Sphingopyxis marina sp. nov. and *Sphingopyxis litoris* sp. nov., isolated from seawater

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Two yellow-pigmented, Gram-negative, aerobic bacterial strains, designated FR1087^T and FR1093^T, were isolated from surface seawater off Jeju Island, Republic of Korea. Both strains required sea salts for growth. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the two isolates belong to the genus *Sphingopyxis*, showing the highest level of sequence similarity with respect to *Sphingopyxis flavimaris* SW-151^T (97.9 %). The two isolates shared 98.5 % sequence similarity. DNA–DNA hybridization between the isolates and the type strain of *Sphingopyxis flavimaris* clearly suggested that strains FR1087^T and FR1093^T represent two separate novel species in the genus *Sphingopyxis*. Several phenotypic characteristics served to differentiate these two isolates from recognized members of the genus *Sphingopyxis*. The data from the polyphasic study presented here indicated that strains FR1087^T and FR1093^T should be classified as representing novel species in the genus *Sphingopyxis*, for which the names *Sphingopyxis marina* sp. nov. and *Sphingopyxis litoris* sp. nov., respectively, are proposed. The type strain of *Sphingopyxis marina* sp. nov. is FR1087^T (=IMSNU 14132^T=KCTC 12763^T=JCM 14161^T) and the type strain of *Sphingopyxis litoris* sp. nov. is FR1093^T (=IMSNU 14133^T=KCTC 12764^T=JCM 14162^T).

Members of the genus *Sphingopyxis* are Gram-negative, non-fermentative, aerobic, non-spore-forming, yellow-pigmented or whitish-brown, motile and contain sphingoglycolipids. Takeuchi *et al.* (2001) proposed that *Sphingomonas* species should be divided into four different genera, namely *Sphingobium*, *Novosphingobium*, *Sphingopyxis* and *Sphingomonas*. The nomenclatural system of Takeuchi *et al.* (2001) has been generally accepted and used (Yabuuchi *et al.*, 2002; Busse *et al.*, 2003; Godoy *et al.*, 2003; Yoon *et al.*, 2005). In this study, we isolated two bacterial strains, designated FR1087^T and FR1093^T, from seawater and subjected them to a polyphasic analysis. The strains clearly differed from recognized *Sphingopyxis* species and from each other and therefore represent two novel species of the genus *Sphingopyxis*.

Strains FR1087^T and FR1093^T were isolated from a sample of coastal surface seawater collected off Jeju Island, Republic of Korea. The seawater sample was diluted with sterilized artificial seawater (Lyman & Fleming, 1940), spread onto a plate that contained marine agar 2216 (MA; Difco) and incubated at 30 °C. The isolates were routinely cultured on MA and maintained as a glycerol suspension (20 %, w/v) at −80 °C.

The 16S rRNA gene was amplified from a single colony by means of PCR with *Taq* polymerase (Takara). The PCR amplification and sequencing were performed as described previously (Chun & Goodfellow, 1995). The pairwise sequence similarity values were determined using the EzTaxon server (Chun *et al.*, 2007). The regions available for all sequences (positions 47–1437; *Escherichia coli* numbering system) were used to construct the phylogenetic trees after the exclusion of ambiguous positions (positions 67–84). Phylogenetic analyses were performed by following the same procedure as that described previously by Yi *et al.* (2005), using jPHYDIT (Jeon *et al.*, 2005; available at http://chunlab.snu.ac.kr/jphydit/) and PAUP 4.0 (Swofford, 1998). Phylogenetic trees were inferred using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) methods. The resultant tree topologies were evaluated by means of bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Sequence comparisons with 16S rRNA gene sequences held in GenBank indicated that the two isolates were closely related to the genus *Sphingopyxis*. Strains FR1087^T and FR1093^T had a sequence similarity of 98.5 %. *Sphingopyxis*

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains FR1087^T and FR1093^T are DQ781320 and DQ781321, respectively.

Table 1. Phenotypic characteristics that serve to differentiate strains FR1087^T and FR1093^T from recognized *Sphingopyxis* species

Taxa: 1, strain FR1087^T; 2, strain FR1093^T; 3, *Sphingopyxis flavimaris*; 4, *Sphingopyxis baekryungensis*; 5, *Sphingopyxis macrogoltabida*; 6, *Sphingopyxis terrae*; 7, *Sphingopyxis alaskensis*; 8, *Sphingopyxis chilensis*; 9, *Sphingopyxis witflariensis*; 10, *Sphingopyxis taejonensis*. The *Escherichia coli* numbering system was used for nucleotide signatures of the 16S rRNA gene. Data were taken from this study and from Godoy *et al.* (2003), Kämpfer *et al.* (2002), Lee *et al.* (2001), Takeuchi *et al.* (1993), Vancanneyt *et al.* (2001), Yoon *et al.* (2005) and Yoon & Oh (2005). +, Positive; −, negative; W, weakly positive; V, variable; ND, not determined. Data in parentheses are for the type strain.

Characteristic	1	2	3	4	5	6	7	8	9	10
Colony colour	Yellow	Yellow	Yellow	Orange	Whitish brown or yellow	Light or deep yellow	Yellow to beige	Yellow	Yellow	Pale yellow
Motility	+	+	+	+	+	V(+)	+	+	+	+
Reduction of nitrate to nitrite	−	−	−	+	−	−	−	−	−	−
Hydrolysis of:										
Aesculin	W	W	−	+	V(+)	V(−)	+	+	−	−
Gelatin	+	−	−	−	V(−)	−	−	−	−	−
Urea	+	+	−	−	−	−	V(+)	−	−	−
Utilization of:										
D-Glucose	+	−	+	+	(+)	(−)	+	+	+	ND
Malate	+	+	−	W	−	+	+	+	−	+
Maltose	−	−	−	−	+	+	+	+	+	−
Mannose	−	−	−	+	−	−	−	+	−	−
Nucleotide signatures of 16S rRNA gene										
134	A	A	A	G	G	G	G	G	G	G
990 : 1215	U : A	U : A	U : A	U : A	U : G	U : G	U : G	U : G	U : A	U : G
Major polyamine*	S, A	S	ND	ND	S	S	ND	S	S	ND
Major fatty acids	$C_{17:0}$, $C_{17:1}\omega8c$, $C_{17:1}\omega6c$, $C_{18:1}\omega7c$	$C_{18:1}\omega7c$, iso-$C_{15:0}$ 2-OH/ $C_{16:1}\omega7c$	$C_{18:1}\omega7c$, iso-$C_{15:0}$ 2-OH/ $C_{16:1}\omega7c$, $C_{17:1}\omega6c$	$C_{18:1}\omega7c$, $C_{17:1}\omega6c$	$C_{18:1}\omega7c$, iso-$C_{15:0}$ 2-OH/ $C_{16:1}\omega7c$	$C_{17:1}\omega6c$, $C_{18:1}\omega7c$	$C_{17:1}\omega6c$, $C_{18:1}\omega7c$	$C_{18:1}\omega7c$, $C_{17:1}\omega6c$	$C_{17:1}\omega6c$, iso-$C_{15:0}$ 2-OH/ $C_{16:1}\omega7c$	$C_{18:1}\omega7c$/ $C_{18:1}\omega9t$/ $C_{18:1}\omega12t$, $C_{16:0}$

*A, Agmatine; S, spermidine.

flavimaris showed the highest level of 16S rRNA gene sequence similarity with respect to strains FR1087^T and FR1093^T (97.9 % for both). Out of five signature nucleotides defined for the genus *Sphingopyxis* (Takeuchi *et al.*, 2001), three were found in the two isolates. They, along with *Sphingopyxis flavimaris*, lacked the remaining two signature nucleotides (Table 1).

The levels of sequence similarity between strain FR1087^T and other *Sphingopyxis* species were in the range 92.8–93.7 % and the corresponding values for strain FR1093^T were 91.8–93.1 %. The neighbour-joining tree showed that the two isolates formed a monophyletic clade with the type strain of *Sphingopyxis flavimaris* (Fig. 1), with 100 % bootstrap support. This relationship was confirmed by all other tree-inferring methods used in this study.

The close taxonomic relationship, i.e. the high levels of 16S rRNA gene sequence similarity among strains FR1087^T and FR1093^T and *Sphingopyxis flavimaris* SW-151^T, led us to employ DNA–DNA relatedness experiments to obtain further genomic comparisons (Stackebrandt & Goebel, 1994). Genomic relatedness was determined using a Cary 300 Bio model UV/VIS spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller (Varian). The experimental details for the DNA–DNA hybridization studies were as described elsewhere (Yi & Chun, 2006). The level of genomic relatedness observed between *Sphingopyxis flavimaris* and strain FR1087^T was 21.2 %, while that between *Sphingopyxis flavimaris* and strain FR1093^T was 36.3 %. The two isolates shared a low DNA–DNA relatedness value (20.7 %). All of these values are below the threshold (70 %) for determining bacterial species (Wayne *et al.*, 1987); the finding strongly suggested that the two isolates belonged to novel genomic species in the genus *Sphingopyxis*.

Growth under anaerobic conditions was checked in an anaerobic chamber on MA and on nitrate-supplemented MA, both of which had been prepared anaerobically under nitrogen gas. Growth at various concentrations of NaCl and sea salts was investigated using sea-salt-free ZoBell's agar [ZoBell, 1941; 15 g Bacto agar (Difco), 5 g Bacto

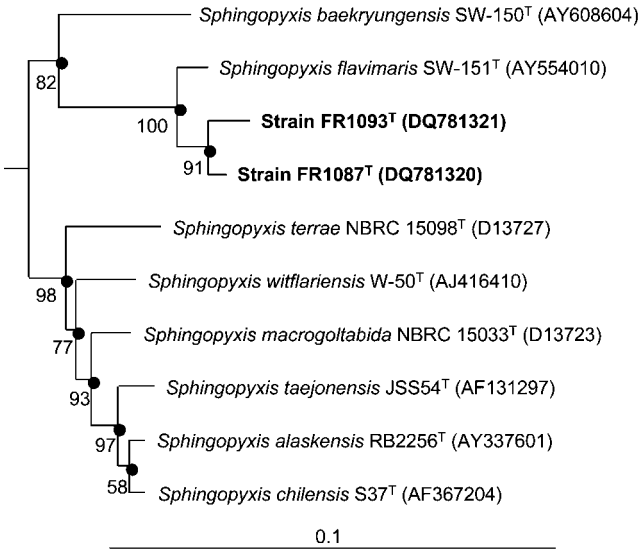

Fig. 1. Neighbour-joining phylogenetic tree for the genus *Sphingopyxis*, showing the positions of strains FR1087^T and FR1093^T. Bootstrap percentages (based on 1000 resampled datasets) are shown at nodes when greater than 50 %. Filled circles indicate that the corresponding nodes (groupings) were also recovered in maximum-likelihood and maximum-parsimony trees. *Helicobacter pylori* ATCC 43504^T (GenBank accession no. U01330) was used as the outgroup (not shown). Bar, 0.1 nucleotide substitutions per position.

peptone (Difco), 1 g yeast extract, 0.1 g ferric citrate in 1 l distilled water]. The temperature range for growth was determined optically using a temperature gradient incubator (TVS 126MA; Advantec) with marine broth 2216 (Difco) and temperatures of 4–45 °C (specifically at 4.0, 10.0, 15.1, 18.3, 21.2, 23.7, 26.5, 29.0, 31.8, 34.4, 37.3, 40.7 and 45 °C). Biochemical tests were performed using API 20NE and API ZYM kits (bioMérieux): the strips were each inoculated with a heavy bacterial suspension in artificial seawater or AUX medium (bioMérieux) supplemented with 2 % (w/v) sea salts. Hydrolysis of casein, starch, Tween 80, hypoxanthine, tyrosine and xanthine was tested on MA using the substrate concentrations described by Cowan & Steel (1965). The two isolates showed differences in terms of the hydrolysis of gelatin and the utilization of glucose. Both isolates utilized urea, unlike other phylogenetic neighbours. The results of the biochemical and physiological tests are given in the species description and are shown in Table 1.

Cellular fatty acids from the test strains were analysed by GLC according to the instructions of the Microbial Identification System (MIDI). For analysis of the cellular fatty acid methyl esters, cells were grown on MA at 30 °C for 6 days. The two strains differed substantially in terms of their major fatty acids: details are given in the species description and are shown in Table 1. Polar lipids were extracted using the procedures described by Minnikin *et al.* (1984) and were identified by using two-dimensional TLC followed by spraying with the appropriate detection reagents (Komagata & Suzuki, 1987). Sphingoglycolipid was found as a major polar lipid in both isolates. Polyamines were extracted and analysed according to the methods of Busse *et al.* (1989) and Flores & Galston (1982). Strain FR1087^T contained spermidine [8.6 μmol (g dry wt)$^{-1}$] and agmatine [4 μmol (g dry wt)$^{-1}$] as major components, whereas strain FR1093^T contained only spermidine [17.78 μmol (g dry wt)$^{-1}$] as the major component.

On the basis of the data from the polyphasic study presented here, it is evident that strains FR1087^T and FR1093^T represent separate novel species in the genus *Sphingopyxis*, for which the names *Sphingopyxis marina* sp. nov. and *Sphingopyxis litoris* sp. nov., respectively, are proposed.

Description of *Sphingopyxis marina* sp. nov.

Sphingopyxis marina (ma.ri′na. L. fem. adj. *marina* of the sea, marine).

Gram-negative and aerobic. Oxidase-positive. Colonies are circular, convex and yellow in colour and 0.7–1.0 mm in diameter after cultivation for 6 days at 30 °C on MA. Spores are not formed. Does not grow without sea salts. Grows at 3–7 % (w/v) sea salts (optimum, 3–5 %) and 15–34.4 °C (optimum, 23.7–31.8 °C). Does not grow under anaerobic conditions on MA or MA supplemented with nitrate. Tyrosine is hydrolysed, but Tween 80, starch, xanthine, hypoxanthine and casein are not. Positive reactions are obtained for arginine dihydrolase, urease, protease and the assimilation of glucose, arabinose, adipate, malate and citrate. Weakly positive for β-glucosidase (with API 20NE). With API ZYM, positive reactions are obtained for alkaline phosphatase, leucine arylamidase and trypsin, weakly positive reactions are obtained for esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase and negative reactions are obtained for α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Sphingoglycolipid is present as the major polar lipid. Major fatty acids are $C_{17:0}$ (20.5 %), $C_{17:1}\omega8c$ (15.38 %), $C_{17:1}\omega6c$ (14.04 %) and $C_{18:1}\omega7c$ (13.6 %). Spermidine and agmatine are present as major polyamine compounds, whereas cadaverine, putrescine and spermine are detected only in small amounts.

The type strain, FR1087^T (=IMSNU 14132^T=KCTC 12763^T=JCM 14161^T), was isolated from surface seawater collected off Jeju Island, Republic of Korea.

Description of *Sphingopyxis litoris* sp. nov.

Sphingopyxis litoris (li.to′ris. L. gen. n. *litoris* of the seashore, of the coast).

Gram-negative and aerobic. Oxidase- and catalase-positive. Colonies are circular, convex and yellow in colour and 0.7–1.0 mm in diameter after 6 days cultivation at 30 °C on MA. Spores are not formed. Does not grow without sea salts. Grows at 2–7 % (w/v) sea salts (optimum, 3–5 %) and 15–34.4 °C (optimum, 23.7–31.8 °C). Does not grow under anaerobic conditions on MA or MA supplemented with nitrate. Tyrosine and starch are hydrolysed, but Tween 80, xanthine, hypoxanthine and casein are not. Positive reactions are obtained for arginine dihydrolase, urease, β-galactosidase and assimilation of malate and citrate. Weakly positive for β-glucosidase (with API 20NE). With API ZYM, positive reactions are obtained for alkaline phosphatase, leucine arylamidase, esterase lipase (C8), trypsin and naphthol-AS-BI-phosphohydrolase, weakly positive reactions are obtained for esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase, α-galactosidase and α-glucosidase and negative reactions are obtained for β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Sphingoglycolipid is present as the major polar lipid. Major cellular fatty acids are $C_{18:1}\omega7c$ (47.82 %) and iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$ (14.1 %; the two fatty acids cannot be separated by GLC with the MIDI system). Spermidine is present as the major polyamine component, whereas agmatine, cadaverine, putrescine and spermine are detected only in small amounts.

The type strain, FR1093^T (=IMSNU 14133^T=KCTC 12764^T=JCM 14162^T), was isolated from surface seawater collected off Jeju Island, Republic of Korea.

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References

Busse, H., El-Banna, T. & Auling, G. (1989). Evaluation of different approaches for identification of xenobiotic-degrading pseudomonads. *Appl Environ Microbiol* **55**, 1578–1583.

Busse, H.-J., Denner, E. B. M., Buczolits, S., Salkinoja-Salonen, M., Bennasar, A. & Kampfer, P. (2003). *Sphingomonas aurantiaca* sp. nov., *Sphingomonas aerolata* sp. nov. and *Sphingomonas faeni* sp. nov., air- and dustborne and Antarctic, orange-pigmented, psychrotolerant bacteria, and emended description of the genus *Sphingomonas*. *Int J Syst Evol Microbiol* **53**, 1253–1260.

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., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y.-W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Flores, H. E. & Galston, A. W. (1982). Analysis of polyamines in higher plants by high performance liquid chromatography. *Plant Physiol* **69**, 701–706.

Godoy, F., Vancanneyt, M., Martinez, M., Steinbuchel, A., Swings, J. & Rehm, B. H. (2003). *Sphingopyxis chilensis* sp. nov., a chlorophenol-degrading bacterium that accumulates polyhydroxyalkanoate, and transfer of *Sphingomonas alaskensis* to *Sphingopyxis alaskensis* comb. nov. *Int J Syst Evol Microbiol* **53**, 473–477.

Jeon, Y. S., Chung, H., Park, S., Hur, I., Lee, J. H. & Chun, J. (2005). jPHYDIT: a JAVA-based integrated environment for molecular phylogeny of ribosomal RNA sequences. *Bioinformatics* **21**, 3171–3173.

Kämpfer, P., Witzenberger, R., Denner, E. B. M., Busse, H.-J. & Neef, A. (2002). *Sphingopyxis witflariensis* sp. nov., isolated from activated sludge. *Int J Syst Evol Microbiol* **52**, 2029–2034.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lee, J.-S., Shin, Y. K., Yoon, J.-H., Takeuchi, M., Pyun, Y.-R. & Park, Y.-H. (2001). *Sphingomonas aquatilis* sp. nov., *Sphingomonas koreensis* sp. nov. and *Sphingomonas taejonensis* sp. nov., yellow-pigmented bacteria isolated from natural mineral water. *Int J Syst Evol Microbiol* **51**, 1491–1498.

Lyman, J. & Fleming, R. H. (1940). Composition of sea water. *J Mar Res* **3**, 134–146.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

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

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.

Swofford, D. L. (1998). PAUP: Phylogenetic analysis using parsimony, version 4. Sunderland, MA: Sinauer Associates.

Takeuchi, M., Kawai, F., Shimada, Y. & Yokota, A. (1993). Taxonomic study of polyethylene glycol-utilizing bacteria: emended description of the genus *Sphingomonas* and new descriptions of *Sphingomonas macrogoltabidus* sp. nov., *Sphingomonas sanguis* sp. nov. and *Sphingomonas terrae* sp. nov. *Syst Appl Microbiol* **16**, 227–238.

Takeuchi, M., Hamana, K. & Hiraishi, A. (2001). Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *Int J Syst Evol Microbiol* **51**, 1405–1417.

Vancanneyt, M., Schut, F., Snauwaert, C., Goris, J., Swings, J. & Gottschal, J. C. (2001). *Sphingomonas alaskensis* sp. nov., a dominant bacterium from a marine oligotrophic environment. *Int J Syst Evol Microbiol* **51**, 73–79.

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.

Yabuuchi, E., Kosako, Y., Fujiwara, N., Naka, T., Matsunaga, I., Ogura, H. & Kobayashi, K. (2002). Emendation of the genus *Sphingomonas* Yabuuchi *et al.* 1990 and junior objective synonymy of the species of three genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, in conjunction with *Blastomonas ursincola*. *Int J Syst Evol Microbiol* **52**, 1485–1496.

Yi, H. & Chun, J. (2006). *Thalassobius aestuarii* sp. nov., isolated from tidal flat sediment. *J Microbiol* **44**, 171–176.

Yi, H., Oh, H.-M., Lee, J.-H., Kim, S.-J. & Chun, J. (2005). *Flavobacterium antarcticum* sp. nov., a novel psychrotolerant bacterium isolated from the Antarctic. *Int J Syst Evol Microbiol* **55**, 637–641.

Yoon, J.-H. & Oh, T.-K. (2005). *Sphingopyxis flavimaris* sp. nov., isolated from sea water of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **55**, 369–373.

Yoon, J.-H., Lee, C.-H., Yeo, S.-H. & Oh, T.-K. (2005). *Sphingopyxis baekryungensis* sp. nov., an orange-pigmented bacterium isolated from sea water of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **55**, 1223–1227.

ZoBell, C. E. (1941). Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *J Mar Res* **4**, 42–75.