Maribaculum marinum gen. nov., sp. nov., isolated from deep seawater

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A taxonomic study was carried out on strain P38^T, which was isolated from an enriched polycyclic aromatic hydrocarbon-degrading consortium from a deep seawater sample collected from the Indian Ocean. Phylogenetic analyses based on 16S rRNA gene sequences showed that strain P38^T formed a distinct evolutionary lineage within the family *Hyphomonadaceae*. Strain P38^T was most closely related to members of the genera *Hyphomonas* (92.3–93.5 % 16S rRNA gene sequence similarity), *Hirschia* (88.8 %), *Maricaulis* (88.3–88.6 %), *Hellea* (87.5 %), *Oceanicaulis* (87.4 %) and *Robiginitomaculum* (86.7 %) of the family *Hyphomonadaceae*. The DNA G+C content of strain P38^T was 61.0 mol% and the predominant cellular fatty acids were $C_{16:0}$ (20 %), $C_{17:0}$ (5.7 %), $C_{18:1}\omega7c$ (37.7 %), $C_{18:0}$ (6.3 %) and $C_{18:1}\omega7c$ 11-methyl (7.1 %). Strain P38^T was distinguishable from members of phylogenetically related genera by differences in several phenotypic properties. On the basis of the phenotypic and phylogenetic data, strain P38^T represents a novel species of a new genus, for which the name *Maribaculum marinum* gen. nov., sp. nov. is proposed. The type strain of *Maribaculum marinum* is P38^T (=CCTCC AB 208227^T=LMG 24711^T=MCCC 1A01086^T).

In an attempt to investigate polycyclic aromatic hydrocarbon-degrading bacteria in deep seawater of the Indian Ocean, many bacterial strains were isolated and characterized taxonomically (Lai *et al.*, 2009). Comparative 16S rRNA gene sequence analysis indicated that one of these isolates, designated strain P38^T, formed a deep branch within the family *Hyphomonadaceae*. The family *Hyphomonadaceae* was first proposed by Lee *et al.* (2005) based on phylogenetic analyses of 16S rRNA gene sequences. At the time of writing, the genus contains six genera: *Hyphomonas* (Moore *et al.*, 1984), *Hirschia* (Schlesner *et al.*, 1990), *Maricaulis* (Abraham *et al.*, 2002), *Oceanicaulis* (Strömpl *et al.*, 2003), *Robiginitomaculum* (Lee *et al.*, 2007) and *Hellea* (Alain *et al.*, 2008). Another genus, *Woodsholea* (Abraham *et al.*, 2004), should be assigned to the family *Hyphomonadaceae* (Alain *et al.*, 2008). Accordingly, the aim of the present work was to determine the exact taxonomic position of strain P38^T.

Deep sea water was sampled at a depth of 2914 m (200 m above the sea floor) at the site of IR-CTD13 (24.2822° S 69.7944° E) on the south-west Indian Ridge during cruise DY-105A of R/V *Da-Yang Yi-Hao* in December 2005. The sample was enriched with polycyclic aromatic hydrocarbons and strains were isolated on 216L marine agar medium following the method described by Lai *et al.* (2009). For morphological and biochemical characterization, strain P38^T was cultivated on 216L agar.

General cell morphology of strain P38^T was studied under an Olympus inverted microscope using a 1-day-old culture. For electron microscopy, exponential-phase cells were harvested, suspended and absorbed onto a Formvar-carbon-coated grid and stained with phosphotungstic acid (Supplementary Fig. S1, available in IJSEM Online). The Gram reaction and tests for catalase and oxidase activities, lipase (Tween 80), amylase and hydrolysis of aesculin were carried out according to Dong & Cai (2001). The optimal growth temperature was determined on 216L agar over the range 4–55 °C. Tolerance of NaCl was tested by using Luria–Bertani medium (Sambrook *et al.*, 1989) supplemented with NaCl (0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 18 and 20 %, w/v). Antibiotic susceptibility tests were performed by the disc diffusion method as described by Shieh *et al.* (2003) with Oxoid discs. Other biochemical tests were performed with API 20NE and API ZYM strips (bioMérieux) and GN2 MicroPlates (Biolog), according to the manufacturers' instructions with the adjustment of NaCl to 3.0 %. To detect bacteriochlorophyll *a* and carotenoids, pigments of strain P38^T were extracted with acetone/methanol (1 : 1, v/v) and absorption spectra were recorded using a scanning UV/visible spectrophotometer

Abbreviation: UPGMA, unweighted pair group method with arithmetic means.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain P38^T is EU819081.

A transmission electron micrograph of strain P38^T and a table detailing the API ZYM characteristics of strain P38^T and related genera are available with the online version of this paper.

(SmartSpec Plus; Bio-Rad). Additionally, the genetic potential for anoxygenic phototrophy was determined by PCR amplification of the photosynthetic reaction centre genes (*pufLM*) using the pufLf and pufMr primer set (Béjà *et al.*, 2002). The results are given in the genus and species descriptions and Table 1.

Genomic DNA was prepared according to the method of Ausubel *et al.* (1995) and the 16S rRNA gene was amplified by PCR with primers that have been described previously (Liu & Shao, 2005). A nearly full-length 16S rRNA gene sequence (1415 nt) of strain P38^T was determined. Sequences of related taxa were obtained from the GenBank database. Phylogenetic analysis was performed using MEGA version 4 (Tamura *et al.*, 2007) after multiple alignment of data using DNAMAN version 5.1 (Lynnon Biosoft). Distances were calculated according to the distance options with Kimura's two-parameter model and clustering with the neighbour-joining method of Saitou & Nei (1987), the minimum-evolution method of Rzhetsky & Nei (1992, 1993) and the unweighted pair group method with arithmetic means (UPGMA) method was determined by using bootstrap values based on 1000 replications. The neighbour-joining tree is shown in Fig. 1. The results obtained with the minimum-evolution and UPGMA methods were similar to those obtained with the neighbour-joining method (not shown).

Phylogenetic analyses showed that strain P38^T formed a distinct evolutionary lineage within the family *Hyphomonadaceae* (Fig. 1). Strain P38^T showed the highest 16S rRNA gene sequence similarity (>97 %) to sequences from strains that have not yet been assigned to any species. Of the sequences from recognized species, strain P38^T was most closely related to those from the genera *Hyphomonas* (92.3–93.5 %), *Hirschia* (88.8 %), *Maricaulis* (88.3–88.6 %), *Hellea* (87.5 %), *Oceanicaulis* (87.4 %) and *Robiginitomaculum* (86.7 %), which belong to the family *Hyphomonadaceae*. All of the 16S rRNA gene sequence divergences between strain P38^T and recognized species were greater than 6.5 % and the distinct phylogenetic relationships revealed that strain P38^T could not be assigned to any of the recognized genera. Consequently, strain P38^T should be considered to represent a novel species in a new genus in the family *Hyphomonadaceae*. In addition, we found that the genus *Woodsholea* (Abraham

Table 1. Characteristics that differentiate strain P38^T from related genera of the family *Hyphomonadaceae*

Taxa: 1, *Maribaculum marinum* gen. nov., sp. nov. P38^T; 2, *Hyphomonas*; 3, *Hirschia*; 4, *Maricaulis*; 5, *Oceanicaulis*; 6, *Robiginitomaculum* (data in columns 2–6 from Lee *et al.*, 2007); 7, *Woodsholea* (Abraham *et al.*, 2004); 8, *Hellea* (Alain *et al.*, 2008). For cellular fatty acid analyses, strains of the genus *Hyphomonas* were grown on MA. Strains of *Hirschia baltica* were grown on M13 medium (Schlesner *et al.*, 1990). Strains of the genera *Maricaulis* and *Oceanicaulis* were grown on SPYEM medium (Abraham *et al.*, 1999). +, Positive; −, negative; tr, trace; V, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Cell morphology								
Budding	−	+	+	−	−	−	+	+
Flagella	−	+*	+	+†	+	−	+	+
Prosthecae	−	+	+	+	+	+‡	+	+
Growth at:								
0.5 % NaCl	−	V§	ND	+‖	−	+	+	+
6.0 % NaCl	+	V¶	ND	+	+	−	+	−
Oxidase	−	+	ND	ND	+	−	+	−
Catalase	+	+	ND	ND	+	+	−	+
Nitrate reduction	−	V	−	V	+	+	−	−
Gelatinase	−	−	+	ND	−	−	−	−
Fatty acid (%)								
$C_{17:0}$	5.7	0–23	1	7–22	10	14	2.2	5.63
$C_{17:1}$	8.9	0–24	1	10–39	1	29	tr	8.78
$C_{18:0}$	6.3	−	2	1–8	22	4	16.9	1.81
$C_{18:1}$	44.8	15–80	52	16–54	30	45	64.5	68.28
G+C content (mol%)	61.0	57–64	45.6	62.5–65.2	61.8	60.3	65.2	46.8

*Data for *Hyphomonas adhaerens*, *Hyphomonas johnsonii* and *Hyphomonas rosenbergii*.
†Data for *Maricaulis maris*.
‡Some cells have a prostheca with a thin, tapered end.
§All species are negative except *Hyphomonas polymorpha*.
‖Data for all species except *Maricaulis maris*.
¶All species are positive except *Hyphomonas polymorpha*.

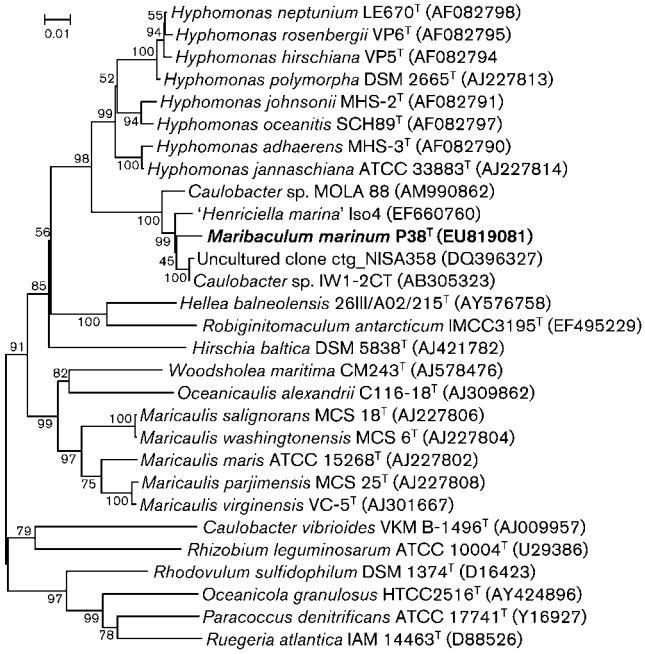

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain P38^T and other members of the family *Hyphomonadaceae*, based on 16S rRNA gene sequences. Bootstrap values based on 1000 replications are shown as percentages at branch nodes. Bar, 0.01 nucleotide substitution rate (K_{nuc}) units.

et al., 2004) probably belongs to the family *Hyphomonadaceae*, as shown previously by Alain *et al.* (2008).

The G+C content of the chromosomal DNA was determined according to the methods described by Mesbah & Whitman (1989) using reversed-phase HPLC. The DNA G+C content of strain P38^T was 61.0 mol%, which is similar to values reported for the genera *Hyphomonas* (57–64 mol%), *Maricaulis* (62.5–65.2 mol%), *Oceanicaulis* (61.8 mol%) and *Robiginitomaculum* (60.3 mol%), but differs by more than 10 mol% from values reported for the genera *Hirschia* (45.6 mol%) and *Hellea* (46.8 mol%).

Fatty acids from whole cells grown on marine agar 2216 (BD Difco) at 28 °C for 48 h were extracted, saponified and esterified. The fatty acid methyl esters were analysed with GC according to the instructions of the MIDI system (Sasser, 1997). The major fatty acids of strain P38^T were $C_{16:0}$ (20 %), $C_{17:0}$ (5.7 %), $C_{18:1}\omega7c$ (37.7 %), $C_{18:0}$ (6.3 %) and $C_{18:1}\omega7c$ 11-methyl (7.1 %). Minor amounts of $C_{12:0}$ 3-OH (3.7 %), $C_{14:0}$ (0.9 %), $C_{16:1}\omega5c$ (4.4 %), $C_{17:1}\omega8c$ (4.8 %), $C_{17:1}\omega6c$ (4.1 %), $C_{19:0}$ cyclo $\omega8c$ (0.9 %), $C_{18:1}$ 2-OH (2.6 %) and $C_{20:0}$ (1.4 %) were also found in strain P38^T. These results differentiated strain P38^T from members of the genus *Hyphomonas*, which do not contain $C_{18:0}$ (Lee *et al.*, 2007), although it should be noted that Abraham *et al.* (2004) detected $C_{18:0}$ in *Hyphomonas polymorpha* DSM 2665^T (trace) and *Hyphomonas jannaschiana* ATCC 33833^T (3.7 %).

Although strain P38^T is related most closely to members of the genus *Hyphomonas* (92.3–93.5 % 16S rRNA gene sequence similarity), with the highest similarity to *Hyphomonas oceanitis* SCH89^T (93.5 %), it cannot be affiliated to the genus *Hyphomonas* because it is non-budding and does not produce prosthecae and is non-motile and oxidase-negative. The low levels of 16S rRNA gene sequence similarity between strain P38^T and all of the other members of the family *Hyphomonadaceae*, together with the differential phenotypic properties shown in Table 1, suggest that strain P38^T represents a novel species in a new genus within the family *Hyphomonadaceae*, for which the name *Maribaculum marinum* gen. nov., sp. nov. is proposed.

Description of *Maribaculum* gen. nov.

Maribaculum (Ma.ri.ba′cu.lum. L. neut. n. *mare* the sea; L. neut. n. *baculum* a stick or rod; N.L. neut. n. *Maribaculum* rod from the sea).

Cells are Gram-negative-staining, short rods or ovoid, non-motile, non-budding and non-prostheca-producing, oxidase-negative and catalase-positive. Multiplication occurs by binary fission. Flagella and holdfasts are not present. Carotenoid, bacteriochlorophyll *a* and the genes for anoxygenic photosynthesis (*pufLM*) are not found. Chemoheterotrophic. The dominant fatty acids are $C_{16:0}$ and $C_{18:1}\omega7c$. The DNA G+C content of the type strain of the type species is 61.0 mol%. The genus is assigned phylogenetically to the family *Hyphomonadaceae* in the order *Rhodobacterales*. The type species is *Maribaculum marinum*.

Description of *Maribaculum marinum* sp. nov.

Maribaculum marinum (ma.ri′num. L. neut. adj. *marinum* of the sea, marine).

Displays the following properties in addition to those given for the genus. Cells are 1.2–1.4 μm long and 0.8–0.9 μm wide. Negative for lipase (Tween 80), amylase, urease, gelatinase, arginine dihydrolase and indole, hydrolysis of aesculin and reduction of nitrate to nitrite. On 216L agar, forms smooth, grey colonies with regular edges that are 2–3 mm in diameter after 72 h incubation at 28 °C. Grows with 2–12 % NaCl (optimum 3–10 %) and at 10–42 °C (optimum 25–37 °C), but not at 4 or 45 °C after 7 days. Unable to ferment glucose. The predominant fatty acids are $C_{16:0}$, $C_{17:0}$, $C_{18:1}\omega7c$, $C_{18:0}$ and $C_{18:1}\omega7c$ 11-methyl. Sensitive to (μg per disc unless otherwise stated) co-trimoxazole (25), ofloxacin (5), tetracycline (30), minomycin (30), penicillin G (10), streptomycin (10), ciprofloxacin (5), neomycin (10), vibramycin (30), piperacillin (100), rocephin (30), kanamycin (30), ampicillin (10), gentamicin (10), rifampicin (5), cephradin (30), chloromycetin (30), carbenicillin (100) and erythromycin (15).

Resistant to cephalexin (30), cephazolin (30), cefobid (30), clindamycin (2), furazolidone (15), lincomycin (2), metronidazole (5), norfloxacin (10), oxacillin (1), polymyxin B (30 U) and vancomycin (30). With API ZYM, positive for acid phosphatase, alkaline phosphatase, cystine aminopeptidase, leucine aminopeptidase, naphthol-AS-BI-phosphoamidase, trypsin, valine aminopeptidase, α-chymotrypsin and α-glucosidase; weakly positive for esterase (C4), esterase lipase (C8) and lipase (C14); negative for *N*-acetyl-β-glucosaminidase, α-fucosidase, α- and β-galactosidase, α-mannosidase, β-glucosidase and β-glucuronidase. With API 20NE, does not utilize adipic acid, capric acid, D-glucose, maltose, D-mannitol, D-mannose, L-arabinose, malic acid, *N*-acetylglucosamine, phenylacetic acid, potassium gluconate or trisodium citrate. With GN2 MicroPlates, positive for utilization of L-aspartic acid, L-glutamic acid, L-ornithine, L-threonine, Tweens 40 and 80, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid and β-hydroxybutyric acid; weakly positive for utilization of acetic acid, *cis*-aconitic acid, citric acid, D-alanine, D-arabitol, cellobiose, dextrin, D-galacturonic acid, D-serine, glucuronamide, glycogen, glycyl L-aspartic acid, glycyl L-glutamic acid, lactulose, L-alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-leucine, L-serine, maltose, pyruvic acid methyl ester, succinic acid monomethyl ester, phenylethylamine, propionic acid, quinic acid, α-D-glucose, γ-aminobutyric acid and γ-hydroxybutyric acid; negative for utilization of 2,3-butanediol, 2-aminoethanol, adonitol, bromosuccinic acid, DL-carnitine, DL-lactic acid, DL-α-glycerol phosphate, D-fructose, D-galactonic acid lactone, D-galactose, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, D-mannitol, D-mannose, melibiose, D-psicose, raffinose, D-saccharic acid, D-sorbitol, trehalose, formic acid, gentiobiose, α-D-glucose 1-phosphate, D-glucose 6-phosphate, glycerol, hydroxy-L-proline, i-erythritol, inosine, itaconic acid, L-arabinose, L-fucose, L-histidine, L-phenylalanine, L-proline, L-pyroglutamic acid, L-rhamnose, malonic acid, *myo*-inositol, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *p*-hydroxyphenylacetic acid, putrescine, sebacic acid, succinamic acid, succinic acid, sucrose, thymidine, turanose, uridine, urocanic acid, xylitol, α-cyclodextrin, α-lactose, α-hydroxybutyric acid and methyl β-D-glucoside. Table 1 and Supplementary Table S1 show characteristics that can be used to distinguish the type strain from related species.

The type strain, P38^T (=CCTCC AB 208227^T=LMG 24711^T=MCCC 1A01086^T), was isolated from deep seawater of the Indian Ocean.

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References

Abraham, W. R., Strömpl, C., Meyer, H., Lindholst, S., Moore, E. R., Christ, R., Vancanneyt, M., Tindall, B. J., Bennasar, A. & other authors (1999). Phylogeny and polyphasic taxonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poindexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. *Int J Syst Bacteriol* **49**, 1053–1073.

Abraham, W. R., Strömpl, C., Bennasar, A., Vancanneyt, M., Snauwaert, C., Swings, J., Smit, J. & Moore, E. R. (2002). Phylogeny of *Maricaulis* Abraham *et al.* 1999 and proposal of *Maricaulis virginensis* sp. nov., *M. parjimensis* sp. nov., *M. washingtonensis* sp. nov. and *M. salignorans* sp. nov. *Int J Syst Evol Microbiol* **52**, 2191–2201.

Abraham, W. R., Strömpl, C., Vancanneyt, M., Bennasar, A., Swings, J., Lunsdorf, H., Smit, J. & Moore, E. R. B. (2004). *Woodsholea maritima* gen. nov., sp. nov., a marine bacterium with a low diversity of polar lipids. *Int J Syst Evol Microbiol* **54**, 1227–1234.

Alain, K., Tindall, B. J., Intertaglia, L., Catala, P. & Lebaron, P. (2008). *Hellea balneolensis* gen. nov., sp. nov., a prosthecate alphaproteobacterium from the Mediterranean Sea. *Int J Syst Evol Microbiol* **58**, 2511–2519.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (editors) (1995). *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, 3rd edn. New York: Wiley.

Béjà, O., Suzuki, M. T., Heidelberg, J. F., Nelson, W. C., Preston, C. M., Hamada, T., Eisen, J. A., Fraser, C. M. & DeLong, E. F. (2002). Unsuspected diversity among marine aerobic anoxygenic phototrophs. *Nature* **415**, 630–633.

Dong, X.-Z. & Cai, M.-Y. (2001). *Determinative Manual for Routine Bacteriology*. Beijing: Scientific Press (English translation).

Lai, Q., Yuan, J., Wu, C. & Shao, Z. (2009). *Oceanibaculum indicum* gen. nov., sp. nov., isolated from deep seawater of the Indian Ocean. *Int J Syst Evol Microbiol* **59**, 1733–1737.

Lee, K.-B., Liu, C.-T., Anzai, Y., Kim, H., Aono, T. & Oyaizu, H. (2005). The hierarchical system of the '*Alphaproteobacteria*': description of *Hyphomonadaceae* fam. nov., *Xanthobacteraceae* fam. nov. and *Erythrobacteraceae* fam. nov. *Int J Syst Evol Microbiol* **55**, 1907–1919.

Lee, K., Lee, H. K., Choi, T. H. & Cho, J. C. (2007). *Robiginitomaculum antarcticum* gen. nov., sp. nov., a member of the family *Hyphomonadaceae*, from Antarctic seawater. *Int J Syst Evol Microbiol* **57**, 2595–2599.

Liu, C. & Shao, Z. (2005). *Alcanivorax dieselolei* sp. nov., a novel alkane-degrading bacterium isolated from sea water and deep-sea sediment. *Int J Syst Evol Microbiol* **55**, 1181–1186.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* **479**, 297–306.

Moore, R. L., Weiner, R. M. & Gebers, R. (1984). Genus *Hyphomonas* Pongratz 1957 nom. rev. emend., *Hyphomonas polymorpha* Pongratz 1957 nom. rev. emend., and *Hyphomonas neptunium* (Leifson 1964) comb. nov. emend. (*Hyphomicrobium neptunium*). *Int J Syst Bacteriol* **34**, 71–73.

Rzhetsky, A. & Nei, M. (1992). A simple method for estimating and testing minimum-evolution trees. *Mol Biol Evol* **9**, 945–967.

Rzhetsky, A. & Nei, M. (1993). Theoretical foundation of the minimum-evolution method of phylogenetic inference. *Mol Biol Evol* **10**, 1073–1095.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Sasser, M. (1997). *Identification of bacteria by gas chromatography of cellular fatty acids.* MIDI Technical Note 101. Newark, DE: MIDI Inc.

Schlesner, H., Bartels, C., Sittig, M., Dorsch, M. & Stackebrandt, E. (1990). Taxonomic and phylogenetic studies on a new taxon of budding, hyphal *Proteobacteria*, *Hirschia baltica* gen. nov., sp. nov. *Int J Syst Bacteriol* **40**, 443–451.

Shieh, W. Y., Chen, Y.-W., Chaw, S.-M. & Chiu, H.-H. (2003). *Vibrio ruber* sp. nov., a red, facultatively anaerobic, marine bacterium isolated from sea water. *Int J Syst Evol Microbiol* **53**, 479–484.

Strömpl, C., Hold, G. L., Lünsdorf, H., Graham, J., Gallacher, S., Abraham, W. R., Moore, E. R. & Timmis, K. N. (2003). *Oceanicaulis alexandrii* gen. nov., sp. nov., a novel stalked bacterium isolated from a culture of the dinoflagellate *Alexandrium tamarense* (Lebour) Balech. *Int J Syst Evol Microbiol* **53**, 1901–1906.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.