NOTE

***Shewanella japonica* sp. nov.**

¹ Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences, 690022 Vladivostok, Pr. 100 Let Vladivostoku 159, Russia

² Laboratory of Microbiology, Faculty of Fisheries, Hokkaido University, 3-1-1 Minatocho, Hakodate 041-8611, Japan

³ Institute of Marine Biology of the Far-Eastern Branch of the Russian Academy of Sciences, 690041 Vladivostok, Russia

⁴ Industrial Research Institute, Swinburne University of Technology, PO Box 218, Hawthorn, Victoria 3122, Australia

⁵ Centre National de la Recherche Scientifique et Université Pierre et Marie Curie, Station Zoologique, Villefranche-sur-Mer 06230, France

Elena P. Ivanova,^{1†} Tomoo Sawabe,² Nataliya M. Gorshkova,¹ Vassillii I. Svetashev,³ Valery V. Mikhailov,¹ Dan V. Nicolau⁴ and Richard Christen⁵

Author for correspondence: Elena P. Ivanova. Tel: +61 3 9214 5137. Fax: +61 3 9214 5050. e-mail: eivanova@groupwise.swin.edu.au

Two strains of agar-digesting bacteria, KMM 3299^T and KMM 3300, respectively isolated from sea water and the mussel *Protothaca jedoensis*, have been characterized. Based on sequencing of the 16S rRNA gene, KMM 3299^T showed the highest similarity (93–95%) to members of the genus *Shewanella*. The G+C contents of the DNAs of these strains were 43–44 mol%. The level of DNA homology between the two strains was conspecific (95%), indicating that they represent a distinct genospecies. These organisms were non-pigmented, Gram-negative, polarly flagellated, facultatively anaerobic, mesophilic, neutrophilic and able to degrade a wide range of high molecular mass polymers, including alginate, carrageenan, laminaran and agar. The novel organisms were susceptible to gentamycin, carbenicillin, lincomycin and oleandomycin. The predominant cellular fatty acids were i-15:0, 16:0, 16:1(n-7), 18:1(n-7). Eicosapentaenoic acid, 20:5(n-3), was detected in the two isolates at levels of 1–8%, depending on the temperature of cultivation. Phylogenetic evidence, together with phenotypic characteristics, showed that the two isolates studied constitute a novel species of the genus *Shewanella*. The name *Shewanella japonica* is proposed; the type strain is KMM 3299^T (= LMG 19691^T = CIP 106860^T).

Keywords: *Shewanella japonica* sp. nov.

The genus *Shewanella* MacDonell and Colwell 1986 currently comprises 12 species of Gram-negative, facultatively anaerobic proteobacteria, associated mainly with aquatic habitats. During the last decade, the bacteria of this genus have received a significant amount of attention due to their important roles in co-metabolic bioremediation of halogenated organic pollutants (Petrovskis *et al.*, 1994), destructive souring of crude petroleum (Semple & Westlake, 1987) and the dissimilatory reduction of magnesium and iron oxides (Myers & Nealson, 1988). Recently, a comprehensive study of the phylogenetic relationships and taxonomy of the genus provided an improved approach for identification of newly isolated wild strains (Venkateswaran *et al.*, 1999).

[†] **Present address:** Swinburne University of Technology, IRIS, 533–545 Burwood Road, Hawthorn, Melbourne, Victoria 3122, Australia.

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

The GenBank accession number for the 16S rDNA sequence of *Shewanella japonica* KMM 3299^T is AF145921.

In this study, a novel agar-digesting bacterium of the genus *Shewanella* is described, isolated from sea water and the mussel *Protothaca jedoensis*, dwelling in the coastal area of the Sea of Japan. The two isolates formed a distinct phylogenetic clade among other previously described shewanellae and constitute a new species.

Sea water samples were collected in 1994 from a depth of 0.5–1.5 m (salinity, 32‰; temperature, 15 °C) at the Pacific Institute of Bio-organic Chemistry of the Marine Experimental Station, Troitz Bay, Gulf of Peter the Great, Sea of Japan. The mussels were collected at a depth of 3–5 m and were prepared aseptically as described elsewhere (Ivanova *et al.*, 1996). All samples were plated onto marine 2216 agar 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, 0.002% (w/v) KH₂PO₄, 0.005% (w/v) MgSO₄ · 7H₂O, 50% (v/v) natural sea water and 50% (v/v) distilled water at pH 7.0. Plates were

incubated aerobically at room temperature (ca. 20–22 °C) for 5, 7 or 10 d and subsequently purified. Strains were stored at –80 °C in marine 2216 broth (Difco) supplemented with 20% (v/v) glycerol. This work was a part of taxonomic investigation of free-living and symbiotrophic marine bacteria of the Far-Eastern region and, in the course of this work, several strains with phenotypes similar to that of the genus *Shewanella* were isolated.

Unless otherwise indicated, phenotypic characteristics were studied by using standard procedures (Baumann *et al.*, 1972; Smibert & Krieg, 1994) as described elsewhere (Ivanova *et al.*, 1998; Sawabe *et al.*, 2000). The tests for utilization of various organic substrates (listed in Table 1) as sole carbon sources at a concentration of 0.1% (w/v) were performed in tubes containing 10 ml liquid BM medium (Baumann *et al.*, 1972). The bacteria were grown with shaking on a rotary shaker at 160 r.p.m. for 72 h at 25 °C. Dissimilatory iron reduction was tested on LM medium (0.02% yeast extract, 0.01% peptone, 0.6% NaCl, 10 mM sodium bicarbonate, 10 mM HEPES) supplemented with carbon substrates as appropriate (5 mM lactate, 5 mM succinate, 5 mM glycerol, 1 mM acetate), 50 mM ferric citrate, 5 mM sodium molybdate and the colour reagent ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4 triazine; pH 7.2]. Plates were inoculated anaerobically at room temperature (ca. 7 d) (with positive and negative controls). Colonies displaying cleared zones were scored as positive for iron reduction.

Electron micrographs of negatively stained cells, using 1.25% uranyl acetate, were obtained using a Zeiss EM 10 CA electron microscope (80 kV).

Haemolytic activity of the strains studied was detected on blood agar (trypticase soy agar, 40%, w/v; sheep blood, 50 ml; water, 950 ml). Haemolytic activity on mouse erythrocytes and cytotoxicity on Ehrlich cells were tested on butanol extracts of the isolates. Bacterial cells from 100 ml culture were extracted with butanol (100 ml) and evaporated under reduced pressure. The extracts were dissolved in water up to 4 mg ml⁻¹. Mouse blood was diluted threefold with saline containing heparin. Erythrocytes were separated by centrifugation at 3000 g for 4 min and washed three times; a 0.2% erythrocyte suspension in saline was prepared. Butanol extracts (40 µl) were added to 1 ml erythrocyte suspension. The mixture was incubated at room temperature for 30 min with shaking. The tubes were then centrifuged for 5 min at 3000 g and the absorbance of the supernatant was measured at 540 nm to detect released haemoglobin. An erythrocyte suspension in saline was used as a blank. Total haemolysis was achieved by suspending the cells in distilled water.

Ascites tumour cells of Ehrlich carcinoma (tetraploid strain) were taken 7–8 d after tumour inoculation into stainless white mice of both sexes. The cell suspension

was diluted fivefold with saline and centrifuged at 3000 g for 10 min. Cells were then resuspended in culture medium 199 containing penicillin and streptomycin (250 U ml⁻¹) to a final concentration of 6–8 × 10⁶ cells ml⁻¹.

Antibacterial activity was assessed by the agar diffusion assay, based on the method described by Barry (1980). Cultures (0.1 ml) of test strains were spread on trypticase soy agar plates in which circular wells (diameter of 10 mm) had been cut. Samples (0.1 ml) were added to the wells and areas of inhibited bacterial growth were measured after incubation for 48 h at 28 °C. Zones of inhibited growth of the indicator strains surrounding the wells were observed. Mean diameters were measured and 10 mm (representing the diameter of the well) subtracted. Antibacterial activities were tested against *Staphylococcus aureus* CIP 103594^T, *Escherichia coli* ATCC 15034, *Proteus vulgaris* IFO 3851, *Enterococcus faecium* CIP 104105, *Bacillus subtilis* ATCC 6051^T and the yeast *Candida albicans* KMM 455.

The analysis of fatty acid (FA) methyl ethers was performed by GLC as described previously by Svetashev *et al.* (1995). Strains were grown on B medium at 28 °C for 24 h or at 12 °C for 4 d.

DNA was extracted from cells grown overnight on B medium following the method of Marmur (1961). The G+C content of the DNA was determined by the thermal denaturation method of Marmur & Doty (1962). Levels of genetic relatedness were determined spectrophotometrically and initial renaturation rates were determined as described by De Ley *et al.* (1970).

Bacterial DNAs for PCR were prepared using the Promega Wizard genomic DNA extraction kit according to the instruction manual. DNA templates (100 ng) were used in a PCR to amplify the small-subunit rRNA genes as described previously by Sawabe *et al.* (1998a, b). PCR conditions were as follows: an initial denaturation step at 94 °C for 180 s, an annealing step at 55 °C for 60 s and an extension step at 72 °C for 90 s. The thermal profile then consisted of 30 cycles. The amplification primers used in this study gave a 1.5 kb PCR product and corresponded to positions 25–1521 of the *E. coli* 16S rDNA sequence. PCR products were purified using a Promega Wizard PCR preps DNA purification kit and sequenced directly by using a *Taq* FS dye terminator sequencing kit (ABI) and the protocol recommended by the manufacturer. DNA sequencing was performed with an Applied Biosystems model 373S automated sequencer. Nine sequencing primers were used (Sawabe *et al.*, 1998a).

The 16S rDNA sequence of *Shewanella japonica* KMM 3299^T was aligned manually by reference to a database of 10000 already aligned bacterial 16S rDNA sequences. Phylogenetic trees were constructed according to three different methods (bioNJ, maximum-likelihood and maximum-parsimony). Only sites that

Table 1. Cellular fatty acid composition of *S. japonica*

Values are given as mean percentages of total fatty acid content. The first value was obtained after cultivation at 12 °C and the second value was obtained after cultivation at 28 °C.

Fatty acid	Relative content (%)
12:0	4.0/3.7
i-13:0	6.3/7.5
13:0	0.5/0.6
i-14:0	0.9/0.8
14:0	4.8/4.2
14:1(n-7)	0.3/0.4
i-15:0	12.5/19.6
a-15:0	2.5/2.3
15:0	1.1/1.1
a-15:1	0.1/0.3
15:1(n-8)	0.1/0.07
15:1(n-6)	0.9/0.1
i-16:0	0.2/0.2
16:0	15.5/16.6
16:1(n-7)	25.94/23.44
i-17:0	0.8/1.6
a-17:0	0.4/0.3
17:0	0.4/0.7
17:1(n-8)	0.2/0.1
17:1(n-6)	1.7/1.4
18:0	0.4/0.7
18:1(n-9)	1.6/1.6
18:1(n-7)	9.1/5.8
18:4(n-3)	0.1/0.06
20:4(n-3)	0.2/0.1
20:5(n-3)	8.3/2.0
Total	98.8/98.8
Branched	23.6/32.2

had no obvious homoplasy were used for these analyses (positions 57–155, 175–799, 806–973, 980–1089, 1095–1405 of the *S. japonica* sequence). For the neighbour-joining (NJ) analysis, matrix distance was calculated according to Kimura's two-parameter correction. Bootstraps were done using 500 replications, bioNJ and Kimura's two-parameter correction. bioNJ was done according to Gascuel (1997), maximum-likelihood data (options QFYG) were obtained using the fastDNAmI program (Olsen *et al.*, 1994) and maximum-parsimony was from PHYLIP (Phylogeny Inference Package, version 3.573c, distributed by J. Felsenstein, Department of Genetics, University of Washington, Seattle, WA, USA). The tree was drawn with NJPLOT (Jeanmougin *et al.*, 1998). For the tree shown in Fig. 2, the following sequences were used: *Aeromonas hydrophila* ATCC 7966^T (X60418), *Aeromonas caviae* ATCC 15468^T (X74674), *Colwellia psychroerythraea* ATCC 27364^T (AF001375), '*Curcubacter baltica*' Baltic 166 (AJ002006), *Feromonas balearica* PAT^T (X93021), *Moritella viscosa*

Fig. 1. Negatively stained cell of *S. japonica* KMM 3299^T.

NVI 88/478^T (AJ13226), *Oceanospirillum japonicum* ATCC 19191^T (AB006766), *Pseudoalteromonas aurantia* ATCC 33046^T (X82135), *Pseudomonas doudoroffii* ATCC 27123^T (AB019390), *Psychromonas antarctica* DSM 10704^T (Y14697), *Shewanella oneidensis* SP-7 (AF039054), '*Shewanella saccharophilus*' GC-29 (AF033028), *Shewanella baltica* NCTC 10735^T (AJ000214), *Shewanella putrefaciens* LMG 26268^T (X81623), '*Shewanella massilia*' (AJ006084), *S. oneidensis* MR-4 (AF005252), *Shewanella frigidimarina* ACAM 591^T (U85903), *Shewanella amazonensis* ATCC 700329^T (AF005248), *Shewanella colwelliana* ATCC 33888 (AF170794), *Shewanella gelidimarina* ACAM 456^T (U85907), *Shewanella pealeana* ANG-SQ1^T (AF011335), *Shewanella benthica* DB21MT-2 (AB008796), *Shewanella violacea* DSS12^T (D21225), *Shewanella hanedai* CIP 103207^T (X82132), *Shewanella woodyi* MS1 (AF003548), *Shewanella algae* ATCC 8073 (AF005250), *S. algae* ATCC 51192 (AF005249), *S. algae* IAM 14159^T (U91546), *Tolumonas auensis* TA 4^T (X92889), *Vibrio cholerae* CECT 514^T (X76337) and *Moritella marina* NCIMB 1144^T (X82142).

The two strains of heterotrophic marine bacteria were isolated from a sea water sample and the mussel *Protothaca jedoensis* (Lischke), a common inhabitant of the coastal area of the Gulf of Peter the Great, Sea of Japan, and the Yellow Sea at depths of 1–5 m. The two strains isolated (KMM 3299^T and KMM 3300) formed circular, smooth, convex colonies with an entire edge that were slightly pinkish and 3–5 mm in diameter after 2–4 d incubation at room temperature (ca. 22–24 °C). Cells of both strains were rod-shaped, 1–2 µm in length and 0.6–0.8 µm in diameter, polarly

Fig. 2. Phylogenetic position of *S. japonica* according to 16S rDNA sequence analysis. The tree shown was obtained by using a neighbour-joining algorithm. Branches also supported by maximum-likelihood and maximum-parsimony are indicated by asterisks and significant bootstrap values are indicated accordingly. The clade of the shewanellae was rooted using a selection of closely related outgroup sequences (members of the γ 3 group). *S. japonica* was not grouped robustly with any other known *Shewanella* species.

Table 2. Characteristics that differentiate *S. japonica* from phylogenetically related species

Data for *S. baltica* and *S. frigidimarina* were taken from Venkateswaran *et al.* (1999) and Bowman *et al.* (1997), respectively. All three taxa are positive for utilization of maltose.

Characteristic	<i>S. japonica</i>	<i>S. baltica</i>	<i>S. frigidimarina</i>
Cell shape	Straight rod	Straight rod	Straight rod
Optimal growth temp. (°C)	25–28	ND	20–22
Growth at 35 °C	+	–	–
Production of:			
Amylase	+	ND	–
Agarase, alginase, κ -carrageenase, laminarinase	+	–	–
Utilization of:			
D-Galactose	+	ND	–
D-Fructose	+	ND	–
Sucrose	+	–	–
Lactose	–	ND	ND
Succinate	+	ND	+
Fumarate	–	ND	+
Citrate	–	+	ND
D-Mannitol	–	ND	+
Glycerol	–	ND	ND
DL-Malate	–	+	ND
DNA G+C content (mol%)	43–44	46	40–43

ND, Data not available.

flagellated (Fig. 1) and Gram-negative. They did not form endospores. The strains were phenotypically similar to *Shewanella* species. Both were able to grow anaerobically by fermentation of glucose, a feature observed in some other species (*S. frigidimarina*, *S. gelidimarina*, *S. hanedai* and *S. benthica*), but could not reduce ferric compounds. Strains KMM 3299^T and KMM 3300 did not require organic growth factors and sodium ions or sea water for growth and grew well at 1–3% NaCl. No growth was detected at 6% NaCl. The temperature range for growth was between 10 and 37 °C, with optimum growth at 20–25 °C. No growth was detected at 40 °C. The pH values for growth ranged from 6.0 to 9.0, with optimum growth at pH 7.5. Both strains were oxidase- and catalase-positive and haemolytic and cytotoxic, but had no antibacterial activities. Both were susceptible to lincomycin, oleandomycin and polymyxin. The Far-Eastern *Shewanella* isolates were also positive for amylase, alginase, agarase, gelatinase, κ -carrageenase, DNase, laminarinase and lipase, but negative for chitinase and elastase. They were able to produce acid oxidatively from a number of carbohydrates. Phenotypic analysis showed that the two isolates were essentially similar to each other and differed only in their susceptibility to gentamycin and carbenicillin.

The DNA G+C content of strain KMM 3299^T was 43.4 ± 0.4 mol% and that of KMM 3300 was 44.3 ± 0.4 mol%. DNA–DNA hybridization data revealed a significant level of DNA relatedness be-

tween KMM 3299^T and KMM 3300 (up to 95%), which is, according to well-recognized criteria, indicative of strains of the same species (Wayne *et al.*, 1987).

Twenty-six FA with 12–20 carbon atoms were detected. The predominant cellular fatty acids were i-15:0, 16:0, 16:1(n-7) and 18:1(n-7). Eicosapentaenoic acid (EPA), 20:5(n-3), was found in the two isolates. The levels of EPA were 1.22–2.93 and 8.05–8.79% during cultivation at 28 and 12 °C, respectively. In addition, the amount of branched FA was greater at 28 °C. The polyenoic acids 18:4(n-3) and 20:4(n-3) were also present in small amounts (Table 1). It is interesting to note that the FA profiles of the two isolates studied were similar to those of other representatives of the genus *Shewanella* (Bowman *et al.*, 1997; Russell & Nichols, 1999). The production of polyunsaturated fatty acids (PUFAs), with regard to their specific role in cold-temperature adaptation, appears to be a characteristic feature of the genus. However, there are species with mesophilic growth temperatures (25–35 °C) (*S. algae*, *S. amazonensis*, *S. oneidensis* and *S. putrefaciens*) for which the ability to synthesize PUFAs or express synthetic enzymes has not been detected so far. To our knowledge, this is the first report of mesophilic strains, from the Sea of Japan, with the ability to synthesize PUFAs. This confirms the hypothesis of Russell & Nichols (1999), that the progenitor of the genus *Shewanella* possessed the ability to synthesize PUFAs, which has disappeared on

several occasions during the divergence of psychrotolerant and/or mesophilic species.

According to phylogenetic analyses, the closest relatives of *S. japonica* were *S. frigidimarina* and *S. baltica* (16S rDNA sequence similarities of 95 and 93 %, respectively) (Fig. 2). Strains KMM 3299^T and KMM 3300 could not, however, be grouped in a robust clade with either *S. frigidimarina* or *S. baltica* and could be distinguished phenotypically from *S. frigidimarina* and *S. baltica* by the presence of amylase and by their ability to utilize sucrose, galactose, fructose and a wide range of high molecular mass substrates (starch, agar, alginate, carrageenan, laminaran) (Table 2).

Based on their conclusions of an intensive review of the literature, Stackebrandt & Goebel (1994) concluded that organisms with less than 97 % similarity over the 16S rRNA gene sequence do not yield DNA reassociation values of more than 60 % and belong to separate species (Wayne *et al.*, 1987). Taking into account the recent phylogenetic and DNA–DNA hybridization data of Venkateswaran *et al.* (1999), showing that different *Shewanella* species share no more than 96.7 % 16S rRNA gene nucleotide sequence similarity, it is concluded that the two isolates represent a new species.

Description of *Shewanella japonica* sp. nov.

Shewanella japonica (ja.po'ni.ca. N.L. adj. *japonica* Japanese, pertaining to the Sea of Japan).

Cells are rod-shaped, 1–2 µm in length and 0.6–0.8 µm in diameter, polarly flagellated and Gram-negative, facultatively anaerobic heterotrophs. Anaerobic growth occurs by fermentation of D-glucose by anaerobic respiration of nitrate. Ferric compounds cannot serve as electron donors. No endospores are formed. Colonies on marine 2216 agar are circular, smooth and convex with an entire edge, slightly pinkish. Organic growth factors and sodium ions or sea water are not required. Does not grow at NaCl concentrations above 3 %. Temperature range for growth is 10–37 °C, with optimum growth at 20–25 °C. No growth detected at 40 °C. The pH range for growth is 6.0–9.0, with optimum growth at pH 7.5. Oxidase- and catalase-positive. Cytotoxic and haemolytic. Exhibits amylase, alginase, agarase, gelatinase, κ -carrageenase, DNase, laminarinase and lipase activities. Chitin and elastin are not hydrolysed. Susceptible to gentamycin, carbenicillin, lincomycin and oleandomycin. Utilizes galactose, fructose, maltose, N-acetylglucosamine, succinate, xylose, glucose, acetate, D-glucosamine, pyruvate and cellobiose as sole sources of carbon. D-Mannose, sucrose, lactose, melibiose, D-gluconate, fumarate, citrate, aconitate, meso-erythritol, D-mannitol, glycerol, γ -aminobutyrate, L-tyrosine, D-sorbitol, DL-malate, α -ketoglutarate, trehalose, gluconate and δ -aminovalerate are not utilized. Major

cellular fatty acids are i-15:0, 16:0 and 16:1(n-7). Produces eicosapentaenoic acid. Based on 16S rRNA nucleotide sequence analysis, the strains belong to the γ -Proteobacteria and are members of the genus *Shewanella*. Isolated from sea water and the mussel *Protothaca jadoensis*. The G + C content of the DNA 43–44 mol %. The type strain is KMM 3299^T (= LMG 19691^T = CIP 106860^T).

Acknowledgements

We are grateful to Dr A. M. Lysenko for genetic analysis. This study was supported by funds from the Russian Fund for Basic Research no. 99-04-48017 and by grant of the State Committee for Science and Technologies of the Russian Federation 99-03-19.

References

- Barry, A. I. (1980). Procedures and theoretical considerations for testing antimicrobial agents in agar media. In *Antibiotics in Laboratory Medicine*, pp. 10–16. Edited by V. Logan. Baltimore: William & Wilkins.
- Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **110**, 402–429.
- 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.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Gascuel, O. (1997). BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Mol Biol Evol* **14**, 685–695.
- Ivanova, E. P., Kiprianova, E. A., Mikhailov, V. V., Levanova, G. F., Garagulya, A. D., Gorshkova, N. M., Yumoto, N. & Yoshikawa, S. (1996). Characterization and identification of marine *Alteromonas nigrifaciens* strains and emendation of the description. *Int J Syst Bacteriol* **46**, 223–228.
- Ivanova, E. P., Kiprianova, E. A., Mikhailov, V. V. & 8 other authors (1998). Phenotypic diversity of *Pseudoalteromonas citrea* from different marine habitats and emendation of the description. *Int J Syst Bacteriol* **48**, 247–256.
- Jeanmougin, F., Thompson, J. D., Gouy, M., Higgins, D. G. & Gibson, T. J. (1998). Multiple sequence alignment with CLUSTAL X. *Trends Biochem Sci* **23**, 403–405.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Myers, C. R. & Nealson, K. H. (1988). Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Science* **240**, 1319–1321.
- Olsen, G. J., Matsuda, H., Hagstrom, R. & Overbeek, R. (1994). fastDNAm1: a tool for construction of phylogenetic trees of

- DNA sequences using maximum likelihood. *Comput Appl Biosci* **10**, 41–48.
- 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.
- Sawabe, T., Sugimura, I., Ohtsuka, M., Nakano, K., Tajima, K., Ezura, Y. & Christen, R. (1998a). *Vibrio halioticoli* sp. nov., a non-motile alginolytic marine bacterium isolated from the gut of the abalone *Haliotis discus hannai*. *Int J Syst Bacteriol* **48**, 573–580.
- Sawabe, T., Makino, H., Tatsumi, M., Nakano, K., Tajima, K., Iqbal, M. M., Yumoto, I., Ezura, Y. & Christen, R. (1998b). *Pseudoalteromonas bacteriolytica* sp. nov., a marine bacterium that is the causative agent of red spot disease of *Laminaria japonica*. *Int J Syst Bacteriol* **48**, 769–774.
- Sawabe, T., Tanaka, R., Iqbal, M. M., Tajima, K., Ezura, Y., Ivanova, E. P. & Christen, R. (2000). Assignment of *Alteromonas elyakovii* KMM 162^T and five strains isolated from spot-wounded fronds of *Laminaria japonica* to *Pseudoalteromonas elyakovii* comb. nov. and the extended description of the species. *Int J Syst Evol Microbiol* **50**, 265–271.
- 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.
- 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.
- Svetashev, V. I., Vysotskii, M. V., Ivanova, E. P. & Mikhailov, V. V. (1995). Cellular fatty acids of *Alteromonas* species. *Syst Appl Microbiol* **18**, 37–43.
- 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). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.