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***Halomonas glaciei* sp. nov. isolated from fast ice of Adelie Land, Antarctica**

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Abstract Eleven psychrophilic bacteria were isolated from a solid layer of fast ice in the middle of Pointe-Geologie Archipelago, Adelie Land, Antarctica. The 11 isolates based on the phenotypic characteristics, chemotaxonomic and phylogenetic analysis have been identified as members of the genus *Halomonas*. All the isolates at the 16S rDNA sequence level were identical, possessed the 15 conserved nucleotides of the family *Halomonadaceae* and four nucleotides of the genus *Halomonas*. Therefore, the 16S rDNA sequence of DD 39 was used for calculating the evolutionary distances and for phylogenetic analysis. It was observed that DD 39 formed a robust cluster with *H. variabilis*, from which it differed by 0.7%. Further DNA–DNA hybridization studies indicated low DNA–DNA homology (15%) between *H. variabilis* and DD 39. Between the 11 Antarctic isolates the homology was >85%. In addition it was observed that DD 39 was different from *H. variabilis* in that it was psychrophilic, could tolerate only up to 15% sodium chloride, could not hydrolyse esculin, could not reduce nitrate, was urease negative, could not utilize glycerol as a carbon source, and was resistant to ampicillin and erythromycin and sensitive to nalidixic acid. In addition, it also exhibited distinct differences with respect to high content of C_{16:1} and low levels of cyclo-C_{17:0} and cyclo-C_{19:0}. DD 39 also differed from all the other reported species of *Halomonas* with respect to many

phenotypic characteristics. It is proposed therefore that DD 39 should be placed in the genus *Halomonas* as a new species that is *Halomonas glaciei*. The type strain of *H. glaciei* is DD 39^T (MTCC 4321; JCM 11692).

Keywords Antarctica · Halotolerant · *Halomonas* · Psychrophiles

Introduction

The family *Halomonadaceae* is a member of the gamma subclass of Proteobacteria (Franzmann et al. 1988). Members of this family are Gram-negative, straight or curved, rod-shaped, slightly or moderately halotolerant, possess ubiquinone-9 (H₂) as the respiratory lipoquinone and contain C_{18:1}, cyclo-C_{19:0} and C_{16:0} as the major fatty acids. The family *Halomonadaceae* currently includes three genera (Arahal et al. 2002), namely *Halomonas* (Vreeland et al. 1980; Dobson and Franzmann 1996), *Chromohalobacter* (Ventosa et al. 1989; Arahal et al. 2001), and *Zymobacter* (Okamoto et al. 1993). All the members of the family belonging to the three different genera possess 15 signature nucleotides characteristic of the family and in addition they have four signature nucleotides which define and differentiate the above three genera.

Currently 23 species of *Halomonas*, three species of *Chromohalobacter*, and one species of *Zymobacter* have been identified mostly from aquatic habitats such as sea water, hypersaline lakes, a solar salt facility, bacon curing brine, and municipal sewage. In all these habitats isolates of *Halomonas* appear to be predominant. Franzmann et al. (1987) were the first to report a psychrotrophic species of *Halomonas* from a hypersaline lake in Antarctica. In the present study a total of 11 isolates of bacteria were isolated from fast ice from Adelie Land, Antarctica and were characterized and identified as belonging to the genera *Halomonas*. Furthermore, the isolates did not identify

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with any of the reported species and have been assigned the status of a new species, namely *Halomonas glaciei*.

Materials and methods

Source of the organism, media and growth conditions

Fast ice samples were collected between May and December, 1997, from a solid layer of fast ice using ice-coring augurs, from a station located 500 m offshore in the middle of Pointe-Geologie Archipelago, Adelie Land (66°40' S; 140°01' E), Antarctica. The ice cores were cut into segments with a sterile blade and samples from the center of the segment were allowed to melt and an aliquot of the melt water (100 µl) was plated on marine agar 2216 (Difco) and incubated for 20 days at 2°C. The appearance of colonies was monitored on a regular basis and representative colonies were picked up and subcultured repeatedly, so as to establish pure cultures of the bacteria. The pure cultures were maintained in Zobell marine broth agar 2216 (HiMedia Laboratories, Bombay, India) medium at 5° or 22°C. The optimum temperature, pH, and salt concentration for growth of the cultures were determined using Zobell marine agar 2216 (HiMedia Laboratories) plates.

Morphology, motility, and biochemical characteristics

All the phenotypic characteristics are listed in Table 1 and described in the Results and Discussion sections, including the ability

of the cultures to utilize a carbon compound as the sole carbon source, the sensitivity to different antibiotics, the isolation of DNA and the mol% G + C content of the DNA was determined as described earlier (Shivaji et al. 1988, 1989a, b, 1991, 1992; Reddy et al. 2000, 2002a, b) using cultures grown at 20°C in the appropriate medium (Hugh and Leifson 1953; Stanier et al. 1966; Holding and Collee 1971; Stolp and Gadkari 1981).

DNA–DNA hybridization

DNA–DNA hybridization was performed by the membrane filter method (Tourova and Antonov 1987) as described previously (Shivaji et al. 1992; Reddy et al. 2000, 2002a, b).

Identification of fatty acids

Fatty acid methyl esters (FAME) prepared from bacterial cell pellets (Sato and Murata 1988) were separated by gas chromatography on a DB-23 capillary column (30 m × 0.25 mm) (J & W Scientific, California, USA) and identified by comparison with fatty acid standards and also by mass spectrometry (Shivaji et al. 1992; Reddy et al. 2000, 2002a, b).

Analysis of isoprenoid quinones

Ubiquinones were extracted according to Collins et al. (1977), separated by thin layer chromatography (TLC) using petroleum ether and diethyl ether (85:15 v/v) (Dumphy et al. 1971), and identified by mass spectrometry (Reddy et al. 2000).

Table 1 Phenotypic characteristics which differentiate the Antarctic strains of *Halomonas*

Isolate	DD 23	DD 24	DD 26	DD 28	DD 29	DD 39	DD 40	DD 45	SJ 23	SJ 37	SS 45
Motility	+	–	+	+	–	+	–	–	+	+	+
Growth at pH 5	–	–	–	–	–	–	–	–	+	–	–
Methyl red test	–	–	–	–	–	–	–	–	+	–	–
Utilization of:											
Glucose	+	+	–	+	+	+	+	–	+	+	+
Fructose	–	–	–	–	–	–	–	–	+	–	–
Glycerol	–	–	–	–	–	–	–	–	+	–	–
Sorbitol	–	–	–	–	+	–	–	–	+	–	–
Xylose	+	–	–	–	–	–	–	–	–	–	–
Arabinose	+	+	–	+	+	+	+	+	+	+	+
Inulin	+	–	–	–	–	–	–	–	–	–	–
Trehalose	+	–	+	–	–	+	–	–	+	–	–
Pyruvate	+	+	–	+	+	+	+	+	+	+	+
Asparagine	–	–	–	–	–	–	–	+	–	–	–
Tyrosine	–	–	–	–	–	–	–	–	+	–	–
Acid production:											
Glucose	–	–	–	–	+	–	–	+	–	–	–
Mannitol	+	–	–	–	–	–	–	–	–	–	–
Arabinose	+	–	+	–	–	–	–	–	–	–	–
Trehalose	+	–	–	–	–	–	–	–	–	–	–
Antibiotics:											
Nalidixic acid	S	R	S	S	S	S	S	S	R	S	S
Nitrofurantoin	S	S	R	S	S	S	R	S	S	S	S
Tobramycin	S	S	R	S	R	S	R	S	S	R	S
Rifampicin	R	R	S	S	S	R	R	S	S	R	R
Streptomycin	S	S	S	S	S	S	R	S	S	S	S
Polymyxin B	S	S	S	S	R	S	S	S	S	S	S
Erythromycin	R	R	R	R	R	R	R	R	R	R	R
Novobiocin	R	R	R	S	S	R	R	S	S	R	R
G + C content of DNA (mol% ± 2/3%)	57	57	59.5	57	57	57	59.5	59.5	60	57	57

+ present, – absent, S sensitive, R resistant

16S rRNA gene sequencing

The 16S rRNA gene (1.5 kb) was amplified from the genomic DNA (Shivaji et al. 2000; Reddy et al. 2000, 2002a, b) using the primers 16S1 (5'AGTTTGATCCTGGCTCA 3') and 16S2 (5'ACGGCT-ACCTTGTTACGACTT 3') corresponding to positions 9–27 and 1,477–1,498, respectively, of *Escherichia coli*. The amplified DNA fragment of 1.5 kb following electrophoresis on a 1% agarose gel was purified using a Clean Genei kit (Bangalore Genei, Bangalore, India) and sequenced using the primers 16S1 and 16S2 and in addition a set of five forward primers pB [(TAACACATGCAAGTCGAACG, (50–70)], pC [(CTACGGGAGGCAGCAGTGGG, (341–361)], pD [(CAGCAGCCGCGGTAATAC, (518–536)], pE [(AAACTCAAAGGAATTGACGG, (908–928)], and pF [(CATGGCTGCTGCAGCTCGT, (1,053–1,073)] and three reverse primers pC [(CCCACCTGCTGCCTCCCGTAG, (361–341)], pE [(CCGTCAATTCCTTTGAGTTT, (928–908)] and pH [(AAGGAGGTGATCCAGCCGCA, (1,542–1,522)], respectively (Woese et al. 1983).

Phylogenetic analysis

The multiple sequence alignment program Clustal V (Higgins et al. 1992) was used to align the 16S rDNA sequence of the bacteria with the sequences of all the species of the family *Halomonadaceae* retrieved from the EMBL database (Fig. 1). The aligned sequences were then manually checked for gaps. The DNADIST program with Kimura-2 factor was used to compute the pairwise evolutionary distances for the above-aligned sequences (Kimura 1980). Further, the original sequence data set was resampled 1,000 times using SEQBOOT and subjected to bootstrap analysis to obtain the confidence values for the rDNA sequence-based genetic affiliations. The multiple distance matrices thus obtained were used to construct phylogenetic trees using distance matrix-based clustering algorithms such as FITCH, KITCH, and UPGMA (Felsenstein 1993). Parsimony analysis was also performed for the aligned sequence data set using DNAPARS. In all the cases, input order of species added to the topology being constructed was randomized using the jumble option with a random seed of seven and ten replications. Majority rule (50%) consensus trees were constructed for the topologies found by each method using CONSENSE. All these analysis were done using the PHYLIP package, version 3.5 (Felsenstein 1993).

SDS-PAGE

Bacterial cell pellets were analyzed on 12% SDS-polyacrylamide gels (Laemmli 1970) to visualize their protein profiles.

Reference strains

H. variabilis (Deutsche Sammlung von Mikroorganismen, DSM 3051^T), *P. stutzeri* (Microbial Type Culture Collection, MTCC 101^T), *Pseudomonas fluorescens* (MTCC), *Pseudomonas putida* (MTCC) and *Sphingobacterium antarcticus* (American Type Culture Collection, ATCC 51970^T) were used as controls in the studies related to morphology, motility, biochemical tests, identification of fatty acids, etc.

Results and discussion

Morphology and growth characteristics

A total of 11 representative colonies of bacteria were isolated and purified from land-fast ice from Adelie Land in Antarctica and were studied in detail to estab-

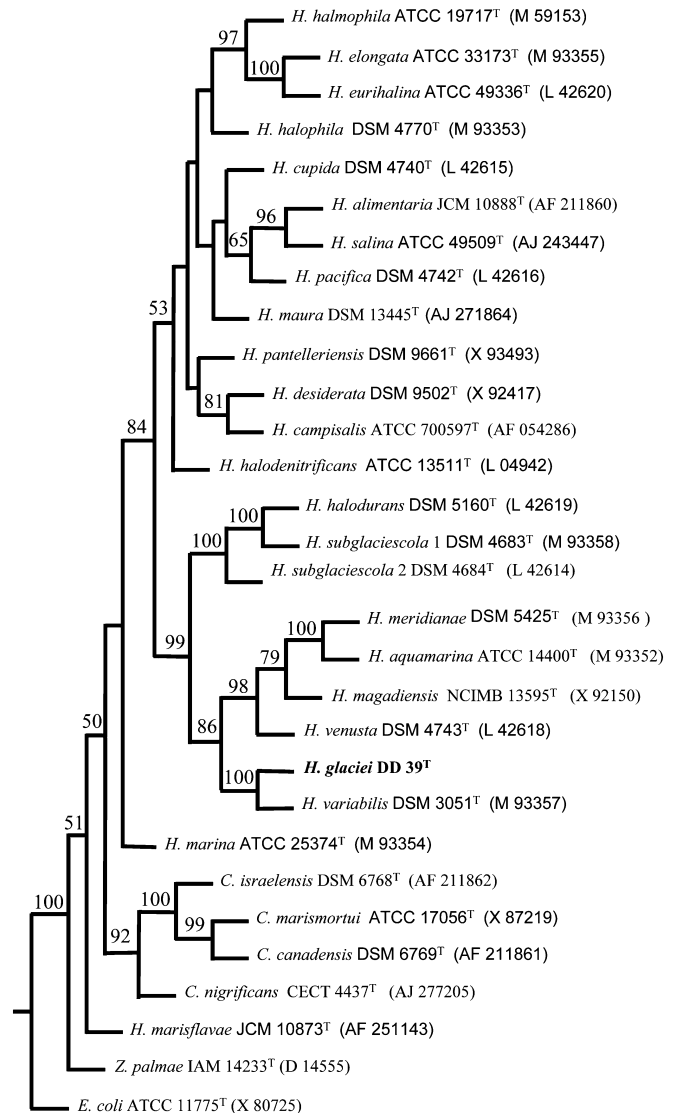


Fig. 1 Phylogenetic relationship between *Halomonas glaciei* sp. nov. (DD 39^T) and other related reference microorganisms in the genera *Halomonas*, *Zymobacter*, and *Chromohalobacter* based on the 16S rDNA sequence analysis using UPGMA. *E. coli* was used as the root species in the tree. The bootstrap values (%) are given at the nodes. The branch lengths indicated in the tree are not to scale

lish their identity. All the colonies were circular (2–3 mm in diameter), convex, white in color, and grew very well in Zobells marine agar between 5° and 30°C; maximum growth was observed at 22°C. Furthermore, although they were able to grow in the absence of NaCl they could tolerate up to 15% NaCl, indicating that the 11 strains were halotolerant. Ten of the 11 isolates were unable to grow at pH 5 (Table 1), but all the isolates could grow at pH 9 and exhibited optimum growth at pH 7.

Biochemical and chemotaxonomic characteristics

Based on their phenotypic characteristics, it was apparent that the 11 isolates which were Gram-negative,

straight or curved rods, halophilic, motile or non-motile, aerobic, catalase and oxidase positive, with a mol% G + C content of DNA ranging from 57 to 60, belonged to the genus *Halomonas* (Vreeland et al. 1980; Franzmann et al. 1988). Earlier studies have indicated that the mol% G + C content of DNA in various species of *Halomonas* ranged from 52% to 64% (see references in Table). In addition, the 11 isolates were negative for gelatinase, urease, lipase, phosphatase, hydrolysis of esculin, hydrolysis of starch, reduction of nitrate, indole production, Voges-Proskauer test, arginine and lysine decarboxylases, citrate utilization, and H₂S production. They did not produce acid from lactose and none of the isolates could produce gas either from glucose, lactose, mannitol, arabinose, or trehalose. Further lactose, sucrose, dulcitol, maltose, mellibiose, mannose, *m*-inositol, adonitol, erythritol, mannitol, melezitol, dextran, leucine, isoleucine, methionine, valine, threonine, histidine, tryptophan, arginine, alanine, phenylalanine, serine, asparagine, tyrosine, and cysteine were not utilized when provided as the sole carbon source. All the isolates were resistant to the antibiotics ampicillin, erythromycin, amoxycillin, lincomycin, nystatin, bacitracin, tetracycline, and carbencillin, and were sensitive to kanamycin, gentamycin, co-trimoxazole, chloramphenicol, and colistin. The important biochemical characteristics that differentiate Antarctic isolates from each other are listed in Table 1.

The major respiratory quinone was ubiquinone-9 and the major fatty acids present were C_{16:0}, C_{16:1}, C_{18:0}, C_{18:1}, cyclopropane octadecanoic acid 2-octyl methyl ester, and octadecanoic acid 11-methoxy methyl ester (Table 2).

Phylogenetic analysis

The 16S rDNA obtained from the 11 isolates was about 1,413 bp in length, corresponding to positions 47–1,460 of *E. coli* 16S rRNA gene. Furthermore, since all the isolates had identical 16S rDNA sequences (and also showed identical protein profiles by

SDS-PAGE), the 16S rDNA sequence of DD 39 was used for calculating the evolutionary distances and for phylogenetic analysis. For this purpose the 16S rDNA sequence of DD 39 was compared with the 16S rDNA sequences of closely related species of *Halomonas*, *Zymobacter*, and *Chromohalobacter*. The topology of the phylogenetic tree (Fig. 1) clearly indicated that all the reported species of *Halomonas* formed four highly stable and coherent clades with high bootstrap values (> 50%), and the interclade resolution was robust. Moreover, the phylogenetic tree was broadly in agreement with those reported recently (Dobson and Franzmann 1996; Mellado et al. 1995; Dobson et al. 1993). Dobson and Franzmann (1996), based on parsimony and distance methods, indicated that all the species of *Halomonas* could be assigned to three subgroups. *Halomonas eurihalina*, *H. elongata*, *H. halomphila*, *H. salina*, *H. pacifica*, *H. cupida*, *H. halophila*, and *H. halodenitrificans* were assigned to subgroup I; *H. subglaciescola*, *H. halodurans*, *H. meridiana*, *H. aquamarina*, *H. venusita*, and *H. variabilis* to subgroup II; and *H. marina* to subgroup III. In the present study, the three subgroups were clearly differentiated and the phylogenetic analysis showed that the DD 39 falls within the phylogenetic affiliation of the genus *Halomonas*. The phylogenetic position of DD 39, as determined by UPGMA, KITSH, FITCH and DNA parsimony methods, indicated that the DD 39 forms a robust cluster with *H. variabilis* of the group-II species of *Halomonas* (Fig. 1), with a bootstrap resampling value of 100%. Therefore, it is obvious that the above 11 isolates are members of the genus *Halomonas* and are closely related to one another and to *H. variabilis*. In fact, the evolutionary distance as calculated by DNADIST indicated that DD 39 is indeed very close to *H. variabilis* and is separated by a distance of 0.7%, a very low value for the species delineation, at the 16S rRNA level and thus it is difficult to assign it to a new species (Stackebrandt et al. 1994). However, a low degree of DNA–DNA homology (15%) between *H. variabilis*

Table 2 Fatty acid composition of the Antarctic strains of *Halomonas*

Fatty acid	DD 23	DD 24	DD 26	DD 28	DD 29	DD 39	DD 40	DD 45	SS 37	SS 45
C _{12:0}	0.7	–	0.2	0.2	0.2	0.5	0.5	0.3	0.3	0.1
C _{14:0}	–	–	0.2	0.1	–	0.3	0.3	0.4	0.3	0.1
C _{14:1}	1.3	1.0	1.0	1.2	–	2.9	2.0	0.3	–	0.3
Iso-C _{15:0}	6.5	4.3	5.5	1.6	1.1	–	0.1	1.2	0.2	3.6
Anteiso-C _{15:0}	13.8	8.8	11.7	4.0	1.9	–	–	16.2	0.3	10.3
C _{15:0}	–	0.3	0.2	0.1	4.0	0.2	0.3	3.6	1.4	0.8
Iso-C _{16:0}	1.4	1.0	1.3	0.8	0.8	–	–	1.7	–	2.0
C _{16:0}	7.7	8.6	8.8	13.1	10.0	11.6	15.8	10.4	13.0	9.5
C _{16:1} (9)	7.0	7.2	8.6	10.0	8.5	13.5	5.7	6.6	6.6	4.8
Iso-C _{17:0}	4.3	3.4	6.0	1.5	1.5	–	–	3.7	1.0	4.4
Anteiso-C _{17:0}	2.0	5.8	8.1	0.8	2.5	–	–	13.4	0.4	13.9
Cyclo-C _{17:0}	2.0	2.8	2.4	3.0	2.5	5.5	4.9	2.6	3.2	2.6
C _{18:0}	3.4	6.6	5.7	9.0	9.5	5.0	8.9	5.5	8.2	6.6
C _{18:1}	21.7	14.7	14.8	18.1	18.3	31.2	30.0	11.2	32.3	11.3
A ^a	7.0	6.0	2.5	3.7	3.7	2.4	–	3.3	0.4	4.3
B ^a	7.1	14.5	10.4	15.7	16.0	10.0	6.0	8.0	12.4	11.8
C ^a	8.5	15.3	12.4	17.4	19.3	10.3	12.0	10.4	14.3	13.3

All the strains were grown in Zobell marine agar (2216) at 25°C

^aA: Hexadecanoic acid 9, 10 dimethoxy methyl ester, B: cyclopropane octadecanoic acid 2-octyl methyl ester, C: octadecanoic acid 11-methoxy methyl ester

and the Antarctic isolates indicated that these isolates could be assigned to a new species. Apart from the low DNA–DNA similarity, the phenotypic differences between DD 39 and *H. variabilis* and other Group II *Halomonas* species (Table 3), clearly support a new species status for the strain DD 39. Thus DD 39 has been assigned to a new species of *Halomonas*, namely *Halomonas glaciei* sp. nov. The remaining ten isolates are strains of *Halomonas glaciei*. This is further evident from the observation that DNA–DNA homology between DD 39 and the remaining ten isolates was >85%, whereas with *H. variabilis* all the isolates exhibited <15% DNA–DNA homology.

Description of *Halomonas glaciei* sp. nov.

Halomonas glaciei (glaciei L. gen. N. meaning of the cold). DD 39 forms a round, convex, smooth, and

translucent colony with a colony diameter of 1–2 mm. The cells are Gram-negative, motile, rod shaped, psychotropic (grows from 4° to 30°C), can tolerate 12% NaCl, and grows at an optimum pH of 7. The bacterium is positive for catalase, oxidase and negative for urease, lipase, gelatinase, phosphatase, arginine dihydrolase, arginine decarboxylase, lysine decarboxylase, indole production, methyl red, Vogues-Proskauer test, aesculine hydrolysis, starch hydrolysis, and does not reduce nitrate to nitrite. It utilizes glucose, arabinose, pyruvate, trehalose, and acetate when used as the sole carbon source and does not utilize citrate, fructose, mannose, glycerol, sorbitol, lactose, sucrose, dulcitol, maltose, mellibiose, melizitol, dextran, leucine, isoleucine, methionine, valine, threonine, histidine, tryptophan, cysteine, adonitol, erythritol, mannitol, phenyl alanine, arginine, alanine, serine, asparagine, glutamic acid, and tyrosine. Does not produce acid or gas from glucose, lactose, mannitol, arabinose, and trehalose. DD 39 is

Table 3 Comparison of the phenotypic characteristics of DD 39 with all the species of Group II *Halomonas*

Characteristics	DD 39 ^a	<i>H. variabilis</i> ^b	<i>H. glaciescoid</i> ^c	<i>H. halodurans</i> ^d	<i>H. meridiana</i> ^e	<i>H. aquamarina</i> ^f	<i>H. megadiensis</i> ^g	<i>H. vensuta</i> ^h
Growth characteristics								
Salt (%)	0 to 15	8.73 to 29.25	0.5 to 20	0 to 20	0 to 15	NA	0 to 20	NA
Optimum (%)	2	11.7	ND	ND	ND	ND	0 to 7	NA
PH	6 to 12	6.5 to 8.4	5 to 9	6	5	6	7 to 11	NA
Optimum pH	7	7.5	–	–	–	–	9.5	NA
Temperature (°C)	4 to 30	15 to 37	–5 to 25	4 to 43	4 to 45	5 to 45	20 to 50	4 to 40
Optimum temperature (°C)	22	33	20	20	37	37	37	37
Enzymology								
Esculine hydrolysis	–	+	–	+	–	–	+	–
Lysine decarboxylase	–	–	–	+	–	ND	ND	ND
Nitrate reduction	–	+	+	–	–	+	+	+
Oxidase	+	+	+	–	–	+	+	+
Phosphatase	–	ND	–	ND	ND	+	–	ND
Starch hydrolysis	–	–	–	ND	ND	+	–	–
Urease	–	+	–	–	+	–	–	+
Major fatty acid composition (mol%)ⁱ								
C _{16:1} (9)	19.5	2.4	23.1	15.2	3.4	6.3	ND	10.7
C _{16:0}	17.1	17.5	32	27.2	15.5	17.4	ND	15.9
Cyclo-C _{17:0}	6.7	11.4	5.8	26.5	4.3	2.1	ND	–
C _{18:1}	18.5	15.1	31.9	19.6	60.1	62.7	ND	68.6
Cyclo-C _{19:0}	5.23	50.9	4.8	7.1	13.3	7	ND	–
16S rDNA identity of 100	99.3	95.75	96	96.9	96.75	95.5	95.5	96.4
DD 39 with other strains (%)								
Mol% G+C	57	61	61	63.2	NA	57	62	52
Carbon source utilization:								
Glycerol	–	+	–	+	–	NA	+	NA
Lactose	–	–	–	NA	–	–	+	–
Mannitol	–	–	NA	+	NA	+	+	+
Sucrose	–	–	–	NA	+	–	NA	+
Alanine	–	–	+	+	NA	–	NA	+
Antibiotic sensitivity								
Ampicillin	R	S	NA	NA	NA	NA	R	NA
Erythromycin	R	S	NA	S	NA	NA	S	NA
Nalidixic acid	S	R	NA	S	NA	NA	NA	NA
Tetracycline	R	R	R	S	NA	NA	R	NA

ND not determined, NA data not available, + present, – absent, S sensitive, R resistant

^aDD 39 was grown in artificial organic lake water peptone broth (Franzmann et al. 1987) at room temperature for fatty acid analysis

^bData from Fendrich (1988)

^cData from Franzmann et al. (1987)

^dData from Hebert and Vreeland (1987)

^eData from Huval et al. (1995)

^fData from Akagawa and Yamasato (1989)

^gData from Duckworth et al. (2000)

^hData from Baumann et al. (1983)

ⁱData from Franzmann and Tindall (1990)

sensitive to the antibiotics kanamycin, gentamycin, co-trimazole, nitrofurantoin, nalidixic acid, tobramycin, streptomycin, polymyxin-B, colistin, and chloramphenicol and resistant to ampicillin, erythromycin, amoxycillin, lincomycin, nystatin, carbencillin, bacitracin, tetracycline, and novobiocin.

The fatty acids and their proportions the bacterium contained were: C_{12:0} (0.5%), C_{14:0} (0.3%), C_{14:1} (2.9%), C_{15:0} (0.2%), C_{16:0} (11.6%), C_{16:1 δ 9} (13.5%), cyclo-C_{17:0} (5.5%), C_{18:0} (5.0%), C_{18:1} (31.2%), hexadecanoic acid 9,10 dimethoxy methyl ester (2.4%), cyclopropane octadecanoic acid 2-octyl methyl ester (10.0%), and octadecanoic acid 11-methoxy methyl ester (10.3%) and the major lipoquinone it contained was ubiquinone-9. The mol% G+C was 57%. Isolated from fast-ice from Adelie Land, Antarctica. The type strain is DD 39^T (MTCC 4321; JCM 11692). The EMBL accession number for the 16S rDNA sequence of DD 39^T is AJ 431369.

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References

- Akagawa M, Yamasato K (1989) Synonymy of *Alcaligenes aquamarinus*, *Alcaligenes faecalis* subsp. *Homari*, and *Deleya aesta*: *Deleya aquamarina* comb. nov. as the type species of the genus *Deleya*. Int J Syst Bacteriol 39:462–466
- Arahal RD, Garcia TM, Ludwig W, Schleifer HK, Ventosa A (2001) Transfer of *Halomonas canadensis* and *Halomonas israelensis* to the genus *Chromohalobacter* as *Chromohalobacter canadensis* comb. nov. and *Chromohalobacter israelensis* comb. nov. Int J Syst Evol Microbiol 51:1443–1448
- Arahal RD, Ludwig W, Schleifer KH, Ventosa A (2002) Phylogeny of the family *Halomonadaceae* based on 23S and 16S rDNA sequence analyses. Int J Syst Evol Microbiol 52:241–249
- Baumann L, Bowditch RD, 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
- Collins MD, Pirouz T, Goodfellow M, Minnikin DE (1977) Distribution of menaquinones in actinomycetes and corynebacteria. J Gen Microbiol 100:221–230
- 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 Gibbans, 1952) into a single genus, *Halomonas* and placement of the genus *Zymobacter* in the family *Halomonadaceae*. Int J Syst Bacteriol 46:550–558
- Dobson SJ, Colwell RR, McMeekin TA, Franzmann PD (1993) Phylogenetic relationship between some members of the genus *Deleya*, *Halomonas* and *Halovibrio*. Int J Syst Bacteriol 43:665–673
- Duckworth AW, Grant WD, Jones BE, Meijer D, Marquez MC, Ventosa A (2000) *Halomonas magadii* sp. nov.: a new member of the genus *Halomonas*, isolated from a soda lake of the east African Rift valley. Extremophiles 4:53–60
- Dumphy PJ, Phillips PG, Brodie AF (1971) Separation and identification of menaquinones from microorganisms. J Lipid Res 12:442–449
- Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.5c. Department of Genetics, University of Washington, Seattle, USA
- Fendrich C (1988) *Halovibrio variabilis* gen. nov., *Pseudomonas halophila* sp. nov. and new Halophilic aerobic coccoid Eubacterium from great salt lake, Utah, USA. Syst Appl Microbiol 11:36–43
- Franzmann PD, Tindall BJ (1990) A chemotaxonomic study of members of the family *Halomonadaceae*. Syst Appl Microbiol 13:142–147
- Franzmann PD, Burton HR, McMeekin TA (1987) *Halomonas subglaciescola*, a new species of halotolerant bacteria isolated from Antarctica. Int J Syst Bacteriol 37:27–34
- Franzmann PD, Wehmeyer U, Stackerbrandt E (1988) *Halomonadaceae* fam. nov., a new family of the class proteobacteria to accommodate the genera *Halomonas* and *Deleya*. Syst Appl Microbiol 11:16–19
- Hebert AM, Vreeland RH (1987) Phenotypic comparison of halotolerant bacteria: *Halomonas halodurans* sp. nov. nom. rev. comb. nov. Int J Syst Bacteriol 37:347–350
- Higgins DG, Bleasby AT, Fuchs R (1992) Clustal V: improved software for multiple sequence alignment. CABIOS 8:189–191
- Holding AJ, Collee JG (1971) Routine biochemical tests. Methods Microbiol 6A:2–32
- Hugh R, Leifson E (1953) The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. J Bacteriol 66:24–26
- Huval JH, Latta R, Wallace R, Kushner DJ, Vreeland RH (1995) Description of two new species of *Halomonas*: *Halomonas israelensis* sp. nov. and *Halomonas canadensis* sp. nov. Can J Microbiol 41:1124–1131
- 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
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685
- Mellado E, Moore ERB, Nieto JS, 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. erukhalina* 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 plamae*, gen. nov. sp. nov. a new ethanol fermenting peritrichous bacterium isolated from palm sap. Arch Microbiol 160:333–337
- Reddy GSN, Aggarwal RK, Matsumoto GI, Shivaji S (2000) *Arthrobacter flavus* sp. nov., a psychrophilic bacterium isolated from a pond in McMurdo Dry Valley, Antarctica. Int J Syst Environ Microbiol 50:1553–1561
- Reddy GSN, Prakash JSS, Matsumoto GI, Sackebrandt E, Shivaji S (2002a) *Arthrobacter roseus* sp. nov., a psychrotolerant bacterium isolated from an Antarctic cyanobacterial mat sample. Int J Syst Environ Microbiol 52:1017–1021
- Reddy GSN, Prakash JSS, Vairamani M, Prabhakar S, Matsumoto GI, Shivaji S (2002b) *Planococcus antarcticus* and *Planococcus psychrophilus* sp. nov. isolated from cyanobacterial mat samples collected from ponds in Antarctica. Extremophiles 6:253–261
- Sato NS, Murata N (1988) Membrane lipids. In: Packer L, Glazer AN (eds) Methods in enzymology, vol 167. Academic Press, New York, pp 251–259
- Shivaji S, Rao NS, Saisree L, Sheth V, Reddy GSN, Bhargava PM (1988) Isolation and identification of *Micrococcus roseus* and *Planococcus* sp. from Schirmacher Oasis, Antarctica. J Biosci 113:409–414
- Shivaji S, Rao NS, Saisree L, Reddy GSN, Seshu Kumar G, Bhargava PM (1989a) Isolates of *Arthrobacter* from the soils of Schirmacher Oasis, Antarctica. Polar Biol 10:225–229
- Shivaji S, Rao NS, Saisree L, Sheth V, Reddy GSN, Bhargava PM (1989b) Isolation and identification of *Pseudomonas* sp. from Schirmacher Oasis, Antarctica. Appl Environ Microbiol 55:767–770
- Shivaji S, Ray MK, Seshu Kumar G, Reddy GSN, Saisree L, Wynn Williams DD (1991) Identification of *Janthinobacterium*

- lividum* from the soils of the islands of Scotia Ridge and from Antarctic peninsula. *Polar Biol* 11:267–272
- Shivaji S, Ray MK, Saisree L, Jagannadham MV, Seshu Kumar G, Reddy GSN, Bhargava PM (1992) *Sphingobacterium antarcticus* sp. nov.: a psychrotrophic bacterium from the soils of Schirmacher Oasis, Antarctica. *Int J Syst Bacteriol* 42:102–116
- Shivaji S, Vijaya Bhanu N, Aggarwal RK (2000) Identification of *Yersinia pestis* as the causative organism of plague in India as determined by 16S rDNA sequencing and RAPD based genomic fingerprinting. *FEMS Microbiol Lett* 189:247–252
- Stackebrandt E, Goebel BM (1994) 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
- Stanier RY, Palleroni NJ, Doudroff M (1966) The aerobic pseudomonas a taxonomic study. *J Gen Microbiol* 43:159–271
- Stolp H, Gadkari D (1981) Non-pathogenic members of the genus *Pseudomonas*. In: Starr MP, Truper HG, Balows A, Schlegel HG (eds) *The prokaryotes*, vol 1. Springer, Berlin Heidelberg New York, pp 719–741
- Tourova TP, Antonov AS (1987) Identification of microorganisms by rapid DNA–DNA hybridisation. *Methods Microbiol* 19:333–355
- Ventosa A, Gutierrez MC, Garcia MT, Ruiz-Berraquero F (1989) Classification of *Chromobacterium marismortui* in a new genus, *Chromohalobacter* gen. nov., as a *Chromohalobacter marismortui* comb. nov. nom. rev. *Int J Syst Bacteriol* 39:382–386
- 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
- Woese CR, Gutell R, Gupta R, Noller HF (1983) Detailed analysis of the higher order structure of 16S-like ribosomal ribonucleic acids. *Microbiol Rev* 47:621–669