Marinomonas ostreistagni sp. nov., isolated from a pearl-oyster culture pond in Sanya, Hainan Province, China

Ken W. K. Lau,¹ Jianping Ren,¹ Natalie L. M. Wai,² Simon C. L. Lau,¹ Pei-Yuan Qian,¹ Po-Keung Wong² and Madeline Wu¹

Correspondence
Ken W. K. Lau
sslwk@ust.hk

¹Department of Biology, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, SAR, P. R. China

²Department of Biology, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, SAR, P. R. China

A Gram-negative, aerobic, halophilic, neutrophilic, rod-shaped, non-pigmented, polar-flagellated bacterium, UST010306-043^T, was isolated from a pearl-oyster culture pond in Sanya, Hainan Province, China in January 2001. This marine bacterium had an optimum temperature for growth of between 33 and 37 °C. On the basis of 16S rRNA gene sequence analysis, the strain was closely related to *Marinomonas aquimarina* and *Marinomonas communis*, with 97.5–97.7 and 97.1 % sequence similarity, respectively. Levels of DNA–DNA relatedness to the type strains of these species were well below 70 %. Analyses of phylogenetic, phenotypic and chemotaxonomic characteristics showed that strain UST010306-043^T was distinct from currently established *Marinomonas* species. A novel species with the name *Marinomonas ostreistagni* sp. nov. is proposed to accommodate this bacterium, with strain UST010306-043^T (= JCM 13672^T = NRRL B-41433^T) as the type strain.

Van Landschoot & De Ley (1983) showed that, based on rRNA gene sequences, the genus *Alteromonas* (Baumann *et al.*, 1972) was composed of four distinct branches and reclassified the branch containing *Alteromonas vaga* and *Alteromonas communis* as the genus *Marinomonas*. At the time of writing, the genus comprises eight species with validly published names: *Marinomonas vaga*, *Marinomonas communis* (Baumann *et al.*, 1972; Van Landschoot & De Ley, 1983), *Marinomonas mediterranea* (Solano & Sanchez-Amat, 1999), *Marinomonas primoryensis* (Romanenko *et al.*, 2003), *Marinomonas pontica* (Ivanova *et al.*, 2005), *Marinomonas ushuaiensis* (Prabakaran *et al.*, 2005), *Marinomonas aquimarina* (Macián *et al.*, 2005) and *Marinomonas dokdonensis* (Yoon *et al.*, 2005).

In this study, we report a novel strain, UST010306-043^T, which was isolated from a sea-water sample from a pearl-oyster culture pond in the HKUST-CAS Joint Laboratory in Sanya City, Hainan Province, China, on 6 January 2001. Based on polyphasic taxonomy, we propose that strain UST010306-043^T represents a novel species, *Marinomonas ostreistagni* sp. nov.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UST010306-043^T is AB242868.

A figure showing a slot-blot DNA–DNA hybridization assay with DNA of strain UST010306-043^T as the probe is available as supplementary material in IJSEM Online.

Sanya City is located at the southern tip of Hainan Island (18° N 109° E), with a tropical oceanic monsoon climate. About 500 ml sea water was sampled from the pearl-oyster culture pond at a depth of 3 m, and was transported in a sterile plastic container to the laboratory within 2 h.

The sea water was filtered through a 1 µm polycarbonate filter (Osmonics). Aliquots of 100 µl filtrate were spread on nutrient agar (Oxoid; prepared with 0.45 µm-filtered sea water) and incubated at room temperature for 2–3 days and then stored at 4 °C. Colonies were purified by being repeatedly restreaked on marine agar 2216 (MA; Difco) plates. Purified strains were stored at –80 °C in marine broth 2216 (MB) with 30 % (v/v) glycerol. Partial 16S rRNA genes were amplified by using the primers GM5F (5'-CCTA-CGGGAGGCAGCAG-3') and 907R (5'-CCGTCAATTCC-TTTRAGTTT-3') (Muyzer *et al.*, 1995) with *Taq* polymerase (Promega) and sequenced using ABI PRISM BigDye Terminator cycle sequencing ready reaction kit version 2.0 and the primer 341F. Strain UST010306-043^T, a single novel isolate related to the genus *Marinomonas*, was selected for further analysis. Colony morphology was observed after incubation for 24 h at 30 °C. The temperature range for growth was evaluated in MB at 4, 16, 20, 25, 30, 33, 37, 40 and 42 °C. Growth at pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 was evaluated in YP-SW broth (0.4 % yeast extract, 0.2 % peptone, 75 % 0.45 µm-filtered sea water), adjusted with 1 M HCl or NaOH. The requirement for sodium ions

was tested in artificial sea water (ASW; 0.1 % $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.1 % KCl, 0.5 % $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.5 % NaCl) (Lewin & Lounsbury, 1969), with NaCl replaced by equal molar KCl and addition of 0.4 % yeast extract. Tolerance to NaCl was tested in ASW supplemented with 0.4 % yeast extract and with NaCl added at final concentrations of 0, 1, 2, 3, 4, 5, 7.5, 10 and 15 %. Anaerobic growth was examined by using the Oxoid anaerobic system for cultures grown on MA supplemented with 0.1 % sodium nitrate or 0.1 % glucose. Cellular morphology was determined from a MB overnight culture using a Zeiss MC100 Spot microscope at a magnification of $\times 1000$. Flagella morphology was examined by using transmission electron microscopy (JOEL 100CXII at 80 kV), using cells placed on a carbon grid and stained with 1 % (w/v) phosphotungstic acid (pH 7.0).

Gram staining was performed according to Collins *et al.* (1989). Tests for requirement of organic growth factors, utilization of various carbon sources and chitin, fermentation of glucose, sucrose or D-mannitol and accumulation of poly- β -hydroxybutyrate were performed according to Baumann & Baumann (1981). Alkaline phosphatase activity, H_2S generation from cysteine or thiosulfate, oxidation of glucose and hydrolysis of starch, casein, gelatin and cellulose were determined according to Smibert & Krieg (1994). Fatty acid methyl ester analysis was performed as described by Svetashev *et al.* (1995), except that the bacterial strains were cultured at 30 °C for 24 h. DNA hydrolysis test and sequencing of the nearly complete 16S rRNA gene were performed as described by Lau *et al.* (2005). Related sequences were identified by MEGABLAST search (Zhang *et al.*, 2000), retrieved from the NCBI nucleotide database, aligned using CLUSTAL_X (Thompson *et al.*, 1997), edited with BioEdit version 5.0.9 (Hall, 1999) and analysed using MEGA version 2.1 (Kumar *et al.*, 2001). A phylogenetic tree was generated by using the neighbour-joining method (Saitou & Nei, 1987) with the Jukes–Cantor substitution model (Jukes & Cantor, 1969) and was evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings. *M. aquimarina* CECT 5080^T and *M. communis* CECT 5003^T were obtained from the Spanish Type Culture Collection.

For DNA–DNA hybridization experiments, genomic DNA was extracted according to Maloy (1990) with modifications: 1.5 ml overnight culture was centrifuged and resuspended in 467 μl TE buffer, containing 30 μl SDS (10 %, w/v) and 3 μl proteinase K (20 mg ml^{-1}), incubated at 37 °C for 1 h and then extracted twice with equal amounts of phenol/chloroform/isoamyl alcohol (25:24:1, by vol.). DNA was precipitated with 0.1 vol. 3 M sodium acetate (pH 5.2) and 0.6 vol. 2-propanol. The precipitate was washed with 0.5 ml 70 % ethanol, dried in a vacuum, dissolved in 100 μl TE buffer with RNase (100 μg ml^{-1}) and incubated at 37 °C for 1 h. DNA was diluted in TE buffer to 0.5–1.0 ng μl^{-1} and run in an agarose gel (1 %) with a λ HindIII marker (100 ng; TaKaRa). Quantification of DNA was done by comparing the band intensities of isolated DNA and that of the marker using the Spot Denso function of the

IS-1000 Digital Imaging System (Alpha Innotech). Slot-blot analysis of DNA was performed according to Brown (2005) with Hybond-N+ membrane (Millipore). One or 10 ng genomic DNA of each strain was loaded per well. One hundred nanogram probe DNA was labelled using the Gene Image random prime labelling module (Amersham), according to the manufacturer's instructions. Overnight hybridization was performed at 60 °C. The membrane was washed three times with $1 \times \text{SSC}/0.1$ % SDS and three times with $0.5 \times \text{SSC}/0.1$ % SDS at 60 °C (each washing step lasted for 10 min), detected using a Gene Image CDP-Star detection module (Amersham) according to the manufacturer's instructions and exposed to a 100NIF X-ray film (Fuji) for 15 min. Levels of hybridization were determined with EagleSight software version 3.2 in the Eagle Eye II system (Stratagene). The DNA G+C content was determined by using the HPLC method (Mesbah *et al.*, 1989), with genomic DNA extracted with a MiniBEST Bacterial Genomic DNA Extraction kit (TaKaRa). Susceptibility to antibiotics was tested by using the disc-diffusion plate method on MA. The following antibiotics were tested: ampicillin (10 μg), polymyxin B (300 U), chloramphenicol (30 μg), tetracycline (30 μg), streptomycin (10 μg), rifampicin (10 μg) and penicillin G (2 U).

Table 1. Whole-cell fatty acid profiles of strain UST010306-043^T and related members of the genus *Marinomonas*

Strains: 1, UST010306-043^T; 2, *M. aquimarina* CECT 5080^T; 3, *M. communis* CECT 5003^T. Data from this study. Values are percentages of total fatty acids. Fatty acids are designated as follows: total number of carbon atoms: number of double bonds, followed by the position of the double bond from the aliphatic end of the molecule. ECL, equivalent chain-length.

Fatty acid	1	2	3
Straight-chain fatty acids:			
10:0	5.4	5.2	3.2
12:0	5.7	5.0	5.4
14:0	4.2	3.0	3.0
16:0	15.6	13.0	10.4
18:0	1.14	1.3	1.4
Unsaturated fatty acids:			
15:1 ω 8c	0.0	0.0	0.2
18:1 ω 7c	39.5	40.4	41.8
Hydroxy fatty acids:			
10:0 3-OH	7.8	9.2	10.5
12:0 3-OH	0.0	0.2	0.0
Summed features:*			
1	19.7	22.4	24.0
2	1.0	0.2	0.0
Unknown ECL 11.799	0.0	0.1	0.0

*1, 16:1 ω 7c/15:0 iso 2-OH; 2, 19:1 ω 6c/19:0 cyclo/unknown ECL 18.846.

Table 2. Characteristics that differentiate strain UST010306-043^T from other members of the genus *Marinomonas*

Taxa: 1, strain UST010306-043^T; 2, *M. aquimarina* (data from Macián *et al.*, 2005); 3, *M. communis* (Baumann *et al.*, 1972); 4, *M. vaga* (Baumann *et al.*, 1972); 5, *M. pontica* 46-16^T (Ivanova *et al.*, 2005); 6, *M. dokdonensis* (Yoon *et al.*, 2005); 7, *M. mediterranea* MMB-1^T (Solano *et al.*, 1997; Solano & Sanchez-Amat, 1999); 8, *M. primoryensis* IAM 15010^T (Romanenko *et al.*, 2003); 9, *M. ushuaiensis* U1^T (Prabakaran *et al.*, 2005). +, Positive; –, negative; ND, data not available; v, variable; w, weakly positive.

Characteristic	1	2	3	4	5	6	7	8	9
Cell shape*	SR	SR	CR	SR	Rod	SR	SR	SR	SR
Pigment	–	–	–	–	–	–	Melanin-like	Yellowish	–
Growth at/in:									
8% NaCl	+	+	+	+	+	+	–	–	–
4 °C	–	–	–	–	+	+	–	+	+
40 °C	+	+	+	–	–	–	–	–	–
NO ₃ [–] reduction to NO ₂ [–]	–	–	–	–	–	–	+	–	–
Oxidase	+	+	+	–	+	+	–	+	–
Hydrolysis of:									
Starch	–	–	–	–	–	–	–	–	+
Gelatin	–	–	–	–	–	–	+	–	–
Tween 80	–	–	–	–	–	+	+	–	–
Utilization of:									
Citrate	+	+	+	+	+	+	+	+	–
Succinate	–	+	+	+	+	+	+	+	–
Malate	–	+	+	+	+	+	+	w	–
D-Mannitol	+	–	+	+	+	ND	ND	+	–
D-Sorbitol	–	–	+	+	–	ND	+	ND	–
L-Histidine	–	+	v	v	+	ND	ND	ND	ND
Sucrose	+	–	–	v	+	v	ND	ND	ND
Maltose	–	–	v	v	+	+	ND	+	w
Ampicillin (10 µg)	Sensitive	ND	ND	ND	ND	Resistant	ND	ND	Resistant
DNA G + C content (mol%)	49.8 ± 0.5	ND	45.9–48.0	46.4–49.3	46.5	45.3–45.7	46.3 ± 0.9	45.3–45.6	43.6

*CR, curved rod; SR, straight rod.

†Data from Ivanova *et al.* (2005).

Morphological, physiological and biochemical features of UST010306-043^T are given in the species description.

Strain UST010306-043^T shared many similarities with other *Marinomonas* species. Strains were all Gram-negative, isolated from marine habitats, had rod-shaped cells, were halophilic, strictly aerobic, heterotrophic, non-spore-forming and non-fermentative, utilized glucose and acetate and did not require complex organic growth factors. Strain UST010306-043^T shared many similarities with *M. aquimarina*; in particular, their similarity in habitat (the former was found in sea water of a pearl-oyster pond and the latter was found in oyster flesh and sea water), growth up to 40 °C but not at 4 °C, oxidative response to glucose and inability to utilize maltose and D-sorbitol. The G + C content of the DNA of strain UST010306-043^T was 49.8 ± 0.5 mol%, which was close to the highest G + C content of members of the genus *Marinomonas* (Sanchez-Amat & Solano, 2005). The fatty acids of strain UST010306-043^T and closely related species are shown in Table 1. The fatty acid profile of strain UST010306-043^T was very similar to that of *M. aquimarina* CECT 5080^T, but differed from that of *M. communis* CECT

Fig. 1. Phylogenetic relationship of strain UST010306-043^T and other members of the genus *Marinomonas* based on 16S rRNA gene sequences. The tree was created using the neighbour-joining method. Numbers at nodes represent percentage bootstrap values from 1000 resampled datasets. *Alteromonas macleodii* IAM 12920^T and *Vibrio cholerae* ATCC 14035^T were used as an outgroup. Bar, 0.02 nucleotide substitutions per position.

5003^T by having a slightly higher percentage of straight-chain fatty acids. Overall, the fatty acid profiles of strain UST010306-043^T and members of the genus *Marinomonas* had a similar pattern; namely, with over 60 % fatty acids distributed among 18:1 ω 7c, 16:0/16:0 iso and 16:1 ω 7c/15:0 iso 2-OH (Table 1; Ivanova *et al.*, 2000, 2005; Yoon *et al.*, 2005; Prabakaran *et al.*, 2005). Characteristics that differentiated strain UST010306-043^T from other species of *Marinomonas* are shown in Table 2.

Phylogenetic analysis showed that strain UST010306-043^T formed a distinct taxon within the genus *Marinomonas* and was closely linked with *M. aquimarina* CECT 5080^T with a bootstrap value of 90 % (Fig. 1). The 16S rRNA gene sequence of strain UST010306-043^T shared 94.1–97.7 % similarity with other members of the genus *Marinomonas*, being most closely related to *M. aquimarina* (97.5–97.7 %) and *M. communis* (97.1 %). Its similarity to other species of *Marinomonas* was less than 97 %, indicating that they were different species (Stackebrandt & Goebel, 1994). As the 16S rRNA gene similarity of strain UST010306-043^T with *M. aquimarina* and *M. communis* was at the borderline for discrimination of species by sequence analysis (Stackebrandt & Goebel, 1994), DNA–DNA hybridization, which has significantly higher resolution power than sequence analysis (Amann *et al.*, 1992), was performed. The results of DNA–DNA hybridization (available as Supplementary Fig. S1 in IJSEM Online) showed that the levels of relatedness of strain UST010306-043^T with *M. aquimarina* CECT 5080^T and *M. communis* CECT 5003^T were below 23 %. This was well below the 70 % threshold value for species discrimination (Wayne *et al.*, 1987), suggesting that strain UST010306-043^T represents a species that is different from *M. aquimarina* or *M. communis*. On the basis of all characteristics described above, a novel species with the name *Marinomonas ostreistagni* sp. nov. is proposed.

Description of *Marinomonas ostreistagni* sp. nov.

Marinomonas ostreistagni (os.tre'i.stag'ni. L. fem. n. *ostrea* oyster; L. gen. neut. n. *stagni* of/from a pond; N.L. gen. neut. n. *ostreistagni* from/of oyster pond).

Cells are Gram-negative rods, 0.6–3.6 μ m in length and 0.4–0.6 μ m in diameter and motile by a single polar flagellum. Cells divide by binary fission. Heterotrophic, strictly aerobic and requires sodium ions for growth. Cell aggregates are detected when cultured in MB. Colonies are non-pigmented, circular, opaque, convex, 1–2 mm in diameter with an entire margin after 24 h incubation at 30 °C on MA. Grows in 1–10 % NaCl but not in 15 % NaCl; optimum NaCl for growth is approximately 3 %. Grows at 16–40 °C, but not at 4 or 42 °C; optimum temperature for growth is 33–37 °C. Grows at pH 5.0–9.0, with optimum growth occurring around pH 7.0. Oxidase-, catalase- and alkaline phosphatase-positive. Oxidizes glucose. Does not produce sulfide from cysteine or thiosulfate and does not degrade extracellular DNA. Does not form endospores and

does not accumulate poly- β -hydroxybutyrate. Utilizes glucose, sucrose, D-mannitol, aesculin, L-alanine, L-asparagine, L-serine, L-glutamic acid, acetate, citrate, pyruvate, propionate, lactate, ethanol and Tween 20, but not galactose, maltose, L-arabinose, trehalose, rhamnose, lactose, melibiose, D-sorbitol, methanol, glycerol, succinate, malate, glycine, L-leucine, L-arginine, L-lysine, L-histidine, L-methionine, L-threonine, valine, starch, gelatin, casein, agar, DNA, Tween 40, Tween 60, Tween 80 or cellulose. Does not ferment glucose, sucrose or D-mannitol. Sensitive to ampicillin (10 μ g), polymyxin B (300 U), chloramphenicol (30 μ g), tetracycline (30 μ g), streptomycin (10 μ g) and rifampicin (10 μ g), but resistant to penicillin G (2 U). Predominant fatty acids are 18:1 ω 7c, 16:0 and 16:1 ω 7c/15:0 iso 2-OH. The DNA G + C content is 49.8 $\pm$ 0.5 mol%.

The type strain is UST010306-043^T (= JCM 13672^T = NRRL B-41433^T), which was isolated from the pearl-oyster culture pond of HKUST-CAS Joint Laboratory, Sanya, Hainan Province, China.

Acknowledgements

We sincerely thank Professor Dr Hans G. Trüper for valuable suggestions for the etymology, Mr Tze Kin Cheung for transmission electron microscopy analysis and Dr Xiancui Li for DNA G + C content analysis. This work was supported by grants R5498, CMI03/04.SC03, CAs-CF03/04.Sc01 and CA04/05.SC01.

References

- Amann, R. I., Lin, C., Key, R., Montgomery, L. & Stahl, D. A. (1992). Diversity among *Fibrobacter* strains: towards a phylogenetic classification. *Syst Appl Microbiol* **15**, 23–31.
- Baumann, P. & Baumann, L. (1981). The marine gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas* and *Alcaligenes*. In *The Prokaryotes*, vol. 1, pp. 1302–1331. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. Schlegel. Berlin: Springer.
- Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **3**, 402–429.
- Brown, T. (2005). Dot and slot blotting of DNA onto a positively charged nylon membrane using a manifold. In *Current Protocols in Molecular Biology*, pp. 2.9.18–2.9.20. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Wiley.
- Collins, C. H., Lyne, P. M. & Grange, J. M. (1989). *Collins and Lyne's Microbiological Methods*. London: Butterworth.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Ivanova, E. P., Zhukova, N. V., Svetashev, V. I., Gorshkova, N. M., Kurilenko, V. V., Frolova, G. M. & Mikhailov, V. V. (2000). Evaluation of phospholipid and fatty acid compositions as chemotaxonomic markers of *Alteromonas*-like proteobacteria. *Curr Microbiol* **41**, 341–345.
- Ivanova, E. P., Onyshchenko, O. M., Christen, R., Lysenko, A. M., Zhukova, N. V., Shevchenko, L. S. & Kiprianova, E. A. (2005). *Marinomonas pontica* sp. nov., isolated from the Black Sea. *Int J Syst Evol Microbiol* **55**, 275–279.

- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Kumar, S., Tamura, K., Jakobsen, I. B. & Nei, M. (2001). MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244–1245.
- Lau, K. W. K., Ng, C. Y. M., Ren, J., Lau, S. C. L., Qian, P.-Y., Wong, P.-K., Lau, T. C. & Wu, M. (2005). *Owenweeksia hongkongensis* gen. nov., sp. nov., a novel marine bacterium of the phylum 'Bacteroidetes'. *Int J Syst Evol Microbiol* **55**, 1051–1057.
- Lewin, R. A. & Lounsbery, D. M. (1969). Isolation, cultivation and characterization of flexibacteria. *J Gen Microbiol* **58**, 145–170.
- Macián, M. C., Arahal, D. R., Garay, E. & Pujalte, M. J. (2005). *Marinomonas aquamarina* sp. nov., isolated from oysters and seawater. *Syst Appl Microbiol* **28**, 145–150.
- Maloy, S. R. (1990). *Experimental Techniques in Bacterial Genetics*. Boston: Jones and Bartlett.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Muyzer, G., Teske, A., Wirsén, C. O. & Jannasch, H. W. (1995). Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Arch Microbiol* **164**, 165–172.
- Prabakaran, S. R., Suresh, K., Manorama, R., Delille, D. & Shivaji, S. (2005). *Marinomonas ushuaiensis* sp. nov., isolated from coastal sea water in Ushuaia, Argentina, sub-Antarctica. *Int J Syst Evol Microbiol* **55**, 309–313.
- Romanenko, L. A., Uchino, M., Mikhailov, V. V., Zhukova, N. V. & Uchimura, T. (2003). *Marinomonas primoryensis* sp. nov., a novel psychrophile isolated from coastal sea-ice in the Sea of Japan. *Int J Syst Evol Microbiol* **53**, 829–832.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sanchez-Amat, A. & Solano, F. (2005). Genus III. *Marinomonas* Van Landschoot and De Ley 1984, 91^{VP} (effective publication: Van Landschoot and De Ley 1983, 3071). In *Bergey's Manual of Systematic Bacteriology*, vol. 2, part B, pp. 284–289. Edited by D. J. Brenner, N. R. Krieg & J. T. Staley. New York: Springer.
- 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.
- Solano, F. & Sanchez-Amat, A. (1999). Studies on the phylogenetic relationships of melanogenic marine bacteria: proposal of *Marinomonas mediterranea* sp. nov. *Int J Syst Bacteriol* **49**, 1241–6.
- Solano, F., García, E., Perez De Egea, E. & Sanchez-Amat, A. (1997). Isolation and characterization of strain MMB-1 (CECT 4803), a novel melanogenic marine bacterium. *Appl Environ Microbiol* **63**, 3499–3506.
- 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.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Van Landschoot, A. & De Ley, J. (1983). Intra- and intergeneric similarities of the rRNA cistrons of *Alteromonas*, *Marinomonas* (gen. nov.) and some other Gram-negative bacteria. *J Gen Microbiol* **129**, 3057–3074.
- 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.
- Yoon, J.-H., Kang, S.-J. & Oh, T.-K. (2005). *Marinomonas dokdonensis* sp. nov., isolated from sea water. *Int J Syst Evol Microbiol* **55**, 2303–2307.
- Zhang, Z., Schwartz, S., Wagner, L. & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *J Comput Biol* **7**, 203–214.