Haloferula chungangensis sp. nov., isolated from marine sediment

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A Gram-stain-negative, non-spore-forming, non-motile, strictly aerobic, rod-shaped bacterial strain, designated CAU 1074^T, was isolated from marine sediment and its taxonomic position was investigated using a polyphasic approach. Strain CAU 1074^T grew optimally at 30 °C and pH 6.5. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CAU 1074^T formed a distinct lineage within the genus *Haloferula* and was most closely related to *Haloferula harenae* KCTC 22198^T (96.0 % similarity). Strain CAU 1074^T contained MK-9 as the major isoprenoid quinone, and iso-$C_{14:0}$, $C_{16:1}\omega9c$ and $C_{16:0}$ as the major fatty acids. The cell wall peptidoglycan contained *meso*-diaminopimelic acid. The major whole-cell sugars were glucose, xylose, mannose and ribose. The polar lipids were composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, aminoglycolipid and two unidentified phospholipids. The DNA G+C content of the strain was 64.0 mol%. On the basis of phenotypic and chemotaxonomic data, and phylogenetic inference, strain CAU 1074^T should be classified as a member of a novel species in the genus *Haloferula*, for which the name *Haloferula chungangensis* sp. nov. is proposed; the type strain is CAU 1074^T (=KCTC 23578^T=CCUG 61920^T). An emended description of the genus *Haloferula* is also provided.

The genus *Haloferula* was described by Yoon *et al.* (2008) and belongs to the phylum *Verrucomicrobia*. At present, this genus consists of six species with validly published names: *Haloferula rosea* isolated from sponge, *Haloferula harenae* from sediment, *Haloferula phyci* from algae, *Haloferula helveola* from seawater and *Haloferula sargassicola* from algae (Yoon *et al.*, 2008), and *Haloferula luteola* from a root (Bibi *et al.*, 2011). Members of the genus *Haloferula* are Gram-stain-negative, rod-shaped, obligately aerobic, non-motile and non-spore-forming, and have menaquinone 9 (MK-9) as the major respiratory quinone and iso-$C_{14:0}$ as the predominant cellular fatty acid (Yoon *et al.*, 2008). During the course of screening bacteria with biotechnological potential from marine samples, a bacterial strain, designated CAU 1074^T, was isolated from marine sediment collected in Jeju Island (33° 22′ 47.30″ N 126° 32′ 59.32″ E) in the Republic of Korea. The purpose of the present study was to establish the taxonomic position of this bacterial strain by using a polyphasic characterization that included the determination of phenotypic and chemotaxonomic properties, and a detailed phylogenetic investigation based on 16S rRNA gene sequences and genetic analysis.

Isolation was performed according to Gordon & Mihm (1962) using marine agar 2216 (MA; Difco), supplemented with cycloheximide (50 mg l^{-1}) and nalidixic acid (20 mg l^{-1}). The crushed sample was diluted with sterilized saline solution. Serial dilutions were spread onto MA plates and incubated under aerobic conditions at 30 °C for 3 days. Pure cultures were preserved at −70 °C in marine broth (MB; Difco) supplemented with 25 % (v/v) glycerol. The

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU 1074^T is JN001489.

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

type strains of the six species of the genus *Haloferula* were used as reference strains in most analyses. *H. harenae* KCTC 22198^T, *H. helveola* KCTC 22199^T, *H. phyci* KCTC 22200^T, *H. rosea* KCTC 22201^T, *H. luteola* KCTC 22447^T and *H. sargassicola* KCTC 22202^T were obtained from the Korean Collection for type Cultures, Taejon, Korea.

Strain CAU 1074^T was cultivated routinely on MA at 30 °C to investigate all morphological, physiological and biochemical characteristics, except for the spore formation assay and fatty acid analysis. The cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. Cell morphology was examined by transmission electron microscopy (JEM 1010; JEOL) using cells from an exponentially growing culture. Gram staining was carried out using the bioMérieux Gram staining kit according to the manufacturer's instructions. A nutrient sporulation medium (NSM) was used to induce the production of spores (Nicholson & Setlow, 1990). After 5 days of growth, spore formation was determined by staining with malachite green as described previously by Conn *et al.* (1957). Motility was checked using a hanging-drop method (Schaal, 1986). Gliding motility was assessed using the hanging-drop method.

Growth in MB at 4, 10, 30, 37, 45 and 55 °C in an aerobic incubator (model MIR-253; Sanyo) and in an anaerobic chamber (Bactron model; Sheldon) was evaluated by measuring the turbidity of the broth after 72 h. The pH range for growth at 30 °C in MB was tested in medium

Table 1. Differential properties of strain CAU 1074^T and the type strains of members of the genus *Haloferula*

Strains: 1, CAU 1074^T; 2, *H. harenae* KCTC 22198^T; 3, *H. helveola* KCTC 22199^T; 4, *H. phyci* KCTC 22200^T; 5, *H. rosea* KCTC 22201^T; 6, *H. luteola* KCTC 22447^T; 7, *H. sargassicola* KCTC 22202^T. +, Positive; −, negative; w, weakly positive. Data are from this study, except where marked.

Characteristic	1	2	3	4	5	6	7
Isolation source*	Sediment	Sediment	Seawater	Algae	Sponge	Plant	Algae
Colony colour	Yellow	Pink	Yellow	Pale orange	Pale pink	Pale yellow	Pale orange
Nitrate reduction	−	w	−	−	w	+	−
Indole production	−	w	−	−	w	+	−
Production of:							
Catalase	+	w	−	−	−	+	+
Oxidase	+	+	+	+	+	+	−
Growth temperature range	4–37	20–37	20–37	20–37	20–37	4–32	4–37
Growth at 4 °C	+	−	−	−	−	+	+
Growth at 37 °C	+	+	+	+	+	−	+
Growth pH range	6.0–9.5	6.0–9.0	6.0–8.5	6.0–9.0	6.5–9.0	6.0–9.5	6.5–9.0
NaCl tolerance (%)	15.0	5.0	6.0	5.0	5.0	4.5	7.0
NaCl requirement	−	+	+	+	+	−	+
Hydrolysis of urea	−	−	−	−	+	−	+
Utilization of:							
α-Cyclodextrin	−	−	−	−	−	−	+
Dextrin	+	+	+	−	+	+	+
Glycogen	−	−	−	−	+	+	+
Glycerol	+	+	−	−	−	−	+
Trehalose	+	+	+	−	+	+	+
Sucrose	+	+	+	−	+	+	+
Cellobiose	−	+	+	−	+	+	+
Acetic acid	−	−	−	+	−	−	+
Thymidine	−	−	−	−	+	+	+
Succinamic acid	−	−	−	+	−	−	+
Activity of:							
Esterase (C4)	+	−	−	−	+	−	−
Valine arylamidase	−	−	−	−	+	−	−
Trypsin	w	−	−	−	+	+	+
α-Galactosidase	−	+	−	+	+	−	−
β-Glucuronidase	−	−	−	−	+	−	−
N-Acetyl-β-glucosaminidase	+	+	−	−	+	−	−
α-Mannosidase	−	−	−	−	+	+	+
DNA G+C content (mol%)*	64.0	63.8	64.9	64.3	63.1	58.5	64.7

*Data taken from Yoon *et al.* (2008) and Bibi *et al.* (2011).

adjusted to pH 4.5–10.0 in increments of 0.5 pH unit by using sodium acetate/acetic acid and Na_2CO_3 buffers. Growth in the absence of NaCl and in the presence of 0–20.0 % NaCl at 1 % intervals was investigated at 30 °C in MB prepared according to the formula of the Difco medium except that NaCl was excluded and that 0.45 % (w/v) $MgCl_2 . 6H_2O$ or 0.06 % (w/v) KCl was added. Catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. Oxidase activity was tested by means of the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Cappuccino & Sherman, 2002). Hydrolysis of casein, gelatin and urea, and nitrate reduction were determined according to Lányí (1987) and Smibert & Krieg (1994). Acid production from carbohydrates, enzyme activity, physiological and biochemical features, and substrate utilization were tested using the API 20E and API ZYM systems (bioMérieux), and the GN2 MicroPlate (Biolog) according to the manufacturers' instructions. API ZYM strips were read after 6 h; API 20NE strips and GN2 MicroPlates were read after 24 h and 48 h, respectively.

For determination of fatty acid composition, the physiological age of the biomass harvested was standardized by observing growth development during incubation of the plates from quadrant streaks and choosing the moment of harvesting according to the standard protocol of the MIDI (Sherlock Microbial Identification System version 6.1). Cell mass of strain CAU 1074^T, *H. harenae* KCTC 22198^T, *H. rosea* KCTC 22201^T, *H. phyci* KCTC 22200^T and *H. helveola* KCTC 22199^T was harvested from tryptic soy agar (TSA; Difco) after cultivation for 72 h at 30 °C. Cellular fatty acid methyl esters were obtained as described previously (Minnikin *et al.*, 1980) and separated using an automated GC system (model 6890N and 7683 autosampler; Agilent). Peaks were identified by using the Microbial Identification software package (MOORE library ver. 5.0; MIDI database TSBA6). Total lipid profiles of strain CAU 1074^T and four reference strains were identified using two-dimensional TLC by the method of Minnikin *et al.* (1984). The plates were sprayed with 10 % ethanolic molybdatophosphoric acid (for total lipids), molybdenum blue (for phospholipids), ninhydrin (for aminolipids) and α-naphthol / sulphuric acid reagent (for glycolipids) (Sigma-Aldrich). The following analyses were performed on strain CAU 1074^T only. Menaquinones were analysed as described previously (Komagata & Suzuki, 1987) using reverse-phase HPLC. Polar lipids were extracted and analysed by two-dimensional TLC according to Minnikin *et al.* (1984). Peptidoglycan was analysed as described by Schleifer (1985), with the modification that a cellulose sheet was substituted for paper chromatography. Whole-cell sugars were analysed by TLC according to the method of Komagata & Suzuki (1987).

Genomic DNA of strain CAU 1074^T was extracted according to the method of Marmur (1961). PCR amplification and sequencing of the 16S rRNA gene of the strain was carried out following established procedures (Nam *et al.*, 2004). The amplified 16S rRNA gene was sequenced directly using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 3730; Applied Biosystems). Multiple alignments of the 16S rRNA sequences of CAU 1074^T with the 16S rRNA sequences from a broad selection of closely related species and calculation of sequence similarity levels were carried out by using the EzTaxon server (http://147.47.212.35:8080/) and CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distance matrices were generated by the neighbour-joining method described by Jukes & Cantor (1969). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) algorithms using the PHYLIP suite of packages (Felsenstein, 1989), and tree topology was evaluated by the bootstrap resampling method with 1000 replicates (Felsenstein, 1985) and the neighbour-joining dataset with the SEQBOOT and CONSENSE modules of the PHYLIP package. The G+C content (mol%) of the genomic DNA was determined using HPLC by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC.

The morphological, cultural, physiological and biochemical characteristics of strain CAU 1074^T are given in Table 1 and in the species description. Overall, the results obtained in this study are in agreement with previously published

Table 2. Cellular fatty acid compositions (%) of strain CAU 1074^T and the type strains of members of the genus *Haloferula*

Strains: 1, CAU 1074^T; 2, *H. harenae* KCTC 22198^T; 3, *H. helveola* KCTC 22199^T; 4, *H. phyci* KCTC 22200^T; 5, *H. rosea* KCTC 22201^T; 6, *H. luteola* KCTC 22447^T; 7, *H. sargassicola* KCTC 22202^T. −, Not detected. Data are from this study.

Fatty acid	1	2	3	4	5	6	7
Saturated							
$C_{14:0}$	6.9	−	1.1	10.2	−	7.6	4.2
$C_{15:0}$	−	6.0	7.8	13.2	2.0	−	3.2
$C_{16:0}$	11.6	8.6	9.8	32.2	4.1	33.3	21.7
Unsaturated							
$C_{13:1}$ AT 12-13	1.3	−	−	−	−	−	−
$C_{15:1}\omega 8c$	3.1	2.0	−	−	−	−	−
$C_{16:1}\omega 9c$	28.6	10.7	10.8	16.2	12.2	16.7	4.1
Branched-chain							
anteiso-$C_{15:0}$	5.3	18.3	11.6	6.7	13.6	9.4	6.1
anteiso-$C_{17:0}$	−	−	−	−	1.4	−	2.2
iso-$C_{14:0}$	35.2	33.7	45.5	20.2	37.6	40.5	36.2
iso-$C_{15:0}$	−	−	1.1	−	−	−	−
iso-$C_{16:0}$	2.4	18.5	13.2	1.3	20.9	5.8	4.7
iso-$C_{14:0}$ 3-OH	2.0	1.9	2.7	3.7	3.2	−	−
iso-$C_{16:1}$ 3-OH	−	−	−	−	1.2	−	−
Hydroxy							
$C_{15:0}$ 2-OH	−	−	−	1.8	1.2	−	−
$C_{16:0}$ 3-OH	1.3	−	−	−	−	−	−

data for the six *Haloferula* species (Bibi *et al.*, 2011; Yoon *et al.*, 2008). However, strain CAU 1074^T differed from its closest relative, *H. harenae* KCTC 22198^T and from the type species of the genus, *H. rosea* KCTC 22201^T (Yoon *et al.*, 2008) by its colony colour, catalase production, growth at 4 °C, NaCl tolerance and requirement, utilization of cellobiose and α-galactosidase activity (Table 1). The novel isolate was rod-shaped, approximately 0.4–0.6 μm in width and 0.9–2.3 μm in length (see Fig. S1 in IJSEM Online). Growth occurs optimally at 30 °C, pH 6.5 and 2 % (w/v) NaCl.

Peptidoglycan of strain CAU 1074^T contained *meso*-diaminopimelic acid. The strain had a MK-9 as the major respiratory quinone. The whole-cell hydrolysate contained glucose, xylose, mannose and ribose. The polar lipid composition comprised diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, aminoglycolipid and two unidentified phospholipids (see Fig. S2 in IJSEM Online). At present, *H. luteola* (Bibi *et al.*, 2011) is the only member of the genus *Haloferula* for which the polar lipid composition is available. An aminoglycolipid was detected in CAU 1074^T, but not found in the polar lipid composition of *H. luteola* strain YC6886^T. However, the polar lipid profile of CAU 1074^T shared with *H. luteola* strain YC6886^T, the major compound diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylglycerol, including the presence of an unidentified phospholipid that exhibited similar chromatographic behaviour. The fatty acid profile of the isolate was very similar to those of type strains of species of the genus *Haloferula*. The isolate contained branched-chain, saturated or unsaturated fatty acids (Table 2). The major fatty acids were iso-$C_{14:0}$ (35.2 %), $C_{16:1}\omega9c$ (28.6 %) and $C_{16:0}$ (11.6 %); significant amounts of $C_{14:0}$ (6.9 %), anteiso-$C_{15:0}$ (5.3 %), $C_{15:1}\omega8c$ (3.1 %), iso-$C_{16:0}$ (2.4 %), iso-$C_{14:0}$ 3-OH (2.0 %), $C_{13:1}$ AT 12-13 (1.3 %) and $C_{16:0}$ 3-OH (1.3 %) were also present.

The nearly complete 16S rRNA gene sequence of strain CAU 1074^T (1467 bp) was determined and compared with the corresponding sequences of other bacterial species in GenBank. Phylogenetic analysis indicated that the strain fell within the genus *Haloferula*. Pairwise analysis showed that the most closely related species to CAU 1074^T were *H. harenae* YM23-227^T (96.0 %), *H. rosea* 06SJR1-1^T (95.7 %),

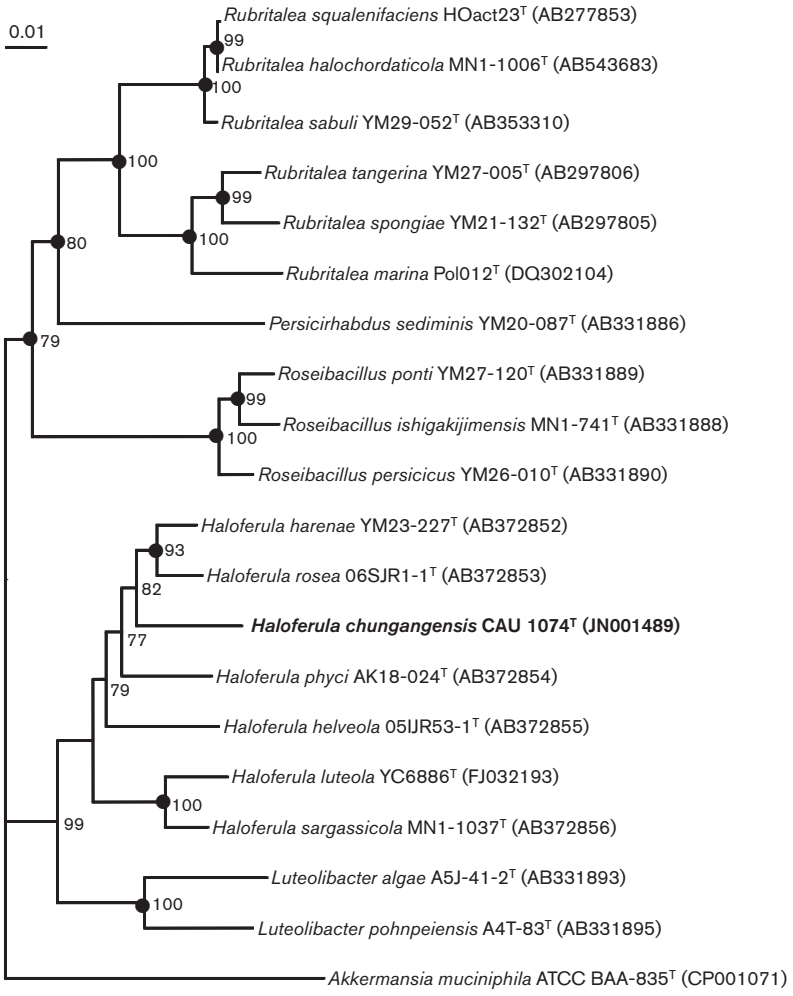

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the relationships between strain CAU 1074^T and type strains of the genus *Haloferula*. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and least-squares algorithms (see Fig. S3 in IJSEM Online). The numbers at the nodes indicate levels of bootstrap support (%) based on neighbour-joining analysis of 1000 resampled datasets; only values >70 % are given. Bar, 0.01 substitutions per nucleotide position.

H. phyci AK18-024^T (94.8 %) and *H. helveola* 05IJR53-1^T (93.4 %) (Fig. 1). The genomic DNA of the strain had a G+C content of 64.0 mol%.

These data together provide sufficient evidence to support the proposal to recognize strain CAU 1074^T as a novel species of the genus *Haloferula*, for which the name *Haloferula chungangensis* sp. nov. is proposed.

Emended description of the genus *Haloferula* Yoon *et al.* 2008 emend. Bibi *et al.*, 2011

This description is as given by Yoon *et al.* (2008) and Bibi *et al.* (2011) with the following amendments. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and aminoglycolipid; unidentified phospholipids may also occur in the type strain of the type species. The whole-cell hydrolysate contains glucose, xylose, mannose and ribose.

Description of *Haloferula chungangensis* sp. nov.

Haloferula chungangensis (chung.ang.en′sis. N.L. fem. adj. *chungangensis* of or belonging to Chung-Ang University where the taxonomic studies on this species were performed).

Cells are Gram-stain-negative, non-motile, strictly aerobic and rod-shaped, approximately 0.4–0.6 × 0.9–2.3 μm in length. Endospores are not observed. Colonies are yellow-coloured and convex with entire margins on MA after 3 days incubation at 30 °C. No colonies are formed under anaerobic growth conditions. Growth occurs at 4–37 °C (optimum, 30 °C) and at pH 6.5–9.5 (optimum, 6.5). NaCl is not required for growth but up to 15.0 % (w/v) NaCl is tolerated. Catalase and oxidase are positive. Hydrolyses urea, but not casein or gelatin. Nitrate is reduced to nitrite. Acid is produced from dextrin, L-arabinose, D-fructose, D-galactose, gentiobiose, α-D-glucose, lactose, maltose, D-mannitol, D-mannose, melibiose, D-psicose, sucrose, trehalose, turanose, pyruvic acid methyl ester, succinic acid monomethyl ester, succinic acid, glycerol (weakly), D-galacturonic acid and DL-α-glycerol phosphate. Activities of alkaline phosphatase, esterase (C4), esterase (C8), lipase (C14), trypsin, and *N*-acetyl-β-glucosaminidase are positive, but negative for leucine arylamidase, valine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-glucuronidase, β-glucosidase, α-glucosidase, α-mannosidase and α-fucosidase. The cell wall peptidoglycan contained *meso*-diaminopimelic acid. The major isoprenoid quinone is MK-9. Whole-cell hydrolysates contain mainly glucose, xylose, mannose and ribose. The polar lipids are composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, aminoglycolipid and two unidentified phospholipids. The predominant cellular fatty acids (>10 % of the total fatty acids) are $C_{14:0}$, $C_{16:1}\omega9c$ and $C_{16:0}$.

The type strain is CAU 1074^T (=KCTC 23578^T=CCUG 61920^T), isolated from a marine sediment sample collected from Jeju Island, Republic of Korea. The DNA G+C content of the type strain is 64.0 mol%.

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References

Bibi, F., Chung, E. J., Yoon, H. S., Song, G. C., Jeon, C. O. & Chung, Y. R. (2011). *Haloferula luteola* sp. nov., an endophytic bacterium isolated from the root of a halophyte, *Rosa rugosa*, and emended description of the genus *Haloferula*. *Int J Syst Evol Microbiol* **61**, 1837–1841.

Cappuccino, J. G. & Sherman, N. (2002). *Microbiology: a Laboratory Manual*, 6th edn. Menlo Park, CA: Benjamin/Cummings.

Conn, H. J., Bartholomew, J. W. & Jennison, M. W. (1957). Staining methods. In *Manual of Microbial Methods*, pp. 30–36. Edited by the Society of American Bacteriologists. New York: McGraw-Hill.

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.

Felsenstein, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.

Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279–284.

Gordon, R. E. & Mihm, J. M. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann N Y Acad Sci* **98**, 628–636.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. H. Munro. New York: Academic Press.

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

Lányí, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Minnikin, D. E., Hutchinson, I. G., Caldicott, A. B. & Goodfellow, M. (1980). Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatogr A* **188**, 221–233.

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.

Nam, S. W., Kim, W., Chun, J. & Goodfellow, M. (2004). *Tsukamurella pseudospumae* sp. nov., a novel actinomycete isolated from activated sludge foam. *Int J Syst Evol Microbiol* **54**, 1209–1212.

Nicholson, W. L. & Setlow, P. (1990). Sporulation, germination and outgrowth. In *Molecular Biological Methods for Bacillus*, pp. 391–450. Edited by C. R. Harwood & S. M. Cutting. Chichester: Wiley.

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

Schaal, K. P. (1986). Genus *Actinomyces* Harz 1877, 133^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1383–1418. Edited by

P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.

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.

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

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.

Yoon, J., Matsuo, Y., Katsuta, A., Jang, J. H., Matsuda, S., Adachi, K., Kasai, H. & Yokota, A. (2008). *Haloferula rosea* gen. nov., sp. nov., *Haloferula harenae* sp. nov., *Haloferula phyci* sp. nov., *Haloferula helveola* sp. nov. and *Haloferula sargassicola* sp. nov., five marine representatives of the family *Verrucomicrobiaceae* within the phylum '*Verrucomicrobia*'. *Int J Syst Evol Microbiol* **58**, 2491–2500.