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ORIGINAL PAPER

Halomonas sediminis sp. nov., a new halophilic bacterium isolated from salt-lake sediment in China

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Abstract A novel Gram-negative, slightly halophilic, catalase- and oxidase-positive, obligately aerobic bacterium, strain YIM-C248^T, was isolated from a sediment sample collected from a salt-lake in the Qaidam Basin in Qinghai, north-west China. Cells were non-sporulating short rods, occurring singly or as doublets, motile with peritrichous flagella. Growth occurred with 1–15% (w/v) NaCl [optimum 2–4% (w/v) NaCl], at pH 6.0–10.0 (optimum pH 7.5) and at 4–35°C (optimum 25–30°C). The major cellular fatty acids were $C_{18:1}\omega7c$, $C_{12:0}$ 3-OH, cyclo $C_{19:0}\omega8c$, $C_{16:0}$ and $C_{16:1}$. The predominant respiratory quinone was Q-9 and the genomic DNA G + C content was 58.6 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YIM-C248^T should be assigned to the genus *Halomonas*. The sequence similarities between the isolate and the type strains of members of the genus *Halomonas* were in the range of 92.5–97.5%. The combination of phylogenetic analysis, DNA–DNA hybridization data, phenotypic characteristics and chemotaxonomic differences supported the view that strain YIM-C248^T represents a new species of the genus *Halomonas*, for which the name *Halomonas sediminis* sp. nov. is proposed, with YIM-C248^T (=CCTCC AA 207031 = KCTC 22167) as the type strain.

Keywords Salt lake · Halophilic · *Halomonas sediminis* sp. nov.

Introduction

The genus *Halomonas*, belonging to the family Halomonadaceae, was first proposed by Vreeland et al. (1980) with the description of *H. elongata*. The members of this genus comprise halophilic/halotolerant, chemoorganotrophic, Gram-negative rods and are widely distributed in saline habitats (Vreeland et al. 1980; Dobson and Franzmann 1996; Mata et al. 2002; Arahal et al. 2007; Xu et al. 2007). In total, 53 species of the genus *Halomonas* have been described at the time of writing. In a recent study of the microbial diversity of the Qaidam Basin in Qinghai, north-west China (Chen et al. 2007a, 2008a, b, c), a novel *Halomonas*-like strain, designated YIM-C248^T, was isolated from a sediment sample collected from the Dachaidamu salt-lake. The lake is located at 37° 46′ N–37° 55′ N and 95° 22′ E–95° 33′ E, and the water temperature was 18°C, pH 6.4–7.8 and had a salinity of 27.4% (w/v). Based on the results of a polyphasic taxonomic study, this strain was proposed to represent a novel species, *Halomonas sediminis* sp. nov.

The GenBank/EMBL/DBBJ accession number for the 16S rRNA gene sequence of strain YIM-C248^T is EU135707.

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Materials and methods

Strains and culture conditions

Strain YIM-C248^T was isolated from a sediment sample by plating 1:10 serial dilutions of the sample on Difco marine agar 2216 (MA, pH 7.5) cultivated at 28°C for 7 days. After primary isolation and purification, the isolate was preserved both on MA slants at 4°C and in Difco marine broth 2216 (MB) supplemented with 20% (v/v) glycerol at −80°C. Unless otherwise indicated, morphological and physiological studies were performed with cells grown on MA (pH 7.5) at 28°C. The reference strain *Halomonas sulfidaeris* DSM 15722^T, which was employed as a control in phenotypic tests (including chemotaxonomic studies) as well as in studies of DNA–DNA relatedness, was obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen).

Phenotypic characterization

Cell morphology was examined by light microscopy (model BH 2; Olympus). Gram staining was carried out using the standard Gram reaction combined with the KOH lysis test method (Gregersen 1978). Flagella were stained according to the method of Leifsson (Smibert and Krieg 1981). Accumulation of poly-β-hydroxybutyrate (PHB) was determined by the Sudan Black staining method (Smibert and Krieg 1994) under a light microscope. Motility was observed as described previously (Chen et al. 2008a). Growth was tested at various temperatures (0, 4°C, and 5–45°C, using increments of 5°C) on MA and at different pH values (5.0–11.0, using increments of 0.5) in MB. Tolerance of/requirement for salts was determined in nutrient broth (NB; Difco; 3.0 g beef extract l^{-1} and 5.0 g peptone l^{-1}) supplemented with modified artificial sea water (ASW) containing (l^{-1}): 0–20% (w/v) NaCl, 5.94 g $MgSO_4 \cdot 7H_2O$, 4.53 g $MgCl_2 \cdot 6H_2O$, 0.64 g KCl and 1.3 g $CaCl_2$ (Lim et al. 2005), and on some other media as controls, i.e. MA and ISP medium 2 agar (Shirling and Gottlieb 1966). Methyl red and Voges–Proskauer tests, H_2S production from L-cysteine, hydrolysis of aesculin, indole production, nitrate and nitrite reduction were tested as recommended by Smibert and Krieg (1994). Hydrolysis of casein, cellulose, DNA, gelatin, starch, Tweens (20, 40, 60 and 80) and urease activity were determined as described by Cowan and Steel (1965). Growth under anaerobic condition, antibiotic susceptibility tests, catalase and oxidase activities were detected as described previously (Chen et al. 2007b). Other enzyme activities were tested by using the API ZYM and 20E systems (bioMérieux) according to the manufacturer's instructions. Acid production from carbohydrates and utilization of substrates as sole carbon or nitrogen sources and other phenotypic characteristics were determined as recommended by Ventosa et al. (1982) and Mata et al. (2002), as well as by employing API 50CH and 20E systems (bioMérieux) and BioLog GN2 MicroPlates (BioLog) according to the manufacturer's instructions. All suspension media were supplemented with ASW containing 2% (w/v) NaCl, and all commercial systems were incubated at 28°C.

Determination of G + C content of DNA, 16S rRNA gene sequencing and phylogenetic analysis, and DNA–DNA hybridization

Genomic DNA was isolated according to Hopwood et al. (1985) and the G + C content was determined using the HPLC method (Mesbah et al. 1989). PCR-mediated amplification of 16S rRNA gene and purification of PCR products were carried out as described previously (Cui et al. 2001). Phylogenetic analysis was performed by using the software package MEGA version 3.1 (Kumar et al. 2004) after multiple alignment of sequence data by CLUSTAL_X (Thompson et al. 1997). Distances (corrected by Kimura's two-parameter model; Kimura 1980) were calculated and clustering was performed with the neighbor-joining method (Saitou and Nei 1987). Maximum-likelihood (Felsenstein 1981) and parsimony (Kluge and Farris 1969) trees (not shown) were generated by using the treeing algorithms contained in the PHYLIP package (Felsenstein 2002). Bootstrap analysis was used to evaluate the tree topology of the neighbor-joining data by means of 1,000 resamplings (Felsenstein 1985). DNA–DNA hybridization was carried out by using photobiotin-labeled probes in microplate wells as described by Ezaki et al. (1989). A microplate spectrofluorimeter (Gemini XPS; Molecular Devices) was employed for fluorescence measurements.

Analysis of isoprenoid quinones and fatty acids

Isoprenoid quinones were analysed by HPLC as described by Groth et al. (1996). Fatty acids were determined for the new isolate as well as for the reference strain *H. sulfidaeris* DSM 15722^T as described by Sasser (1990) using the Microbial Identification System (MIDI; Microbial ID) with cells grown in MB in flasks on a rotary shaker at 200 rotations min^{-1} at 28°C for 3 days.

Results and discussion

Phenotypic characteristics

Colonies were pale-yellow pigmented, flat and non-translucent with glistening surfaces and circular/slightly

irregular margins, 2–3 mm in diameter after incubation for 3–5 days at 28°C on MA. Cells were Gram-negative, catalase- and oxidase-positive, non-sporulating, obligately aerobic rods, approximately 0.8–1.5 μm wide and 2.0–3.5 μm long, motile with peritrichous flagella. This strain was slightly halophilic, with growth occurring at 1–15% (v/v) NaCl [optimum 2–4% (v/v) NaCl] (Kushner 1993). The results of other phenotypic tests were listed in the species description and in Table 1.

DNA G + C content, phylogenetic analysis based on 16S rRNA gene sequence comparison and DNA–DNA relatedness

The DNA G + C content of strain YIM-C248^T was 58.6 mol%. The almost-complete 16S rRNA gene sequence (1,479 bp; EU135707) was determined. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM-C248^T was closely related to the type strains of members of the genus *Halomonas*. The 16S rRNA gene sequence similarities between the isolate and the type strains of members of the genus *Halomonas* were in the range of 92.5–97.5%. Phylogenetically, strain YIM-C248^T was most closely related to the type strain of *H. sulfidaeris* (sequence similarity 97.5%), followed by the type strains of *H. variabilis* (96.6%), *H. boliviensis* (96.4%) and *H. neptunia* (96.3%), but strain YIM-C248^T occupied a distinctly separated branch in the phylogenetic tree (three methods, Fig. 1). To establish the precise taxonomic position of strain YIM-C248^T, DNA–DNA hybridizations were performed between the novel isolate and the type strain of *H. sulfidaeris*, and the level of DNA–DNA relatedness between them was 10.6%, which was far below the threshold value of about 70% recommended by Wayne et al. (1987) for assigning strains to the same species. It is therefore evident based on the results of phylogenetic analysis and DNA–DNA hybridization that strain YIM-C248^T represents a previously unknown species of the genus *Halomonas* (Wayne et al. 1987; Stackebrandt and Goebel 1994).

Chemotaxonomic characteristics

Chemotaxonomic data for strain YIM-C248^T are compatible with its assignment to the genus *Halomonas*. The strain contained Q-9 (86.7%) as the predominant respiratory quinone, with Q-7 (8.0%), Q-6 (2.0%), Q-10 (1.7%) and Q-8 (1.1%) present in minor amounts. The fatty acid profile of strain YIM-C248^T was similar to that of the type strain of *H. sulfidaeris* (Table 2). The major fatty acids of this strain were $C_{18:1}\omega7c$, $C_{12:0}$ 3-OH, cyclo $C_{19:0}\omega8c$, $C_{16:0}$ and $C_{16:1}$.

Taxonomic conclusion

The results of phylogenetic analysis and chemotaxonomic studies supported the view that strain YIM-C248^T should be assigned to the genus *Halomonas*. However, the pale-yellow pigmentation and the comparatively narrow salt concentration range for growth, differentiated the new isolate markedly from recognized *Halomonas* species (Table 1). Strain YIM-C248^T could also be distinguished obviously from its closest phylogenetic neighbor *H. sulfidaeris* by their discriminative fatty acid profiles, in which there were significant amounts of cyclo $C_{19:0}\omega8c$ and cyclo $C_{17:0}$, whereas the amount of $C_{18:1}\omega7c$ was noticeably reduced in the fatty acid pool of strain YIM-C248^T, and four fatty acids, i.e. anteiso $C_{15:0}$, iso $C_{16:0}$, 11-methyl $C_{18:1}\omega7c$ and $C_{20:2}\omega6,9c$ were only detected for the new isolate (Table 2). Moreover, strain YIM-C248^T could be distinguished clearly from its closest phylogenetic neighbor by some other discriminative taxonomic markers, such as its ability to produce acid from L-arabinose and to utilize citrate, D-glucose, L-alanine and L-serine as sole carbon sources (Table 1), together with its inability to respire on nitrate or nitrite in anaerobiosis and the low level of DNA–DNA relatedness between them. Overall, the polyphasic taxonamic results presented above allowed us to assign the novel isolate to a new species, for which we propose the name *Halomonas sediminis* sp. nov.

Description of *Halomonas sediminis*

Halomonas sediminis (se.di'mi.nis. L. gen. n. *sediminis* of sediment). Cells are Gram-negative rods, approximately 0.8–1.5 μm wide and 2.0–3.5 μm long, occurring singly or as doublets, motile with peritrichous flagella. Devoid of endospores. Strictly aerobic, catalase- and oxidase-positive. Cells accumulate poly-β-hydroxybutyrate but do not produce exopolysaccharide. Colonies are pale-yellow pigmented, flat and non-translucent with glistening surfaces and circular/slightly irregular margins, 2–3 mm in diameter after incubation for 3–5 days at 28°C on Difco marine agar 2216. Slightly halophilic, growth occurs with 1–15% (v/v) NaCl [optimum 2–4% (v/v) NaCl]. Growth happens at pH 6.0–10.0 and at 4–35°C, with an optimum growth at pH 7.5 and 25–30°C. Under aerobic conditions, nitrate and nitrite are reduced. Negative for respiration on nitrate, nitrite or fumarate in anaerobiosis. Tweens 40 and 60 are hydrolyzed, but aesculin, casein, cellulose (carboxymethylcellulose and filter paper), DNA, gelatin, starch, Tweens 20 and 80 are not. H_2S is not produced from L-cysteine. Indole and urease are not produced. Methyl red and Voges–Proskauer tests are negative. Acid is produced from L-arabinose, gentiobiose, glycogen, D-glucose and D-turanose, but not from *N*-acetylglucosamine, D-adonitol, amygdaline, D-arabinose,

Table 1 Differentiating characteristics of strain YIM-C248^T from its closest phylogenetic relatives

Characteristics	1	2	3	4	5	6	7	8	9
Colony pigmentation	Pale yellow	Cream	Cream	Cream	Cream	Cream	Cream	White	Cream-beige
Exopolysaccharide	−	−[a]	ND	ND	−	ND	ND	ND	ND
Poly-β-hydroxybutyrate	+	+[a]	ND	ND	+	ND	ND	ND	ND
Oxidase	+	+	+	ND	+	−	−	−	−
Optimum temperature (°C)	25–30	20–35	30	25–30	32	30	30	25–30	25–30
Temperature range (°C)	4–35	−1 to 35	−1 to 35	0–45	15–37	4–48	4–48	5–45	5–45
pH range	6–10	5–10	5–12	6–11	6–9	6–10	6–10	6–10	6–10
Optimum salts (%, w/v)	2–4	2–3	2–3	5	10	1–5	1–5	10–15	8–12
Salt range (%, w/v)	1–15	0.5–24	0.5–27	0–25	1–25	0–20	0–15	1–20	1–20
Nitrate reduction	+	+	+	+	−	ND	ND	−	−
Nitrite reduction	+	−	−	ND	−	ND	ND	−	−
Respiration on nitrate	−	+	+	ND	−	ND	ND	−	−
Facultative anaerobic	−	+	+	−	−	−	−	−	−
H_2S production	−	−	−	−	+	+	+	−	−
Phenylalanine deaminase	−	+[a]	+[b]	ND	−	+	−	ND	ND
Hydrolysis of casein	−	+	−	ND	−	+	+	−	−
Acid production from									
L-Arabinose	+	−	−	ND	−	+	+	+	+
D-Galactose	−	+	+	ND	−	+	+	+	+
Lactose	−	+	−	ND	−	ND	ND	−	−
D-Maltose	−	+	−	ND	−	+	+	+	+
D-Xylose	−	+	−	ND	ND	ND	ND	−	+
Inositol	−	+[b]	ND	ND	ND	−	+	−	+
Utilization of									
D-Cellobiose	−	−	+	−	+	−	−	ND	ND
Citrate	+	−	+	−	+	+	+	+	+
D-Glucose	+	−	+	+	+	ND	ND	+	+
Glycerol	−	−	+	−	+	+	+	+	+
D-Mannose	−	−	+	+	+	−	−	−	+
L-Alanine	+	−	+	ND	+	+	+	+	+
L-Serine	+	−	−	ND	+	ND	ND	−	−
DNA G + C content (mol%)	58.6	56.0	57.3	51.4	61	56.7	57.6	61.0	62.0

Strains: *1 Halomonas sediminis* YIM-C248^T (data from this study), *2 H. sulfidaeris* Esulfide1^T (Kaye et al. 2004), *3 H. neptunia* Eplume1^T (Kaye et al. 2004), *4 H. boliviensis* LC1^T (Quillaguamán et al. 2004), *5 H. variabilis* DSM 3051^T (Fendrich 1988; Mata et al. 2002), *6 H. arcis* AJ282^T (Xu et al. 2007), *7 H. subterranea* ZG16^T (Xu et al. 2007), *8 H. janggokensis* M24^T (Kim et al. 2007), *9 H. gomseomensis* M12^T (Kim et al. 2007)

+ Positive, − negative

ND no data available

[a] Data from our study

[b] Data from Xu et al. (2007)

D-arabitol, L-arabitol, arbutine, D-cellobiose, dulcitol, erythritol, esculin, D-fructose, D-fucose, L-fucose, D-galactose, glycerol, inositol, inulin, D-lactose, D-lyxose, D-maltose, D-mannitol, D-mannose, D-melezitose, D-melibiose, methyl-α-D-glucopyranoside, methyl-α-D-mannopyranoside, methyl-β-D-xylopyranoside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, D-raffinose, L-rhamnose, D-ribose, sucrose, salicin, D-sorbitol, L-sorbose, starch, D-tagatose, D-trehalose, xylitol, D-xylose or L-xylose. The following substances are utilized as sole carbon and energy sources: acetate, citrate, dextrin, glycogen, D-gluconic acid, D-glucose, gentiobiose,

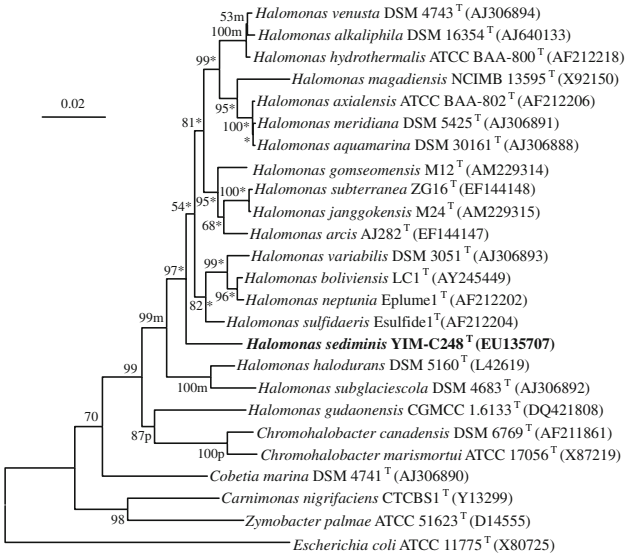

Fig. 1 Phylogenetic tree based on 16S rRNA gene sequence analysis and constructed using the neighbor-joining method showing the phylogenetic positions of strains YIM-C248^T and related taxa. The *m* or *p* labels indicate branches that were also found with the maximum-likelihood (Felsenstein 1981) or parsimony (Kluge and Farris 1969) algorithms, respectively; *asterisks* indicate branches that were recovered with all the three methods. *Numbers at nodes* indicate bootstrap values (>50%) based on neighbor-joining analysis of 1,000 re-sampled datasets. *Bar* 2 substitutions per 100 nucleotides

Table 2 Fatty acid composition of *Halomonas sediminis* YIM-C248^T and *Halomonas sulfidaeris* DSM 15722^T

Fatty acid (%)[a]	*H. sediminis* YIM-C248^T	*H. sulfidaeris* DSM 15722^T
10:0	5.4	2.0
10:0 3-OH	2.7	0.3
12:0	1.0	1.3
12:0 3-OH	16.8	15.0
15:0 anteiso	2.0	–
16:0 iso	1.0	–
16:1[b]	6.9	9.5
16:0	8.1	8.3
17:0 cyclo	4.5	0.3
18:1ω7*c*	29.1	59.2
18:1 ω7*c* 11-methyl	2.1	–
19:0 cyclo ω8*c*	15.7	0.9
20:2 ω6, 9*c*	1.8	–

– Not detected

[a] Fatty acids that represented <1.0% in the two strains have been omitted

[b] Double bond positions in $C_{16:1}$ fatty acids were either ω6*c* or ω7*c*

myo-inositol, itaconic acid, α-keto butyric acid, α-keto glutaric acid, D-melibiose, β-methyl-D-glucoside, D-psicose, D-sorbitol, succinamic acid, sucrose, L-threonine, D-turanose, xylitol and Tween 40, and the following substances are utilized as sole carbon, nitrogen and energy sources: D-alanine, L-alanine, L-aspartic acid, L-proline and L-serine. The following substances are not utilized: adonitol, aesculin, L-arabinose, D-cellobiose, ethanol, formate, D-fructose, fumarate, D-galactose, gluconate, glycerol, lactose, malonate, maltose, D-mannitol, D-mannose, D-melezitose, propionate, D-raffinose, L-rhamnose, D-ribose, salicin, starch, succinate, trehalose, D-xylose, L-cysteine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-ornithine, L-phenylalanine or L-valine. Constitutive enzymes expressed are alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and valine arylamidase, but acid phosphatase, arginine dihydrolase, α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, gelatinase, *N*-acetyl-β-glucosaminidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase (C14), lysine decarboxylase, α-mannosidase, *o*-nitrophenyl-β-D-galactopyranosidase, ornithine decarboxylase, phenylalanine deaminase, trypsin or tryptophane deaminase are not observed. Cells are susceptible to ampicillin (30 μg), carbenicillin (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), nalidixic acid (20 μg), nitrofurantion (30 μg), polymixin B (30 μg), rifampicin (5 μg), streptomycin (10 μg) and tetracycline (30 μg), but not to novobiocin (30 μg) or tobramycin (10 μg). The major fatty acids are $C_{18:1}\omega7c$, $C_{12:0}$ 3-OH, cyclo $C_{19:0}\omega8c$, $C_{16:0}$ and $C_{16:1}$. The predominant respiratory quinone is Q-9 and the DNA G + C content is 58.6 mol% (HPLC method).

The type strain YIM-C248^T was isolated from a sediment sample collected from the Dachaidamu salt-lake in the Qaidam Basin in Qinghai, north-west China. Strain YIM-C248^T has been deposited in the KCTC (Korean Collection for Type Cultures) as strain KCTC 22167 and the China Center for Type Culture Collection, Wuhan, China as strain CCTCC AA 207031.

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References

Arahal DR, Vreeland RH, Litchfield CD, Mormile MR, Tindall BJ, Oren A, Bejar V, Quesada E, Ventosa A (2007) Recommended minimal standards for describing new taxa of the family Halomonadaceae. Int J Syst Evol Microbiol 57:2436–2446

Chen YG, Jiang Y, Li WJ, Cui XL, Xu LH (2007a) Microbial diversity and screening of antitumor activity of actinomycete strains from saline and alkaline environments in the Qinghai Province, PR China. Acta Microbiol Sin 47:757–762

Chen YG, Cui XL, Pukall R, Li HM, Yang YL, Xu LH, Wen ML, Peng Q, Jiang CL (2007b) *Salinicoccus kunmingensis* sp. nov., a moderately halophilic bacterium isolated from a salt mine in Yunnan, south-west China. Int J Syst Evol Microbiol 57:2327–2332

Chen YG, Cui XL, Fritze D, Chai LH, Schumann P, Wen ML, Wang YX, Xu LH, Jiang CL (2008a) *Virgibacillus kekensis* sp. nov., a moderately halophilic bacterium isolated from a salt lake in China. Int J Syst Evol Microbiol 58:647–653

Chen YG, Cui XL, Kroppenstedt RM, Stackebrandt E, Wen ML, Xu LH, Jiang CL (2008b) *Nocardiopsis quinghaiensis* sp. nov., isolated from saline soil in China. Int J Syst Evol Microbiol 58:699–705

Chen YG, Cui XL, Li WJ, Xu LH, Wen ML, Peng Q, Jiang CL (2008c) *Salinicoccus salitudinis* sp. nov., a new moderately halophilic bacterium isolated from a saline soil sample. Extremophiles 12:197–203

Cowan ST, Steel KJ (1965) Manual for the identification of medical bacteria. Cambridge University Press, London

Cui XL, Mao PH, Zeng M, Li WJ, Zhang LP, Xu LH, Jiang CL (2001) *Streptomonospora salina* gen. nov., sp. nov., a new member of the family Nocardiopsaceae. Int J Syst Evol Microbiol 51:357–363

Dobson SJ, Franzmann PD (1996) Unification of the genera *Deleya* (Baumann et al. 1983), *Halomonas* (Vreeland et al. 1980), and *Halovibrio* (Fendrich 1988) and the species *Paracoccus halodenitrificans* (Robinson and Gibbons 1952) into a single genus, *Halomonas*, and placement of the genus *Zymobacter* in the family Halomonadaceae. Int J Syst Bacteriol 46:550–558

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

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 (2002) PHYLIP (Phylogeny Inference Package), version 3.6a. Distributed by the auther. Department of Genome Science, University of Washington, Seattle

Fendrich C (1988) *Halovibrio variabilis* gen. nov. sp. nov., *Pseudomonas halophila* sp. nov. and a new halophilic aerobic coccoid Eubacterium from Great Salt Lake, Utah, USA. Syst Appl Microbiol 11:36–43

Gregersen T (1978) Rapid method for distinction of Gram-negative from Gram-positive bacteria. Eur J Appl Microbiol Biotechnol 5:123–127

Groth I, Schumann P, Weiss N, Martin K, Rainey FA (1996) *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. Int J Syst Bacteriol 46:234–239

Hopwood DA, Bibb MJ, Chater KF, Kieser T, Bruton CJ, Kieser HM, Lydiate DJ, Smith CP, Ward JM (1985) Preparation of chromosomal, plasmid and phage DNA. In: Hopwood DA, Bibb MJ, Chater KF, Kieser T, Bruton CJ, Kieser HM, Lydiate DJ, Smith CP, Ward JM, Schrempf H (eds) Genetic manipulation of *Streptomyces*: a laboratory manual. F. Crowe and Sons, Norwich, pp 79–80

Kaye JZ, Márquez MC, Ventosa A, Baross JA (2004) *Halomonas neptunia* sp. nov., *Halomonas sulfidaeris* sp. nov., *Halomonas axialensis* sp. nov. and *Halomonas hydrothermalis* sp. nov.: halophilic bacteria isolated from deep-sea hydrothermal-vent environments. Int J Syst Evol Microbiol 54:499–511

Kim KK, Jin L, Yang HC, Lee ST (2007) *Halomonas gomseomensis* sp. nov., *Halomonas janggokensis* sp. nov., *Halomonas salaria* sp. nov. and *Halomonas denitrificans* sp. nov., moderately halophilic bacteria isolated from saline water. Int J Syst Evol Microbiol 57:675–681

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kluge AG, Farris FS (1969) Quantitative phyletics and the evolution of anurans. Syst Zool 18:1–32

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163

Kushner DJ (1993) Growth and nutrition of halophilic bacteria. In: Vreeland RH, Hochstein LI (eds) The biology of halophilic bacteria. CRC, Boca Raton, pp 87–103

Lim JM, Jeon CO, Song SM, Kim CJ (2005) *Pontibacillus chungwhensis* gen. nov., sp. nov., a moderately halophilic Gram-positive bacterium from a solar saltern in Korea. Int J Syst Evol Microbiol 55:165–170

Mata JA, Martínez-Cánovas MJ, Quesada E, Bèjar V (2002) A detailed phenotypic characterization of the type strains of *Halomonas* species. Syst Appl Microbiol 25:360–375

Mesbah M, Premachandran U, Whitman WB (1989) Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. Int J Syst Bacteriol 39:159–167

Quillaguamán J, Hatti-Kaul R, Mattiasson B, Alvarez MT, Delgado O (2004) *Halomonas boliviensis* sp. nov., an alkalitolerant, moderate halophile isolated from soil around a Bolivian hypersaline lake. Int J Syst Evol Microbiol 54:721–725

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

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. USFCC Newsl 20:16

Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340

Smibert RM, Krieg NR (1981) General characterization. In: Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, Krieg NR, Phillips GB (eds) Manual of methods for general microbiology. American Society for Microbiology, Washington, pp 409–443

Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. American Society for Microbiology, Washington, pp 607–654

Stackebrandt E, Goebel BM (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

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Ventosa A, Quesada E, Rodriguez-Valera F, Ruiz-Berraquero F, Ramos-Cormenzana A (1982) Numerical taxonomy of moderately halophilic Gram-negative rods. J Gen Microbiol 128:1959–1968

Vreeland RH, Litchfield CD, Martin EL, Elliot E (1980) *Halomonas elongata*, a new genus and species of extremely salt tolerant bacteria. Int J Syst Bacteriol 30:485–495

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE et al

(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

Xu XW, Wu YH, Zhou Z, Wang CS, Zhou YG, Zhang HB, Wang Y, Wu M (2007) *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. Int J Syst Bacteriol 57:1619–1624