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Halomonas marisflavae sp. nov., a halophilic bacterium isolated from the Yellow Sea in Korea

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A halophilic Gram-negative bacterial strain, SW32^T, which was isolated from a sample from the Yellow Sea of Korea, was subjected to a polyphasic taxonomic study. This organism grew optimally in the presence of 0·5–12% NaCl. On the basis of phenotypic and phylogenetic data, strain SW32^T appeared to be a member of the genus *Halomonas*. Strain SW32^T formed a distinct evolutionary lineage within the phylogenetic clade comprising *Halomonas* species and the genera *Zymobacter* and *Carnimonas*. The 16S rDNA sequence of strain SW32^T contains 19 signature characteristics of the genus *Halomonas* and the family *Halomonadaceae*. Strain SW32^T possessed a single polar flagellum, ubiquinone-9 as the predominant respiratory lipoquinone and $C_{18:1}$, $C_{16:0}$ and $C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2OH as the major fatty acids. The DNA G+C content was 59 mol%. Levels of 16S rDNA similarity between strain SW32^T and the type strains of all validly described *Halomonas* species were 92·0–93·8%. Strain SW32^T exhibited 16S rDNA similarity values of 92·7% to *Zymobacter palmae* IAM 14233^T and 91·6% to *Carnimonas nigrificans* CECT 4437^T. These data indicate that strain SW32^T was related enough to members of the genus *Halomonas* to be placed as a new species within that genus. Therefore the name *Halomonas marisflavae* sp. nov. is proposed for strain SW32^T. The type strain of the new species is strain SW32^T (= KCCM 80003^T = JCM 10873^T).

Keywords: *Halomonas marisflavae* sp. nov., polyphasic taxonomy, marine bacterium

INTRODUCTION

The family *Halomonadaceae* was originally created by Franzmann *et al*. (1989) to accommodate the genera *Halomonas* (Vreeland *et al*., 1980) and *Deleya* (Baumann *et al*., 1983). However, *Halomonas* and *Deleya*, together with the genus *Halovibrio* (Fendrich, 1988), lacked differential phenotypic, particularly chemotaxonomic, characters (Franzmann & Tindall, 1990; Skerratt *et al*., 1991). Phylogenetic analyses based on 16S rRNA gene sequences showed that the members belonging to the three genera form a monophyletic group (Dobson *et al*., 1993) and *Volcaniella eurihalina* is closely related to *Halomonas elongata*, the type species of the genus *Halomonas* (Mellado *et al*., 1995). Accordingly, the genera *Halomonas*, *Deleya* and *Halovibrio* and the species *Paracoccus halodenitrificans* were recently unified into a single genus *Halomonas* (Dobson & Franzmann, 1996) and *Volcaniella eurihalina* was transferred to the genus *Halomonas* as *Halomonas eurihalina* (Mellado *et al*., 1995).

The genus *Zymobacter*, which was proposed by Okamoto *et al*. (1993), has been included in the family *Halomonadaceae* (Dobson & Franzmann, 1996). The genus *Chromohalobacter*, with only a single species, *Chromohalobacter marismortui* (Ventosa *et al*., 1989), falls within the phylogenetic radiation occupied by *Halomonas* species, although Mellado *et al*. (1995) concluded that there are enough phenotypic differences to warrant classification of *Chromo-*

Abbreviations: FAME, fatty acid methyl ester; MA, marine agar; TSA(B), trypticase soy agar (broth).

The GenBank accession number for the 16S rDNA sequence of strain SW32^T is AF251143.

halobacter as a genus separate from the *Halomonas/Deleya* complex. A new genus, *Carnimonas*, which contains only a single species, *Carnimonas nigrificans*, and forms a monophyletic group with *Zymobacter palmae*, has been described (Garriga *et al*., 1998). Despite its chemotaxonomic and physiological similarities, the authors did not include the genus *Carnimonas* within the family *Halomonadaceae* because it lacked 2 out of 15 descriptive signature characteristics in its 16S rRNA sequence (Garriga *et al*., 1998). Thus, the family *Halomonadaceae* currently possesses three genera; *Halomonas* (Vreeland *et al*., 1980; Dobson & Franzmann, 1996), *Chromohalobacter* (Ventosa *et al*., 1989) and *Zymobacter* (Okamoto *et al*., 1993).

Members of the family *Halomonadaceae* are Gram-negative, either slight or moderate halophiles with straight or curved, rod-shaped cells. The family *Halomonadaceae* is characterized by having ubiquinone-9 as the predominant respiratory lipoquinone and $C_{18:1}$ plus cyclo $C_{19:0}$ and $C_{16:0}$ as the major fatty acids. This family is a member of the γ-subclass of the class *Proteobacteria*.

Recently, a halophilic Gram-negative bacterial organism, strain SW32, was isolated from a water sample of the Yellow Sea, Korea. The 16S rDNA sequence analysis indicated that this organism lies at the periphery of the phyletic radiation encompassed by the genus *Halomonas* and is phylogenetically related to the genera *Zymobacter* and *Carnimonas*. This observation suggested that other useful taxonomic data were also required for reliable classification of strain SW32^T. Accordingly, the aim of the present study was to unravel the taxonomic status of strain SW32^T with a combination of phenotypic characters and detailed phylogenetic analysis.

METHODS

Bacterial strain and cultural conditions. Strain SW32^T was isolated from a water sample of the Yellow Sea in Boryung City, Korea, by the dilution plating technique on marine agar (MA) (Difco). For the investigation of morphological and physiological characteristics, strain SW32^T was cultivated on MA and broth (Difco) and trypticase soy agar (TSA) and broth (TSB) (BBL) at 28 °C. Cell biomass for respiratory lipoquinone analysis and DNA extraction was obtained from a marine broth culture. Strain SW32^T was cultivated at 28 °C on a horizontal shaker at 150 r.p.m. and the broth culture was checked for purity microscopically before being harvested by centrifugation. For fatty acid methyl ester (FAME) analysis, cell mass of strain SW32^T was obtained from agar plates after growing for 3 d at 28 °C on MA and TSA.

Morphological and physiological characterization. The morphology of cells was examined by phase-contrast microscopy and transmission electron microscopy (TEM). The presence or absence of flagella was examined using TEM with cells from an exponentially growing culture. The cells were negatively stained with 1 % (w/v) phosphotungstic acid and, after air-drying, grids were examined with a model CM-20 transmission electron microscope (Philips). Gram reaction was determined using the bioMérieux Gram Strain kit according to the manufacturer's instructions. Catalase activity was determined by bubble formation in a 3 % hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1 % tetramethyl-*p*-phenylenediamine. Nitrate reduction and hydrolysis of aesculin were determined as described by Lanyi (1987). Hydrolysis of casein, gelatin, hypoxanthine, starch, Tween 80, tyrosine and xanthine, and production of urease were determined as described by Cowan & Steel (1965). Acid production from carbohydrates was determined by using the API 50CH system (bioMérieux). Other physiological tests were performed with the API 20NE system (bioMérieux). Growth under anaerobic conditions was determined after incubation in an anaerobic chamber with MA that was prepared anaerobically. Tolerance of NaCl was measured in TSB containing no NaCl or 0·5–30 % (w/v) NaCl. Growth at various temperatures was measured on MA.

Chemotaxonomic characterization. The presence or absence of diaminopimelic acid in the peptidoglycan was determined as described by Komagata & Suzuki (1987). Respiratory lipoquinones were extracted and purified as described by Komagata & Suzuki (1987). The purified ubiquinones were dissolved in acetone and separated by reverse-phase HPLC. For quantitative analysis of cellular fatty acid compositions, a loop of cell mass was harvested and FAMEs were prepared and identified following the instructions of the Microbial Identification System (MIDI).

Determination of G+C content. Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al*., 1996) with the exception that ribonuclease T1 was used together with ribonuclease A. The G+C content was determined by the method of Tamaoka & Komagata (1984). DNA was hydrolysed and the resultant nucleotides were analysed by reverse-phase HPLC.

16S rDNA sequencing and phylogenetic analysis. 16S rDNA was amplified by PCR using the two universal primers as described previously (Yoon *et al*., 1998). The PCR product was purified with a QIAquick PCR purification kit (Qiagen) and the 16S rDNA was sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). The purified sequencing reaction mixtures were automatically electrophoresed using an Applied Biosystems model 310 automatic DNA sequencer. Alignment of sequences was carried out with CLUSTAL W software (Thompson *et al*., 1994). Gaps at the 5′ and 3′ ends of the alignment were omitted from further analyses. Phylogenetic trees were inferred by using three tree-making algorithms, neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods in the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining method were calculated with the algorithm of Jukes & Cantor (1969) with the DNADIST program. The stability of relationships was assessed by a bootstrap analysis of 1000 data sets by using the programs SEQBOOT and CONSENSE of the PHYLIP package.

RESULTS

Morphology

Strain SW32^T is a Gram-negative rod or oval. Cells are 0·9–1·3 μm wide and 1·7–2·3 μm long after 3 d of growth on MA at 28 °C. Cells are motile by means of

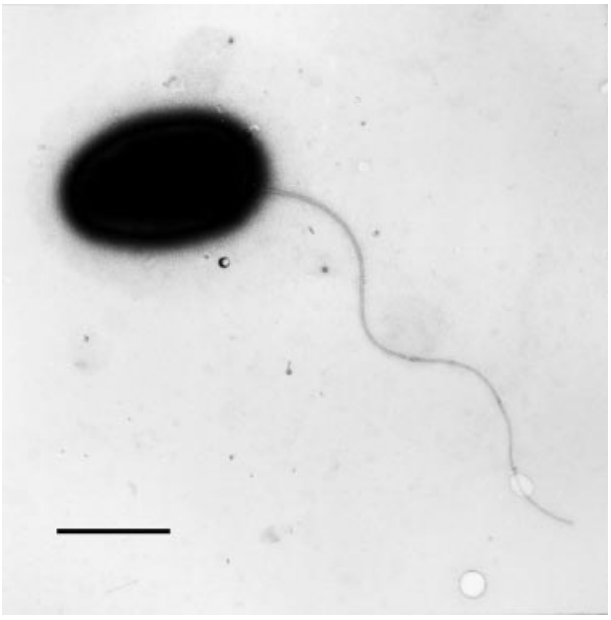

Fig. 1. Transmission electron micrograph of strain SW32^T from an exponentially growing culture. Bar, 1 μm.

a single polar flagellum (Fig. 1). No spores were observed by phase-contrast microscopy. On TSA and MA, colonies appear yellow-orange, smooth, glistening and circular/slightly irregular. Colonies are convex on TSA and flat/slightly convex on MA.

Cultural and physiological characteristics

Strain SW32^T grew well in TSA and nutrient agar as well as MA. Strain SW32^T grew at 4–37 °C with an optimum temperature of 25–30 °C, but no growth occurred at temperatures above 39 °C. Optimum pH for the growth was pH 7·0–8·0 and growth was inhibited at pH values below 4·5. Strain SW32^T grew optimally in the presence of 0·5–12 % (w/v) NaCl. It grew in the presence of 27 % NaCl. Growth did not occur in the presence of 0 % NaCl and more than 28 % NaCl. Strain SW32^T grew under anaerobic conditions on MA. Strain SW32^T had catalase activity but no oxidase and urease activities. Aesculin and gelatin were hydrolysed, but no hydrolysis of casein, hypoxanthine, starch, Tween 80, tyrosine and xanthine was observed. Nitrate was not reduced to nitrite. Arginine was not deaminated and indole was not produced. The following substrates were assimilated: glucose, arabinose, mannose, mannitol, gluconate, malate and citrate. Acids were produced from glycerol, erythritol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, gentiobiose, D-turanose, D-lyxose and D-fucose and weakly produced from adonitol, mannitol and xylitol.

Chemotaxonomic characteristics and DNA base composition

Strain SW32^T did not contain any diaminopimelic acid as the diagnostic diamino acid in the cell wall peptidoglycan. The predominant respiratory lipoquinone found in strain SW32^T was ubiquinone with nine isoprene units (Q-9) (about 95 %). The fatty acid profile of strain SW32^T was similar on both TSA and MA and contained major amounts of saturated and unsaturated fatty acids (Table 1). The major fatty acids were $C_{18:1}$, $C_{16:0}$ and $C_{16:1}$ $\omega7c$ and/or iso-$C_{15:0}$ 2OH (Table 1). The DNA G+C content of strain SW32^T, determined by HPLC, was 59 mol%.

Phylogenetic analysis

An almost complete 16S rDNA sequence of 1495 nt (approx. 96 % of the *Escherichia coli* sequence) was determined for strain SW32^T. This sequence contained four signature nucleotides defined for the genus *Halomonas* and 15 signature nucleotides associated with the family *Halomonadaceae* as described by Dobson & Franzmann (1996) (Table 2). The phylogenetic analyses showed that strain SW32^T falls within the radiation of the cluster comprising the members of the family *Halomonadaceae* and the genus *Carnimonas*, and forms a distinct evolutionary lineage at the periphery of the phyletic radiation occupied by *Halomonas* species (Fig. 2). In the tree based on the neighbour-joining algorithm, strain SW32^T clustered to the clade comprising *Halomonas* species with a bootstrap resampling value of 68·2 %, and the relationship between this cluster and the cluster comprising the genera *Zymobacter* and *Carnimonas* was supported by bootstrap analysis at a confidence level

Table 1. Cellular fatty acid profiles of strain SW32^T on TSA and MA

Medium	Fatty acid composition (%)										
	$C_{10:0}$	$C_{12:0}$	$C_{12:0}$ 2OH	$C_{12:0}$ 3OH	$C_{14:0}$	$C_{16:0}$	$C_{17:0}$ cyclo	$C_{18:0}$	$C_{18:1}$*	$C_{19:0}$ cyclo $\omega8c$	Summed feature 4†
TSA	1·1	2·2	1·9	9·1	0·9	28·0	–	1·1	42·5	0·8	12·4
MA	–	–	1·4	6·7	–	31·6	2·2	–	42·3	4·6	11·3

* Unresolved mixture of $C_{18:1}$ $\omega7c$, $C_{18:1}$ $\omega9t$ and $C_{18:1}$ $\omega12t$.

† Summed feature represents $C_{16:1}$ $\omega7c$ and/or iso-$C_{15:0}$ 2OH, which could not be separated by GLC with the MIDI system.

Table 2. 16S rDNA signature nucleotides characteristic of the family *Halomonadaceae* and the genus *Halomonas*

Data of the genera *Halomonas* and *Zymobacter* are from Dobson & Franzmann (1996) and data for the genus *Carnimonas* are from Garriga *et al*. (1998). The large box shows the signature that defines the family *Halomonadaceae* and the small box shows the signature that defines the genus *Halomonas*. Positions are according to the *Escherichia coli* 16S rRNA numbering system.

Position	Strain SW32^T	*Halomonas*	*Zymobacter*	*Carnimonas*
76–93	6 bp stem	6 bp stem	6 bp stem	6 bp stem
484	A	A	A	G
486	C	C	C	U
640	G	G	G	G
660	A	A	A	A
668	A	A	A	A
669	A	A	A	A
737	U	U	U	U
738	U	U	U	U
745	U	U	U	U
776	U	U	U	U
1124	U	U	U	U
1297	U	U	U	U
1298	C	C	C	C
1423	A	A	A	A
1424	C	C	U	U
1439	U	U	C	C
1462	A	A	C	C
1464	C	C	U	C

of 99·4 % (Fig. 2). The phylogenetic position of strain SW32^T was also confirmed in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. The 16S rDNA similarity values between strain SW32^T and the type strains of all validly described *Halomonas* species were in the range of 92·0–93·8 %. Strain SW32^T exhibited sequence similarity values of 92·3 and 92·7 % to *Chromohalobacter marismortui* ATCC 17056^T and *Zymobacter palmae* IAM 14233^T, respectively. The sequence similarity value between strain SW32^T and *Carnimonas nigrificans* CECT 4437^T was 91·6 %.

DISCUSSION

The phylogenetic inference based on 16S rDNA sequences shows that strain SW32^T forms an intermediate evolutionary lineage between one cluster containing *Halomonas* species and the other cluster consisting of the genera *Zymobacter* and *Carnimonas* (Fig. 2). However, its 16S rDNA sequence contains 4 signature nucleotides that define the genus *Halomonas* as well as 15 signature nucleotides characteristic of the family *Halomonadaceae* (Table 2). The results obtained in phenotypic and chemotaxonomic analyses make it possible to assign strain SW32^T to the genus *Halomonas*. The members of the family *Halomonadaceae* and the genus *Carnimonas* show no differences in their predominant respiratory lipoquinone, which is ubiquinone-9. While the major elements of their cellular fatty acid profiles are similar (Garriga *et al*., 1998; Franzmann & Tindall, 1990; Okamoto *et al*., 1993), the three genera for which FAME analysis is available also have noteworthy differences in the composition of some fatty acids. The fatty acid profile of strain SW32^T is similar to those of members belonging to the genus *Halomonas*, but distinguished from those of the genera *Carnimonas* and *Zymobacter* (Dobson & Franzmann, 1996; Franzmann & Tindall, 1990; Garriga *et al*., 1998). The levels of $C_{16:0}$ are 28·0 or 31·6 % in strain SW32^T (Table 1) and 16–32 % in the members belonging to the genus *Halomonas*, whereas its mean level in *Zymobacter palmae* is 51 % and its level in the type strain of *Carnimonas nigrificans* is 40 % (Dobson & Franzmann, 1996; Franzmann & Tindall, 1990; Garriga *et al*., 1998; Okamoto *et al*., 1993). The level of $C_{18:1}$ in strain SW32^T is similar to the levels observed in *Halomonas* species, but much higher than the levels of *Zymobacter palmae* (mean level 11 %) and *Carnimonas nigrificans* CECT 4437^T (13 %) (Berendes *et al*., 1996; Garriga *et al*., 1998; Franzmann & Tindall, 1990; Okamoto *et al*., 1993).

In addition, strain SW32^T is different from the genera *Zymobacter* and *Carnimonas* in some physiological properties, such as growth temperature, hydrolysis of

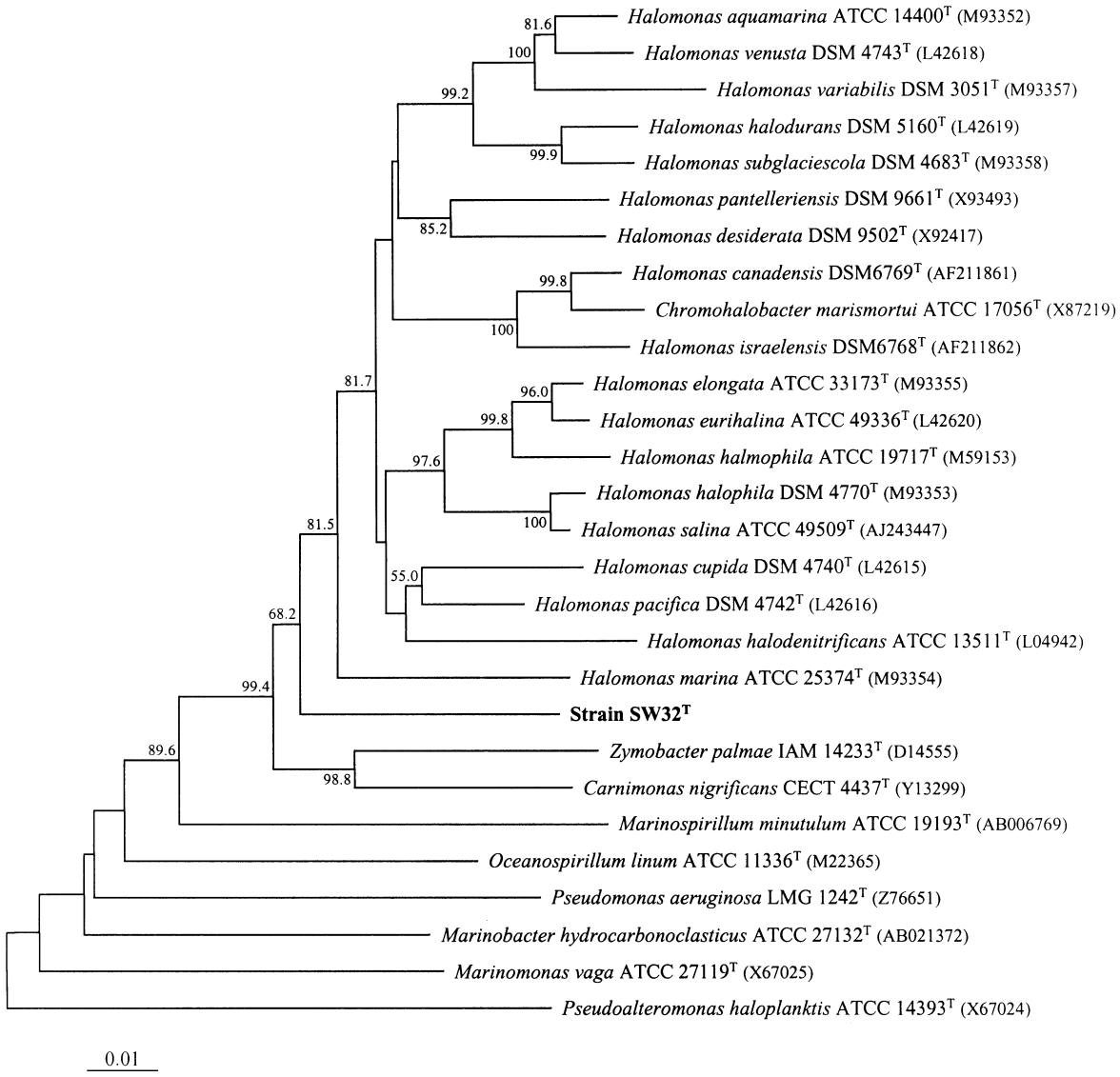

Fig. 2. Neighbour-joining tree showing the phylogenetic positions of strain SW32^T, members of the family *Halomonadaceae* and some related taxa based on 16S rDNA sequences. Scale bar represents 0·01 substitutions per nucleotide position. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at the branch points.

starch and salt tolerance (Garriga *et al.*, 1998; Okamoto *et al.*, 1993). There is also a cellular morphological difference between strain SW32^T and the genera *Zymobacter* and *Carnimonas*. While cells of strain SW32^T have a single polar flagellum (Fig. 1), *Carnimonas nigrificans* has no flagellum (Garriga *et al.*, 1998) and *Zymobacter palmae* has peritrichously flagellated cells (Okamoto *et al.*, 1993). Therefore, both phenotypic and phylogenetic data indicate that it is appropriate to classify strain SW32^T as a member of the genus *Halomonas*. It is currently recognized that the genus *Halomonas* may be a heterogeneous complex that comprises several genera. It should be considered that the placement of strain SW32^T into the genus *Halomonas* may be changed if the genus *Halomonas* is taxonomically re-evaluated.

From the results of 16S rDNA similarity as well as the formation of a phylogenetic lineage distinct from the *Halomonas* species described previously, strain SW32^T can be clearly distinguished from other *Halomonas* species. The 16S rDNA similarity values of strain SW32^T to all other bacterial organisms as well as *Halomonas* species are less than 93·8 %. Strain SW32^T showed phenotypic properties that are different from those of some other *Halomonas* species (Table 3). There are widely accepted criteria for delineating species in current bacteriology, stating that strains with a level of DNA relatedness less than 70 % or with greater than 3 % difference in 16S rDNA similarity are considered as being different species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Sequence similarity values obtained for strain SW32^T and the type

Table 3. Phenotypic characteristics of strain SW32^T and some other *Halomonas* species

1, Strain SW32^T; 2, *Halomonas elongata*; 3, *Halomonas eurihalina*; 4, *Halomonas halophila*; 5, *Halomonas salina* (data for ATCC 49509^T); 6, *Halomonas desiderata*; 7, *Halomonas cupida*; 8, *Halomonas pacifica*; 9, *Halomonas halodenitrificans*; 10, *Halomonas marina*. Data from Romano *et al.* (1996), Mellado *et al.* (1995), Baumann *et al.* (1972, 1983), Vreeland *et al.* (1980), Quesada *et al.* (1984, 1990), Valderrama *et al.* (1991), Kocur (1984), Kersters & De Ley (1984) and Palleroni (1984). Symbols: P, polar; Pr, peritrichous; +, positive reaction; −, negative reaction; W+, weakly positive reaction; V, variable reaction; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10
Cell morphology	Rods or ovals	Rods	Short rods	Rods	Short rods	Rods	Rods	Rods	Cocci or short rods	Rods
Flagellum	P	P or Pr	Absent	P or Pr	Absent	Pr	Pr	Pr	Absent	P
Motility	+	+	−	+	−	+	+	+	−	+
Oxidase	−	−	−	+	+	+	−	+	+	−
Catalase	+	+	+	+	+	+	ND	ND	+	ND
Nitrate reduction	−	+	+	+	+	+	+	−	+	−
Growth in the presence of 0·5% NaCl	+	+	V	−	−	+	+	+	−	+
Growth in the presence of 30% NaCl	−	+	−	+	−	−	−	−	−	−
Hydrolysis of:										
Aesculin	+	V	V	+	−	ND	+	+	−	−
Casein	−	−	−	−	−	−	ND	ND	ND	ND
Starch	−	−	ND	−	−	−	−	−	−	−
Gelatin	+	V	+	−	−	−	ND	ND	−	−
Urea	−	V	+	+	+	−	ND	ND	−	ND
Tween 80	−	ND	ND	−	−	−	ND	ND	−	−
Acid production from:										
Arabinose	+	−	−	−	−	ND	ND	ND	ND	ND
Glucose	+	+	−	+	−	ND	ND	ND	−	ND
Lactose	+	−	−	−	−	ND	ND	ND	ND	ND
Mannitol	W+	−	−	−	−	ND	ND	ND	ND	ND
G+C content (mol%)	59	60–61	59–66	67	64	66	60–63	67–68	64–66	62–64

strains of all validly described *Halomonas* species are low enough to exclude the possibility of assigning strain SW32^T into one of these species. Accordingly, strain SW32^T should be given taxonomic status distinct from the *Halomonas* species described previously, although no DNA–DNA hybridization study was performed. On the basis of these data, we propose the creation of a new species, *Halomonas marisflavae* sp. nov., for strain SW32^T. The properties of the new species are summarized below.

Description of *Halomonas marisflavae* sp. nov.

Halomonas marisflavae (ma.ris.fla′vae. L. gen. neut. n. *maris* of the sea; L. neut. adj. *flavum* yellow; L. gen. neut. n. *marisflavae* of the Yellow Sea, Korea).

Cells are rods or oval measuring 0·9–1·3 μm wide and 1·7–2·3 μm long after 3 d culture on MA at 28 °C. Gram staining reaction is negative. Non-spore-forming. Cells are motile by means of a single polar flagellum. Colonies are yellow-orange, smooth, glistening, circular/slightly irregular on both TSA and MA. Colonies are convex on TSA and flat/slightly convex on MA. Grows in the presence of 0·5–27% (w/v) NaCl. Optimal NaCl concentration for growth is 0·5–12% (w/v). Growth occurs at 4–37 °C with an optimum of 25–30 °C. Optimal pH for growth is 7·0–8·0. Growth is inhibited at pH values below 4·5. Anaerobic growth occurs on MA. Catalase-positive. Oxidase- and urease-negative. Aesculin and gelatin are hydrolysed. Casein, hypoxanthine, starch, Tween 80, tyrosine and xanthine are not hydrolysed. Nitrate is not reduced to nitrite. Indole is not produced. Arginine is not deaminated. Glucose, arabinose, mannose, mannitol, gluconate, malate and citrate are assimilated. Acids are produced from glycerol, erythritol, L-arabinose, ribose, D-xylose, galactose, D-glucose, D-fructose, D-mannose, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, gentiobiose, D-turanose, D-lyxose and D-fucose and weakly produced from adonitol, mannitol and xylitol. The predominant respiratory lipoquinone is ubiquinone-9. The major fatty acids are $C_{18:1}$, $C_{16:0}$ and $C_{16:1}$ $\omega7c$ and/or iso-$C_{15:0}$ 2OH. The G+C content is 59 mol% (determined by HPLC). Isolated from a water sample of the Yellow Sea, Korea. The type strain is strain SW32^T, which has been deposited at the Korean Culture Center of Microorganisms as KCCM 80003^T and at the Japan Collection of Microorganisms as JCM 10873^T.

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REFERENCES

Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **110**, 402–429.

Baumann, L., Bowditch, R. D. & Baumann, P. (1983). Description of *Deleya* gen. nov. created to accommodate the marine species *Alcaligenes aestus*, *A. pacificus*, *A. cupidus*, *A. venustus*, and *Pseudomonas marina*. *Int J Syst Bacteriol* **33**, 793–802.

Berendes, F., Gottschalk, G., Heine-Dobbernack, E., Moore, E. R. B. & Tindall, B. J. (1996). *Halomonas desiderata* sp. nov., a new alkaliphilic, halotolerant and denitrifying bacterium iso-

lated from a municipal sewage works. *Syst Appl Microbiol* **19**, 158–167.

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

Dobson, S. J. & Franzmann, P. D. (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.

Dobson, S. J., McMeekin, T. A. & Franzmann, P. D. (1993). Phylogenetic relationships between some members of the genera *Deleya*, *Halomonas*, and *Halovibrio*. *Int J Syst Bacteriol* **43**, 665–673.

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

Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) version 3.5. Seattle: Department of Genetics, University of Washington.

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

Franzmann, P. D. & Tindall, B. J. (1990). A chemotaxonomic study of members of the family *Halomonadaceae*. *Syst Appl Microbiol* **13**, 142–147.

Franzmann, P. D., Wehmeyer, U. & Stackebrandt, E. (1989). *Halomonadaceae* fam. nov., a new family of the class *Proteobacteria* to accommodate the genera *Halomonas* and *Deleya*. *Syst Appl Microbiol* **11**, 16–19.

Garriga, M., Ehrmann, M. A., Arnau, J., Hugas, M. & Vogel, R. F. (1998). *Carnimonas nigrificans* gen. nov., sp. nov., a bacterial causative agent for black spot formation on cured meat products. *Int J Syst Bacteriol* **48**, 677–686.

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

Kersters, K. & De Ley, J. (1984). Genus *Alcaligenes* Castellani and Chalmers 1919, 936[AL]. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 361–373. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.

Kocur, M. (1984). Genus *Paracoccus* Davis 1969, 384[AL]. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 399–402. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Komagata, K. & Suzuki, K. (1987). Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–203.

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

Mellado, E., Moore, E. R. B., Nieto, J. J. & Ventosa, A. (1995). Phylogenetic inferences and taxonomic consequences of 16S ribosomal DNA sequence comparison of *Chromohalobacter marismortui*, *Volcaniella eurihalina*, and *Deleya salina* and reclassification of *V. eurihalina* as *Halomonas eurihalina* comb. nov. *Int J Syst Bacteriol* **45**, 712–716.

Okamoto, T., Taguchi, H., Nakamura, K., Ikenaga, H., Kuraishi, H. & Yamasato, K. (1993). *Zymobacter palmae* gen. nov., sp. nov., a new ethanol fermenting peritrichous bacterium isolated from palm sap. *Arch Microbiol* **160**, 333–337.

Palleroni, N. J. (1984). Genus I. *Pseudomonas* Migula 1894, 237[AL]. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 141–199. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Quesada, E., Ventosa, A., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1984). *Deleya halophila*, a new species of moderately halophilic bacteria. *Int J Syst Bacteriol* **34**, 287–292.

Quesada, E., Valderrama, M. J., Bejar, V., Ventosa, A., Gutierrez, M. C., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1990). *Volcaniella eurihalina* gen. nov., sp. nov., a moderately halophilic nonmotile Gram-negative rod. *Int J Syst Bacteriol* **40**, 261–267.

Romano, I., Nicolaus, B., Lama, L., Manca, M. C. & Gambacorta, A. (1996). Characterization of a haloalkalophilic strictly aerobic bacterium, isolated from Pantelleria Island. *Syst Appl Microbiol* **19**, 326–333.

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

Skerratt, J. H., Nichols, P. D., Mancuso, C. A., James, S. R., Dobson, S. J., McMeekin, T. A. & Burton, H. (1991). The phospholipid ester-linked fatty acid composition of members of the family *Halomonadaceae* and genus *Flavobacterium*. A chemotaxonomic guide. *Syst Appl Microbiol* **14**, 8–13.

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.

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., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL w: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Valderrama, M. J., Quesada, E., Bejar, V., Ventosa, A., Gutierrez, M. C., Ruiz-Berraquero, F. & Ramos-Cormenzana, A. (1991). *Deleya salina* sp. nov., a moderately halophilic Gram-negative bacterium. *Int J Syst Bacteriol* **41**, 377–384.

Ventosa, A., Gutierrez, M. C., Garcia, M. T. & Ruiz-Berraquero, F. (1989). Classification of '*Chromobacterium marismortui*' in a new genus, *Chromohalobacter* gen. nov., as *Chromohalobacter marismortui* comb. nov., nom. rev. *Int J Syst Bacteriol* **39**, 382–386.

Vreeland, R. H., Litchfield, C. D., Martin, E. L. & Elliot, E. (1980). *Halomonas elongata*, a new genus and species of extremely salt-tolerant bacteria. *Int J Syst Bacteriol* **30**, 485–495.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 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.

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.