Oceanisphaera ostreae sp. nov., isolated from seawater of an oyster farm, and emended description of the genus *Oceanisphaera* Romanenko *et al.* 2003

Won-Chan Choi,¹ So-Jung Kang,¹ Yong-Taek Jung,¹ Tae-Kwang Oh¹ and Jung-Hoon Yoon^{1,2}

Correspondence
Jung-Hoon Yoon
jhyoon69@skku.edu

¹Korea Research Institute of Bioscience and Biotechnology (KRIBB), PO Box 115, Yusong, Daejeon, South Korea

²Department of Food Science and Biotechnology, Sungkyunkwan University, Jangan-gu, Suwon, South Korea

A Gram-stain-negative, motile, non-spore-forming and short rod- or rod-shaped bacterial strain, T-w6^T, was isolated from seawater of an oyster farm in the South Sea, Korea. Strain T-w6^T grew optimally at 25 °C and in the presence of 2% (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain T-w6^T joined the cluster comprising *Oceanisphaera* species with a bootstrap resampling value of 90.8%, and this cluster joined the clade comprising members of the genera *Oceanimonas* and *Zobellella* with a bootstrap resampling value of 100%. Strain T-w6^T exhibited 16S rRNA gene sequence similarity of 95.9 and 96.6% to the type strains of *Oceanisphaera litoralis* and *Oceanisphaera donghaensis*, respectively. Strain T-w6^T and the type strains of *Oceanisphaera litoralis* and *Oceanisphaera donghaensis* had Q-8 as the predominant ubiquinone and iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, C_{18:1}ω7c and C_{16:0} as the major fatty acids. The major polar lipids were phosphatidylglycerol and phosphatidylethanolamine. The DNA G+C content of strain T-w6^T was 56.6 mol%. Mean DNA–DNA relatedness of strain T-w6^T with *Oceanisphaera litoralis* DSM 15406^T and *Oceanisphaera donghaensis* KCTC 12522^T was 13 and 10%, respectively. Phenotypic properties of strain T-w6^T demonstrated that this strain could be distinguished from the other *Oceanisphaera* species. On the basis of the data presented, strain T-w6^T is considered to represent a novel species of the genus *Oceanisphaera*, for which the name *Oceanisphaera ostreae* sp. nov. is proposed; the type strain is T-w6^T (=KCTC 23422^T =CCUG 60525^T). An emended description of the genus *Oceanisphaera* is also presented.

The genus *Oceanisphaera* was first proposed by Romanenko *et al.* (2003), who described a single novel species, *Oceanisphaera litoralis*. Subsequently, another *Oceanisphaera* species, *Oceanisphaera donghaensis*, was described (Park *et al.*, 2006). Phylogenetic analysis based on 16S rRNA gene sequences showed that the genus *Oceanisphaera* belongs to the *Gamma-proteobacteria* (Romanenko *et al.*, 2003). Members of the genus *Oceanisphaera* have been isolated from marine bottom sediments (Romanenko *et al.*, 2003; Park *et al.*, 2006). In this study, a bacterial strain, designated T-w6^T, which was isolated from seawater of an oyster farm on the southern coast of

Korea, is described. Comparative 16S rRNA gene sequence analysis indicated that, phylogenetically, strain T-w6^T is affiliated with the genus *Oceanisphaera*. The aim of the present work was to determine the exact taxonomic position of strain T-w6^T by using a polyphasic characterization that included the determination of chemotaxonomic and phenotypic properties and a detailed phylogenetic investigation based on 16S rRNA gene sequences.

Strain T-w6^T was isolated by means of the standard dilution plating technique at 25 °C on marine agar 2216 (MA; Difco). The type strains of the two *Oceanisphaera* species were used as reference strains for phenotypic characterization, DNA–DNA hybridization and analyses of isoprenoid quinones and fatty acids. *Oceanisphaera litoralis* DSM 15406^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany, and *Oceanisphaera*

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain T-w6^T is HQ340607.

Two supplementary figures are available with the online version of this paper.

donghaensis KCTC 12522^T was obtained from the Korean Collection for Type Cultures, Daejeon, South Korea. Morphological, physiological and biochemical characteristics of strain T-w6^T were investigated following routine cultivation on MA at 25 °C. Cell morphology was examined by light microscopy (E600; Nikon) and transmission electron microscopy (CM-20; Philips). Flagellation was determined by using transmission electron microscopy with cells from exponentially growing cultures. For this purpose, cells were negatively stained with 1 % (w/v) phosphotungstic acid and grids were examined after being air-dried. The Gram reaction was determined by using a commercial Gram stain kit (bioMérieux) according to the manufacturer's instructions. Endospores were investigated by using Schaeffer–Fulton (Murray *et al.*, 1994) and malachite green (Smibert & Krieg, 1994) staining methods. Growth in the absence of NaCl and in the presence of 0.5, 1.0, 2.0 and 3.0 % (w/v) NaCl was investigated by using trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded. Growth at 2.0–10.0 % (w/v) NaCl (in increments of 1.0 %) was investigated in marine broth 2216 (MB; Difco). Growth at 4, 10, 20, 25, 28, 30, 37, 40 and 45 °C was measured on MA. The pH range for growth was determined in MB that was adjusted to pH 4.5–9.5 (in increments of 0.5 pH units) by the addition of HCl or Na₂CO₃; the pH was verified after autoclaving. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber (1029; Forma) on MA and on MA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere (N₂/CO₂/H₂, 86:7:7). Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Hydrolysis of casein, starch, hypoxanthine, tyrosine and xanthine was tested on MA by using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin, Tweens 20, 40, 60 and 80 and urea and nitrate reduction were investigated as described previously (Lányi, 1987), with the modification that artificial seawater was used for preparation of media. The artificial seawater contained (l⁻¹ distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested as described by Baumann & Baumann (1981) using supplementation with 2 % (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1 % (v/v) vitamin solution (Staley, 1968). Susceptibility to antibiotics was tested on MA plates using the following antibiotic discs: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), neomycin (30 µg) and carbenicillin (100 µg). Enzyme activities were investigated using the API ZYM system (bioMérieux).

Cell biomass of strain T-w6^T for DNA extraction and for analyses of isoprenoid quinones and polar lipids was

obtained from cultures grown for 3 days in MB at 25 °C. Cell biomass of *Oceanisphaera litoralis* DSM 15406^T and *Oceanisphaera donghaensis* KCTC 12522^T for DNA extraction and isoprenoid quinone analysis was obtained from cultures grown under the same conditions. Chromosomal DNA was isolated and purified as described previously (Yoon *et al.*, 1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid methyl ester analysis, cell mass of strain T-w6^T, *Oceanisphaera litoralis* DSM 15406^T and *Oceanisphaera donghaensis* KCTC 12522^T was harvested from MA plates after incubation for 3 days at 25 °C. Fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Polar lipids were extracted in accordance with procedures described by Minnikin *et al.* (1984) and were identified by two-dimensional TLC followed by spraying with the appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984), with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain T-w6^T are given in the species description or in Table 1. The almost-complete 16S rRNA gene sequence of strain T-w6^T determined in this study comprised 1465 nt (approx. 96 % of the *Escherichia coli* 16S rRNA gene sequence). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain T-w6^T joined the cluster comprising the type strains of the two *Oceanisphaera* species and one *Oceanisphaera* species with a name that has not been validly published by a bootstrap resampling value of 90.8 % (Fig. 1). The cluster comprising strain T-w6^T and members of the genus *Oceanisphaera* joined the cluster comprising members of the genera *Oceanimonas* and *Zobellella* with a bootstrap resampling value of 100 % (Fig. 1). The relationship between strain T-w6^T and the type strains of *Oceanisphaera* species was also maintained in trees constructed using the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain T-w6^T exhibited 16S rRNA gene sequence similarity of 95.9, 96.6 and 95.8 % to the type strains of *Oceanisphaera*

Table 1. Differential phenotypic characteristics of *Oceanisphaera ostreae* sp. nov. T-w6^T and the type strains of the two *Oceanisphaera* species

Strains: 1, *Oceanisphaera ostreae* sp. nov. T-w6^T (data from this study); 2, *Oceanisphaera litoralis* DSM 15406^T; 3, *Oceanisphaera donghaensis* KCTC 12522^T. All data are from this study except for Gram-staining, morphology, NaCl requirement for growth, growth at 4 and 40 °C, catalase, oxidase, nitrate reduction, hydrolysis of substrates and DNA G+C content of the reference strains, which are from Romanenko *et al.* (2003) and Park *et al.* (2006). +, Positive; –, negative; w, weakly positive. All strains are Gram-stain-negative. All strains are positive for motility, growth at 4 °C, catalase, oxidase, nitrate reduction, utilization of acetate, succinate and pyruvate, activity of alkaline phosphatase and leucine arylamidase and susceptibility to chloramphenicol, gentamicin, neomycin and polymyxin B. All strains are negative for hydrolysis of aesculin and gelatin, utilization of L-arabinose, cellobiose, formate, D-galactose, D-glucose, L-glutamate, maltose, D-mannose, salicin, sucrose, trehalose and D-xylose, activity of esterase lipase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase, and susceptibility to ampicillin, lincomycin, novobiocin, oleandomycin, penicillin G and tetracycline.

Characteristic	1	2	3
Cell morphology	Short rods or rods	Cocci	Cocci
NaCl requirement for growth	–	+	+
Growth at 40 °C	–	+	+
Urea hydrolysis	+	+	–
Utilization of:			
D-Fructose	–	+	–
Citrate	–	+	–
Benzoate	–	+	–
L-Malate	+	+	–
Esterase (C8) activity (API ZYM)	–	–	w
Susceptibility to antibiotics			
Carbenicillin	–	w	–
Cephalothin	–	–	w
Kanamycin	+	–	+
Streptomycin	+	–	–
DNA G+C content (mol%)	56.6	56.4	56.6

litoralis and *Oceanisphaera donghaensis* and ‘*Oceanisphaera arctica*’ V1-41, respectively. It exhibited 16S rRNA gene sequence similarity of 94.3–96.4 % to members of the genera *Oceanimonas* and *Zobellella* and less than 92.1 % similarity to strains of other species used in the phylogenetic analysis.

The predominant isoprenoid quinone detected in strain T-w6^T was ubiquinone-8 (Q-8). In this study, the type strains of *Oceanisphaera litoralis* and *Oceanisphaera donghaensis* also had Q-8 as the predominant isoprenoid quinone. The cellular fatty acid profiles of strain T-w6^T and the type strains of two *Oceanisphaera* species analysed in this study are shown in Table 2. The major fatty acids (>10 % of total

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Oceanisphaera ostreae* sp. nov. T-w6^T, other *Oceanisphaera* species and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Pseudomonas aeruginosa* LMG 1242^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

fatty acids) found in strain T-w6^T were iso-C_{15:0} 2-OH and/or C_{16:1}ω7c, C_{18:1}ω7c and C_{16:0} (Table 2). The fatty acid profiles were similar in all strains, although there were differences in the proportions of some fatty acids (Table 2). The major polar lipids detected in strain T-w6^T were phosphatidylglycerol and phosphatidylethanolamine; a minor amount of diphosphatidylglycerol was also present (Supplementary Fig. S1, available in IJSEM Online). This polar lipid profile was the same as those of the type strains of *Oceanisphaera litoralis* and *Oceanisphaera donghaensis* (Romanenko *et al.*, 2003; Park *et al.*, 2006). The DNA G+C content of strain T-w6^T was 56.6 mol%. Hence, it is appropriate to classify strain T-w6^T as a member of the genus *Oceanisphaera* based on the absence of chemotaxonomic differences between strain T-w6^T and *Oceanisphaera* species, as well as the phylogenetic data based on 16S rRNA gene sequences.

Strain T-w6^T exhibited mean DNA–DNA relatedness of 13 and 10 % to *Oceanisphaera litoralis* DSM 15406^T and *Oceanisphaera donghaensis* KCTC 12522^T, respectively. Strain T-w6^T could be distinguished from *Oceanisphaera litoralis* and *Oceanisphaera donghaensis* by several phenotypic characteristics, most of which were determined under the same conditions and by the same methods (Table 1). The phylogenetic and genetic distinctiveness and differential phenotypic properties of strain T-w6^T are sufficient to show that this strain is separate from other *Oceanisphaera* species (Stackebrandt & Goebel, 1994). Therefore, on the basis of the phenotypic, chemotaxonomic, phylogenetic and genetic data, strain T-w6^T is considered to represent a novel species of the genus *Oceanisphaera*, for which the name *Oceanisphaera ostreae* sp. nov. is proposed.

Table 2. Cellular fatty acid compositions of *Oceanisphaera ostreae* sp. nov. T-w6^T and the type strains of the two *Oceanisphaera* species

Strains: 1, *Oceanisphaera ostreae* T-w6^T; 2, *Oceanisphaera litoralis* DSM 15406^T; 3, *Oceanisphaera donghaensis* KCTC 12522^T. All data are from this study. Values are percentages of total fatty acids. Fatty acids that represent <0.5% in all strains were omitted. —, Not detected; ECL, equivalent chain length.

Fatty acid	1	2	3
Straight-chain			
C _{12:0}	7.1	6.5	6.4
C _{14:0}	1.2	0.3	0.4
C _{15:0}	1.0	0.7	1.0
C _{16:0}	15.6	20.4	20.6
C _{17:0}	0.6	0.4	0.3
Branched			
iso-C _{16:0}	—	1.2	1.7
iso-C _{17:0}	—	—	0.7
Unsaturated			
C _{17:1ω8c}	1.0	0.4	0.3
C _{18:1ω7c}	21.7	22.3	17.6
Unknown			
ECL 14.502	1.0	0.5	0.5
Summed features*			
2	5.9	5.4	5.7
3	43.7	40.7	42.3

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

Emended description of the genus *Oceanisphaera* Romanenko et al. 2003

The description of the genus *Oceanisphaera* is as given by Romanenko et al. (2003) with the following amendments. Cells are cocci, short rods or rods. The predominant ubiquinone is Q-8.

Description of *Oceanisphaera ostreae* sp. nov.

Oceanisphaera ostreae (os.tre' a.e. L. gen. n. *ostreae* of/from an oyster).

Cells are Gram-stain-negative, non-spore-forming and short rods or rods (0.5–1.0 × 1.2–5.0 µm). Motile by means of a single polar flagellum (Supplementary Fig. S2). Colonies on MA are circular to slightly irregular, slightly convex, smooth, glistening, light greyish yellow and 1.0–1.5 mm in diameter after incubation for 3 days at 25 °C. Optimal growth occurs at 25 °C; grows at 4 and 37 °C, but not at 40 °C. Optimal pH for growth is between 7.0 and 8.0; grows at pH 5.0, but not at pH 4.5. Optimal growth occurs in the presence of 2% (w/v) NaCl; grows in 0–10.0% (w/v) NaCl. Growth does not occur under anaerobic conditions on MA or on MA

supplemented with nitrate. Hypoxanthine, xanthine, tyrosine and Tweens 20, 40, 60 and 80 are hydrolysed, but casein and starch are not. Acid is produced from D-ribose, but not from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, myo-inositol, lactose, maltose, D-mannitol, D-mannose, melezitose, melibiose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose or D-xylose. The predominant ubiquinone is Q-8. The major fatty acids (>10% of total fatty acids) are iso-C_{15:0} 2-OH and/or C_{16:1ω7c}, C_{18:1ω7c} and C_{16:0}. The major polar lipids are phosphatidylglycerol and phosphatidylethanolamine; diphosphatidylglycerol is present as a minor component. Other phenotypic properties are shown in Table 1.

The type strain is T-w6^T (=KCTC 23422^T =CCUG 60525^T), isolated from seawater of an oyster farm in the South Sea, Korea. The DNA G+C content of the type strain is 56.6 mol% (HPLC).

Acknowledgements

This work was supported by the 21C Frontier Program of Microbial Genomics and Applications from the Ministry of Education, Science and Technology (MEST) of the Republic of Korea (grant 11-2008-00-002-00).

References

- Baumann, P. & Baumann, L. (1981). The marine Gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas*, and *Alcaligenes*. In *The Prokaryotes*, pp. 1302–1331. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.
- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* 51, 1997–2006.
- Cohen-Bazire, G., Sistrom, W. R. & Stanier, R. Y. (1957). Kinetic studies of pigment synthesis by non-sulfur purple bacteria. *J Cell Comp Physiol* 49, 25–68.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 39, 224–229.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 19, 161–207.
- Lányi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 19, 1–67.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* 85, 1183–1184.
- 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.
- Murray, R. G. E., Doetsch, R. N. & Robinow, F. (1994). Determinative and cytological light microscopy. In *Methods for General and Molecular Bacteriology*, pp. 21–41. Edited by P. Gerhardt, R. G. E. Murray,

W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Park, S.-J., Kang, C.-H., Nam, Y.-D., Bae, J.-W., Park, Y.-H., Quan, Z.-X., Moon, D.-S., Kim, H.-J., Roh, D.-H. & Rhee, S.-K. (2006). *Oceanisphaera donghaensis* sp. nov., a halophilic bacterium from the East Sea, Korea. *Int J Syst Evol Microbiol* **56**, 895–898.

Romanenko, L. A., Schumann, P., Zhukova, N. V., Rohde, M., Mikhailov, V. V. & Stackebrandt, E. (2003). *Oceanisphaera litoralis* gen. nov., sp. nov., a novel halophilic bacterium from marine bottom sediments. *Int J Syst Evol Microbiol* **53**, 1885–1888.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

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.

Staley, J. T. (1968). *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* **95**, 1921–1942.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.

Yoon, J.-H., Kim, H., Kim, S.-B., Kim, H.-J., Kim, W. Y., Lee, S. T., Goodfellow, M. & Park, Y.-H. (1996). Identification of *Saccharomonospora* strains by the use of genomic DNA fragments and rRNA gene probes. *Int J Syst Bacteriol* **46**, 502–505.

Yoon, J.-H., Lee, S. T. & Park, Y.-H. (1998). Inter- and intraspecific phylogenetic analysis of the genus *Nocardioides* and related taxa based on 16S rDNA sequences. *Int J Syst Bacteriol* **48**, 187–194.

Yoon, J.-H., Kim, I.-G., Shin, D.-Y., Kang, K. H. & Park, Y.-H. (2003). *Microbulbifer salipaludis* sp. nov., a moderate halophile isolated from a Korean salt marsh. *Int J Syst Evol Microbiol* **53**, 53–57.