 2003, 2006, 2007; Simidu *et al.*, 1990; Skerratt *et al.*, 2002; Stenström & Molin, 1990; Toffin *et al.*, 2004; Venkateswaran *et al.*, 1998, 1999; Xiao *et al.*, 2007; Yang *et al.*, 2006, 2007; Yoon *et al.*, 2004a, b; Ziemke *et al.*, 1998; Zhao *et al.*, 2005, 2006, 2007), clinical samples (Brink *et al.*, 1995; Debois *et al.*, 1975; Holmes *et al.*, 1975; Levin, 1972; Nozue *et al.*, 1992), oilfield fluids (Semple & Westlake, 1987) and activated sludge (Xu *et al.*, 2005).

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain C4^T is EU290154.

A scanning electron micrograph and GN2 MicroPlate (Biolog) data for strain C4^T are available as supplementary material with the online version of this paper.

Members of this genus are known to play important roles in co-metabolic bioremediation of halogenated organic pollutants (Petrovskis *et al.*, 1994), destruction of crude petroleum (Semple & Westlake, 1987), the dissimilatory reduction of manganese and iron oxides (Myers & Nealson, 1988) and production of high proportions of polyunsaturated fatty acids (Russell & Nichols, 1999). They have also been implicated as opportunistic pathogens of humans and aquatic animals (Aguirre *et al.*, 1994; Brink *et al.*, 1995) and as the causal agents of proteinaceous food spoilage (Jørgensen & Huss, 1989).

In the course of our study on the microbial diversity of seawater, a rod-shaped bacterial strain, designated C4^T, was isolated and was the subject of a taxonomic investigation. On the basis of evidence from polyphasic taxonomic studies, strain C4^T is considered to represent a novel species in the genus *Shewanella*.

Strain C4^T was isolated from seawater collected from the South Sea near Gwangyang (34° 51' N 128° 47' E), Republic of Korea, using the standard dilution plating technique. Isolation was achieved on marine agar (MA; Difco) (Yang *et al.*, 2006) at 30 °C for 7 days. The isolate was cultured routinely on MA and maintained at –80 °C as a suspension in marine broth (MB; Difco) containing glycerol (20%, w/v).

Bacterial DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). The resulting sequence of strain C4^T was aligned manually

against sequences obtained from GenBank. Phylogenetic trees were inferred from the regions available for all sequences (positions 38–1450; *Escherichia coli* numbering system) using the Fitch–Margoliash (Fitch & Margoliash, 1967) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the program JPHYDIT (available at <http://plaza.snu.ac.kr/~jchun/jphydit/>) and PAUP 4.0 (Swofford, 1998) as described previously (Chun *et al.*, 2000).

Preliminary sequence comparison with 16S rRNA gene sequences held in GenBank indicated that our isolate was closely related to strains of the genus *Shewanella*. The newly determined sequence was then aligned manually against representatives of the genus *Shewanella*. Strain C4^T showed the highest 16S rRNA gene sequence similarity to *Shewanella waksmanii* KMM 3823^T (96.1%), followed by *Shewanella fidelis* KMM 3582^T (96.0%), *Shewanella surugensis* DSM 17177^T (95.8%), *Shewanella pneumatophori* JCM 13187^T (95.8%), *Shewanella schlegeliana* JCM 11561^T (95.7%), *Shewanella haliotis* DW01^T (95.6%) and *Shewanella algae* ATCC 51192^T (95.6%); similarities to other members of the genus *Shewanella* were 92.7–95.6%. To elucidate the

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain C4^T and members of the genus *Shewanella*. Numbers at nodes are the levels of bootstrap support (%) based on neighbour-joining analyses of 1000 resampled datasets. The sequence of *Vibrio cholerae* ATCC 14035^T (Z21856) was used as an outgroup (not shown). Bar, 0.01 nt substitutions per position.

phylogenetic relationship between the novel isolate and other members of the genus *Shewanella*, phylogenetic trees were constructed using two different tree-making algorithms. The neighbour-joining tree (Fig. 1) showed that strain C4^T formed a distinct branch with the clade comprising *S. algae* ATCC 51192^T and *S. haliotis* DW01^T, which was also observed in the Fitch–Margoliash tree (data not shown). On the basis of 16S rRNA gene sequence similarity data and the results of the phylogenetic analysis, it is clear that our isolate represents a novel genomic species within the genus *Shewanella* (Wayne *et al.*, 1987).

As 16S rRNA gene sequence similarities between strain C4^T and recognized *Shewanella* species were below the level indicative of relatedness at the species level (97%; Stackebrandt & Goebel, 1994), no DNA–DNA hybridization tests were needed to define a novel species.

Growth on various standard bacteriological media was tested on nutrient agar (NA; Difco), tryptic soy agar (TSA; Difco) and R2A agar (Difco) according to the manufacturer's instructions. Cells grown at 30 °C for 2 days were observed using a differential interference microscope (BX50; Olympus) and a scanning electron microscope (S-4800; Hitachi). Cells of strain C4^T grown on MA at 30 °C for 3–7 days were used for physiological and biochemical tests. Motility was examined by observing cells grown in wet mounts using a phase-contrast microscope (DS-Fi1; Nikon). The requirement for sea salts (0–10%; Sigma) for

growth was tested using synthetic ZoBell medium (15 g Bacto agar, 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate in 1 l distilled water). The pH range for growth was determined in ZoBell medium that was adjusted to various pH values (pH 3–11, in increments of 1 pH unit) by the addition of HCl and NaOH. Growth at various temperatures (4–50 °C) and growth in an anaerobic chamber (CO₂/H₂/N₂, 10:10:80 by vol.; Sheldon Manufacturing) were assessed using MA and incubation times of up to 1 week. Catalase and oxidase activities were determined using 3% (v/v) hydrogen peroxide and Kovacs' reagent (Kovacs, 1956), respectively. Acid production from glucose was tested as described by Yamaguchi & Yokoe (2000). Nitrate reduction was tested on MB containing 0.2% KNO₃ (Skerman, 1967). H₂S production was determined on Kligler iron agar (Difco) with 2% (w/v) sea salts. Hydrolysis of carboxymethylcellulose (1%, w/v), casein (2%, w/v, skim milk), egg yolk (10%, w/v), starch (0.2%, w/v) and Tween 80 (1%, w/v) was tested as described by Smibert & Krieg (1994) using MA as the basal medium. Hydrolysis of L-tyrosine (0.5%, w/v) and xylan (1%, w/v) was tested using MA as the basal medium (Barrow & Feltham, 1993). DNase activity was determined with DNase test agar (Difco) containing 2% (w/v) sea salts. Other biochemical tests and enzymic activities were performed using API 20E and API ZYM kits (bioMérieux) and the GN2 MicroPlate (Biolog) prepared according to the manufacturer's instructions except that bacterial strains

Table 1. Phenotypic characteristics that differentiate strain C4^T from type strains of phylogenetically related *Shewanella* species

Strains: 1, C4^T; 2, *S. algae* ATCC 51192^T; 3, *S. fidelis* KMM 3582^T; 4, *S. haliotis* DW01^T; 5, *S. waksmanii* KMM 3823^T. Data are from Ivanova *et al.* (2003a, b), Khashe & Janda (1998), Kim *et al.* (2007), Nozue *et al.* (1992) and this study. All strains are positive for catalase, oxidase, production of H₂S and gelatinase. All strains are negative for utilization of citrate and lactose. +, Positive; –, negative; w, weakly positive; ND, no data available; R, resistant; S, sensitive.

Characteristic	1	2	3	4	5
Source	Seawater	Salt marsh	Sediment	Abalone	Sipuncula
Colony colour	Pale brown	Pink	Pinkish	Pink–orange	Greenish brown
Growth at 4 °C	+	–	+	–	+
Growth at 42 °C	–	+	–	+	–
Arginine dihydrolase	+	–	ND	–	ND
Lysine decarboxylase	+	–	ND	–	ND
Ornithine decarboxylase	–	+	ND	+	ND
Acidification of glucose	w	w	+	–	+
Assimilation of:					
Mannose	–	+	–	–	+
Caprate	–	+	ND	+	ND
Malate	–	–	ND	+	ND
Glucose	–	–	+	–	+
Fructose	–	+	–	–	–
Antibiotic resistance					
Ampicillin (10 µg)	S	R*	ND	R	ND
Polymyxin B (300 IU)	S	S*	S	R	S
DNA G + C content (mol%)	40.8†	54.0	45.0	53.7	43.0

*Data from this study.

†Mean value from two DNA analyses.

were suspended in distilled water supplemented with 2% (w/v) sea salts. Antibiotic resistance was determined by the disc diffusion method using commercial antibiotic-impregnated discs (BBL Becton Dickinson). The results were interpreted according to the guidelines set forth by the CLSI (2003).

Cells of strain C4^T were facultatively anaerobic, Gram-negative, motile and rod-shaped (Supplementary Fig. S1, available in IJSEM Online). Colonies grown on MA plates for 5 days at 30 °C were circular, convex with entire margins, smooth, opaque, pale brown in colour and approximately 2.0–3.0 mm in diameter. The isolate grew well on MA and R2A, but not on TSA or NA. On MA, strain C4^T was able to grow at 4–37 °C. Detailed results of physiological and biochemical analyses are given in Table 1 and the species description. It is evident from Table 1 that there are several phenotypic characteristics that readily separate strain C4^T from phylogenetically related species.

Cellular fatty acids of strain C4^T grown on MA for 2 days at 30 °C were prepared (in duplicate) and analysed as methyl esters by GLC according to the instructions of the Microbial Identification system (MIDI). For G+C content calculations, DNA samples were prepared in duplicate and the G+C content was determined by using the thermal denaturation method of Marmur & Doty (1962).

The cellular fatty acid profile of strain C4^T is given in Table 2. Significant differences in the contents of predominant fatty acids were found between strain C4^T and strains of related species, i.e. summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0} and iso-C_{15:0}. The DNA G+C content of strain C4^T was 40.8±0.4 mol%, which is lower than those of *S. algae* and *S. haliotis*.

Phylogenetic, genomic, chemotaxonomic and phenotypic data clearly indicate that strain C4^T represents a novel genomic species within the genus *Shewanella*, for which the name *Shewanella marina* sp. nov. is proposed.

Description of *Shewanella marina* sp. nov.

Shewanella marina (ma.ri'na. L. fem. adj. *marina* marine, of the sea, where the type strain was isolated).

Cells are rod-shaped, Gram-negative and facultatively anaerobic. Cells grow best on media such as MA and R2A. Colonies on MA are circular, convex with entire margins, smooth, opaque, pale brown in colour and approximately 2.0–3.0 mm in diameter after 5 days at 30 °C (pH 7). Cells are motile rods, 0.4–0.5 × 1.4–2.0 μm in size. Growth occurs in 2–9% (w/v) NaCl (optimum, 3%), at pH 5–10 (optimum, pH 7) and at 4–37 °C (optimum, 30 °C). Oxidase- and catalase-positive. Acetoin is produced. Casein, DNA and Tween 80 are hydrolysed, but carboxymethylcellulose, egg yolk, aesculin, starch, tyrosine and xylan are not. In the API ZYM gallery: alkaline phosphatase, acid phosphatase, esterase

Table 2. Cellular fatty acid compositions (%) of strain C4^T and type strains of phylogenetically related *Shewanella* species

Strains: 1, C4^T; 2, *S. algae* ATCC 51192^T; 3, *S. fidelis* KMM 3582^T; 4, *S. haliotis* DW01^T; 5, *S. waksmanii* KMM 3823^T. Data are from Ivanova *et al.* (2003a, b), Khashe & Janda (1998), Kim *et al.* (2007), Nozue *et al.* (1992) and this study. –, Fatty acid not present; tr, trace amount (<1%).

Fatty acid	1*	2	3	4	5
Straight-chain saturated					
C _{12:0}	3.7	1.9	1.2	2.2	2.0
C _{13:0}	tr	tr	1.5	tr	2.3
C _{14:0}	1.4	1.2	2.3	1.9	1.7
C _{15:0}	2.5	3.7	8.7	2.8	5.3
C _{16:0}	16.3	11.6	12.9	13.4	6.2
C _{17:0}	2.3	3.0	1.5	2.5	2.9
C _{18:0}	2.4	3.3	tr	6.5	tr
C _{12:0} 3-OH	2.0	2.4	–	2.0	–
Branched saturated					
iso-C _{13:0}	4.4	3.3	10.7	4.1	10.3
iso-C _{14:0}	2.0	tr	tr	1.1	tr
iso-C _{15:0}	7.7	20.7	22.3	17.7	33.4
iso-C _{16:0}	tr	tr	–	tr	tr
iso-C _{17:0}	tr	1.8	tr	1.6	4.7
iso-C _{13:0} 3-OH	1.3	2.9	–	2.5	tr
Monounsaturated					
C _{15:1} ω6c	–	–	1.0	–	tr
C _{16:1} ω7c	–	–	20.0	–	9.8
C _{16:1} ω9c	2.0	1.3	–	1.1	–
C _{17:1} ω6c	tr	tr	1.9	tr	1.2
C _{17:1} ω8c	9.5	11.8	6.3	10.7	6.2
C _{18:1} ω7c	5.3	4.8	5.6	5.4	2.9
C _{18:1} ω9c	5.1	4.1	tr	4.5	tr
C _{20:5} ω3c	–	–	–	–	6.7
Summed feature 1†	tr	1.9	–	1.5	–
Summed feature 2†	2.6	1.2	–	1.1	–
Summed feature 3†	19.4	13.5	–	12.5	–

*Mean values from two determinations.

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

(C4), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and trypsin are positive; esterase lipase (C8), cystine arylamidase and valine arylamidase are weakly positive; and *N*-acetyl-β-glucosaminidase, α-chymotrypsin, α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, β-glucosidase, lipase (C14) and α-mannosidase are negative. The following carbon sources are utilized (positive with the Biolog GN2 system): DL-lactic acid, L-arabinose (weak), D-glucose (weak), L-rhamnose (weak), citric acid (weak), malonic acid (weak), propionic acid (weak), L-alanine (weak), L-alanyl glycine (weak) and α-hydroxybutyric acid (weak).

The remaining substrates of the Biolog GN2 system are not utilized (Supplementary Table S1, available in IJSEM Online). Cells are sensitive to (μg per disc, unless otherwise indicated) amikacin (30), ampicillin (10), chloramphenicol (30), gentamicin (10), erythromycin (15), kanamycin (30), nalidixic acid (30) and polymyxin B (300 IU), but resistant to penicillin (10 IU), streptomycin (10), tetracycline (30) and vancomycin (30). Other physiological and biochemical characteristics are given in Table 1. Major fatty acids are summed feature 3 ($\text{C}_{16:1}\omega 7\text{c}$ and/or iso- $\text{C}_{15:0}$ 2-OH), $\text{C}_{16:0}$, $\text{C}_{17:1}\omega 8\text{c}$ and iso- $\text{C}_{15:0}$; the complete fatty acid composition of the type strain is given in Table 2.

The type strain, C4^T (=KCTC 22185^T=JCM 15074^T), was isolated from seawater collected from the South Sea, Republic of Korea. The DNA G+C content of the type strain is 40.8 mol%.

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References

- Aguirre, A. A., Balazs, G. H., Zimmerman, B. & Spraker, T. R. (1994). Evaluation of Hawaiian green turtles (*Cheledonia mydas*) for potential pathogens associated with fibropapillomas. *J Wildl Dis* **30**, 8–15.
- 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.
- Barrow, G. I. & Feltham, R. K. A. (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd edn. Cambridge: Cambridge University Press.
- Bowman, J. P. (2005). Genus XIII. *Shewanella* MacDonell and Colwell 1986, 355^{VP} (Effective publication: MacDonell and Colwell 1985, 180). In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 480–491. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: 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.
- Brettar, I., Christen, R. & Höfle, M. G. (2002). *Shewanella denitrificans* sp. nov., a vigorously denitrifying bacterium isolated from the oxic-anoxic interface of the Gotland Deep in the central Baltic Sea. *Int J Syst Evol Microbiol* **52**, 2211–2217.
- Brink, A. J., van Straten, A. & van Rensburg, A. J. (1995). *Shewanella* (*Pseudomonas*) *putrefaciens* bacteremia. *Clin Infect Dis* **20**, 1327–1332.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Chun, J., Bae, K. S., Moon, E. Y., Jung, S.-O., Lee, H. K. & Kim, S.-J. (2000). *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete isolated from a saltern. *Int J Syst Evol Microbiol* **50**, 1909–1913.
- CLSI (2003). Performance standards for antimicrobial disk susceptibility tests, 8th edn. Approved Standard M2-A8. Wayne, PA: Clinical Laboratory Standards Institute.
- Debois, J., Degreef, H., Vandepitte, J. & Spaepen, J. (1975). *Pseudomonas putrefaciens* as a cause of infection in humans. *J Clin Pathol* **28**, 993–996.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279–284.
- Gao, H., Obrastova, A., Stewart, N., Popa, R., Fredrickson, J. K., Tiedje, J. M., Nealson, K. H. & Zhou, J. (2006). *Shewanella loihica* sp. nov., isolated from iron-rich microbial mats in the Pacific Ocean. *Int J Syst Evol Microbiol* **56**, 1911–1916.
- Gauthier, G., Gauthier, M. & Christen, R. (1995). Phylogenetic analysis of the genera *Alteromonas*, *Shewanella*, and *Moritella* using genes coding for small-subunit rRNA sequences and division of the genus *Alteromonas* into two genera, *Alteromonas* (emended) and *Pseudoalteromonas* gen. nov., and proposal of twelve new species combinations. *Int J Syst Bacteriol* **45**, 755–761.
- Gram, L. & Huss, H. H. (1996). Microbiological spoilage of fish and fish products. *Int J Food Microbiol* **33**, 121–137.
- Gram, L., Trolle, G. & Huss, H. H. (1987). Detection of specific spoilage bacteria from fish stored at low (0 °C) and high (20 °C) temperatures. *Int J Food Microbiol* **4**, 65–72.
- Hirota, K., Nodasaka, Y., Orikasa, Y., Okuyama, H. & Yumoto, I. (2005). *Shewanella pneumatophori* sp. nov., an eicosapentaenoic acid-producing marine bacterium isolated from the intestines of Pacific mackerel (*Pneumatophorus japonicus*). *Int J Syst Evol Microbiol* **55**, 2355–2359.
- Holmes, B., Lapage, S. P. & Malnick, H. (1975). Strains of *Pseudomonas putrefaciens* from clinical material. *J Clin Pathol* **28**, 149–155.
- 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., Nedashkovskaya, O. I., Zhukova, N. V., Nicolau, D. V., Christen, R. & Mikhailov, V. V. (2003a). *Shewanella waksmanii* sp. nov., isolated from a sipuncula (*Phascolosoma japonicum*). *Int J Syst Evol Microbiol* **53**, 1471–1477.
- Ivanova, E. P., Sawabe, T., Hayashi, K., Gorshkova, N. M., Zhukova, N. V., Nedashkovskaya, O. I., Mikhailov, V. V., Nicolau, D. V. & Christen, R. (2003b). *Shewanella fidelis* sp. nov., isolated from sediments and sea water. *Int J Syst Evol Microbiol* **53**, 577–582.
- Ivanova, E. P., Gorshkova, N. M., Bowman, J. P., Lysenko, A. M., Zhukova, N. V., Sergeev, A. F., Mikhailov, V. V. & Nicolau, D. V. (2004a). *Shewanella pacifica* sp. nov., a polyunsaturated fatty acid-producing bacterium isolated from sea water. *Int J Syst Evol Microbiol* **54**, 1083–1087.
- Ivanova, E. P., Nedashkovskaya, O. I., Sawabe, T., Zhukova, N. V., Frolova, G. M., Nicolau, D. V., Mikhailov, V. V. & Bowman, J. P. (2004b). *Shewanella affinis* sp. nov., isolated from marine invertebrates. *Int J Syst Evol Microbiol* **54**, 1089–1093.
- Jørgensen, B. R. & Huss, H. H. (1989). Growth and activity of *Shewanella putrefaciens* isolated from spoiling fish. *Int J Food Microbiol* **9**, 51–62.

- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Khashe, S. & Janda, J. M. (1998). Biochemical and pathogenic properties of *Shewanella alga* and *Shewanella putrefaciens*. *J Clin Microbiol* **36**, 783–787.
- Kim, D., Baik, K. S., Kim, M. S., Jung, B.-M., Shin, T.-S., Chung, G.-H., Rhee, M. S. & Seong, C. N. (2007). *Shewanella haliotis* sp. nov., isolated from the gut microflora of abalone, *Haliotis discus hannai*. *Int J Syst Evol Microbiol* **57**, 2926–2931.
- Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* **178**, 703.
- Lee, O. O., Lau, S. C. K., Tsoi, M. M. Y., Li, X., Plakhotnikova, I., Dobretsov, S., Wu, M. C. S., Wong, P.-K., Weinbauer, M. & Qian, P.-Y. (2006). *Shewanella ircinia* sp. nov., a novel member of the family Shewanellaceae, isolated from the marine sponge *Ircinia dendroides* in the Bay of Villefranche, Mediterranean Sea. *Int J Syst Evol Microbiol* **56**, 2871–2877.
- Leonardo, M. R., Moser, D. P., Barbieri, E., Brantner, C. A., MacGregor, B. J., Paster, B. J., Stackebrandt, E. & Neilson, K. H. (1999). *Shewanella pealeana* sp. nov., a member of the microbial community associated with the accessory nidamental gland of the squid *Loligo pealei*. *Int J Syst Bacteriol* **49**, 1341–1351.
- Levin, R. E. (1972). Correlation of DNA base composition and metabolism of *Pseudomonas putrefaciens* isolates from food, human clinical specimens, and other sources. *Antonie van Leeuwenhoek* **38**, 121–127.
- 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.
- Makemson, J. C., Fulayfil, N. R., Landry, W., Van Ert, L. M., Wimpee, C. F., Widder, E. A. & Case, J. F. (1997). *Shewanella woodyi* sp. nov., an exclusively respiratory luminous bacterium isolated from the Alboran Sea. *Int J Syst Bacteriol* **47**, 1034–1039.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Miyazaki, M., Nogi, Y., Usami, R. & Horikoshi, K. (2006). *Shewanella surugensis* sp. nov., *Shewanella kaireitica* sp. nov. and *Shewanella abyssi* sp. nov., isolated from deep-sea sediments of Suruga Bay, Japan. *Int J Syst Evol Microbiol* **56**, 1607–1613.
- Myers, C. R. & Neilson, K. H. (1988). Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Science* **240**, 1319–1321.
- Neilson, K. H., Myers, C. R. & Wimpee, B. (1991). Isolation and identification of manganese reducing bacteria, and estimates of microbial manganese reducing potential in the Black Sea. *Deep Sea Res* **38**, 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 clinical specimens and emendation of the description of *S. alga* Simidu *et al.*, 1990, 335. *Int J Syst Bacteriol* **42**, 628–634.
- Petrovskis, E. A., Vogel, T. M. & Adriaens, P. (1994). Effects of electron acceptors and donors on transformation of tetrachloromethane by *Shewanella putrefaciens* MR-1. *FEMS Microbiol Lett* **121**, 357–363.
- Russell, N. J. & Nichols, D. S. (1999). Polyunsaturated fatty acids in marine bacteria – a dogma rewritten. *Microbiology* **145**, 767–779.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- 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.
- Satomi, M., Vogel, B. F., Gram, L. & Venkateswaran, K. (2006). *Shewanella hafniensis* sp. nov. and *Shewanella morhuae* sp. nov., isolated from marine fish of the Baltic Sea. *Int J Syst Evol Microbiol* **56**, 243–249.
- Satomi, M., Vogel, B. F., Venkateswaran, K. & Gram, L. (2007). Description of *Shewanella glacialipiscicola* sp. nov. and *Shewanella algidipiscicola* sp. nov., isolated from marine fish of the Danish Baltic Sea, and proposal that *Shewanella affinis* is a later heterotypic synonym of *Shewanella colwelliana*. *Int J Syst Evol Microbiol* **57**, 347–352.
- Semple, K. M. & Westlake, D. W. S. (1987). Characterization of iron-reducing *Alteromonas putrefaciens* strains from oil field fluids. *Can J Microbiol* **35**, 925–931.
- Simidu, U., Kita-Tsukamoto, K., Yasumoto, T. & Yotsu, M. (1990). Taxonomy of four marine bacterial strains that produce tetrodotoxin. *Int J Syst Bacteriol* **40**, 331–336.
- Skerman, V. B. D. (1967). *A Guide to the Identification of the Genera of Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.
- Skerratt, J. H., Bowman, J. P. & Nichols, P. D. (2002). *Shewanella olleyana* sp. nov., a marine species isolated from a temperate estuary which produces high levels of polyunsaturated fatty acids. *Int J Syst Evol Microbiol* **52**, 2101–2106.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- 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.
- Stenström, I. M. & Molin, G. (1990). Classification of the spoilage flora of fish, with special reference to *Shewanella putrefaciens*. *J Appl Bacteriol* **68**, 601–618.
- Swofford, D. L. (1998). PAUP*: Phylogenetic analysis using parsimony (and other methods), version 4. Sunderland, MA: Sinauer Associates.
- Toffin, L., Bidault, A., Pignet, P., Tindall, B. J., Slobodkin, A., Kato, C. & Prieur, D. (2004). *Shewanella profunda* sp. nov., isolated from deep marine sediment of the Nankai Trough. *Int J Syst Evol Microbiol* **54**, 1943–1949.
- Venkateswaran, K., Dollhopf, M. E., Aller, R., Stackebrandt, E. & Neilson, K. H. (1998). *Shewanella amazonensis* sp. nov., a novel metal-reducing facultative anaerobe from Amazonian shelf muds. *Int J Syst Bacteriol* **48**, 965–972.
- Venkateswaran, K., Moser, D. P., Dollhopf, M. E., Lies, D. P., Saffarini, D. A., MacGregor, B. J., Ringelberg, D. B., White, D. C., Nishijima, M. & 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., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & 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.
- Xiao, X., Wang, P., Zeng, X., Bartlett, D. H. & Wang, F. (2007). *Shewanella psychrophila* sp. nov. and *Shewanella piezotolerans* sp. nov.,

isolated from west Pacific deep-sea sediment. *Int J Syst Evol Microbiol* 57, 60–65.

Xu, M., Guo, J., Cen, Y., Zhong, X., Cao, W. & Sun, G. (2005). *Shewanella decolorationis* sp. nov., a dye-decolorizing bacterium isolated from activated sludge of a waste-water treatment plant. *Int J Syst Evol Microbiol* 55, 363–368.

Yamaguchi, S. & Yokoe, M. (2000). A novel protein-deamidating enzyme from *Chryseobacterium proteolyticum* sp. nov., a newly isolated bacterium from soil. *Appl Environ Microbiol* 66, 3337–3343.

Yang, S.-H., Kwon, K. K., Lee, H.-S. & Kim, S.-J. (2006). *Shewanella spongiae* sp. nov., isolated from a marine sponge. *Int J Syst Evol Microbiol* 56, 2879–2882.

Yang, S.-H., Lee, J.-H., Ryu, J.-S., Kato, C. & Kim, S.-J. (2007). *Shewanella donghaensis* sp. nov., a psychrophilic, piezosensitive bacterium producing high levels of polyunsaturated fatty acid, isolated from deep-sea sediments. *Int J Syst Evol Microbiol* 57, 208–212.

Yoon, J. H., Kang, K. H., Oh, T. K. & Park, Y. H. (2004a). *Shewanella gaetbuli* sp. nov., a slight halophile isolated from a tidal flat in Korea. *Int J Syst Evol Microbiol* 54, 487–491.

Yoon, J. H., Yeo, S. H., Kim, I. G. & Oh, T. K. (2004b). *Shewanella marisflavi* sp. nov. and *Shewanella aquimarina* sp. nov., slightly halophilic organisms isolated from sea water of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* 54, 2347–2352.

Zhao, J.-S., Manno, D., Beaulieu, C., Paquet, L. & Hawari, J. (2005). *Shewanella sediminis* sp. nov., a novel Na⁺-requiring and hexahydro-1,3,5-trinitro-1,3,5-triazine-degrading bacterium from marine sediment. *Int J Syst Evol Microbiol* 55, 1511–1520.

Zhao, J.-S., Manno, D., Leggiadro, C., O'Neil, D. & Hawari, J. (2006). *Shewanella halifaxensis* sp. nov., a novel obligately respiratory and denitrifying psychrophile. *Int J Syst Evol Microbiol* 56, 205–212.

Zhao, J.-S., Manno, D., Thiboutot, S., Ampleman, G. & Hawari, J. (2007). *Shewanella canadensis* sp. nov. and *Shewanella atlantica* sp. nov., manganese dioxide- and hexahydro-1,3,5-trinitro-1,3,5-triazine-reducing, psychrophilic marine bacteria. *Int J Syst Evol Microbiol* 57, 2155–2162.

Ziemke, F., Höfle, M. G., Lalucat, J. & Rosselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* 48, 179–186.