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Mesonia algae gen. nov., sp. nov., a novel marine bacterium of the family *Flavobacteriaceae* isolated from the green alga *Acrosiphonia sonderi* (Kütz) Kornm

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The taxonomic position of four heterotrophic, aerobic, Gram-negative, non-motile and moderately halophilic marine bacteria, isolated from the green alga *Acrosiphonia sonderi* (Kütz) Kornm, was established. 16S rDNA sequence analysis indicated that the strains studied are members of the family *Flavobacteriaceae*, in which they form a distinct lineage. On the basis of phenotypic, chemotaxonomic, genotypic and phylogenetic data, the novel bacteria were classified as *Mesonia algae* gen. nov., sp. nov. The type strain is KMM 3909^T (=KCTC 12089^T=CCUG 47092^T).

Molecular techniques used to study complex marine ecosystems have essentially enriched our knowledge of the taxonomic diversity and structure of the microbial population, including uncultured and cultured micro-organisms (Britschgi & Giovannoni, 1991; Fuhrman *et al.*, 1993; Suzuki *et al.*, 1997; Brambilla *et al.*, 2001). Bacteria that belong to the phylum *Cytophaga–Flavobacterium–Bacteroides* (CFB) are often found in natural microbial communities of marine environments (Glöckner *et al.*, 1999; Bano & Hollibaugh, 2002; Kirchman, 2002). In recent years, isolation procedures supported by phylogenetic methods have led to the description of the novel genera *Gelidibacter*, *Psychroserpens*, *Polaribacter*, *Cellulophaga*, *Zobellia*, *Tenacibaculum*, *Arenibacter*, *Muricauda*, *Aequorivita* and *Reichenbachia* (Bowman *et al.*, 1997; Gosink *et al.*, 1998; Johansen *et al.*, 1999; Barbeyron *et al.*, 2001; Bruns *et al.*, 2001; Ivanova *et al.*, 2001; Suzuki *et al.*, 2001; Bowman & Nichols, 2002; Nedashkovskaya *et al.*, 2003). Among recently described marine bacteria of the family *Flavobacteriaceae* that have been isolated from unique Antarctic saline lakes are representatives of the genera *Psychroflexus* and *Salegentibacter*, which are characterized by the ability to

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Abbreviations: CCUG, Culture Collection University of Göteborg, Göteborg, Sweden; CFB, *Cytophaga–Flavobacterium–Bacteroides* group; KCTC, Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology, Daejon, Republic of Korea; KMM, Collection of Marine Micro-organisms of the Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of Russian Academy of Sciences, Vladivostok, Russia.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of *Mesonia algae* KMM 3909^T, KMM 3910, KMM 3936 and KMM 3937 are AF536383, AF536384, AF536385 and AF536386, respectively.

A micrograph showing rod-shaped cells of *Mesonia algae* is available as supplementary material in IJSEM Online.

grow in nutrient media that contain up to 15–20 % NaCl (Dobson *et al.*, 1993; Bowman *et al.*, 1998; McCammon & Bowman, 2000). In the course of the study of the microbial community associated with the common green alga *Acrosiphonia sonderi*, which inhabits coastal waters of the Sea of Japan, we isolated another novel, moderately halophilic bacterium that forms a phylogenetic cluster with species of the genera *Salegentibacter* and *Psychroflexus*. Phenotypic and genotypic characteristics and fatty acid and menaquinone compositions, in combination with phylogenetic findings, support the establishment a novel genus, for which the name *Mesonia* gen. nov. is proposed. In the present work, we describe a novel member of the family *Flavobacteriaceae*, which is designated *Mesonia algae* gen. nov., sp. nov.

Strains KMM 3909^T, KMM 3910, KMM 3936 and KMM 3937 were isolated from samples of the green alga *Acrosiphonia sonderi* that were collected in Troitsa Bay, Gulf of Peter the Great, Sea of Japan, during June 2000. For strain isolation, 0·1 ml homogenates of algal fronds were transferred onto plates of marine agar 2216 (Difco). After primary isolation and purification, strains were cultivated at 28 °C on the same medium and stored at −80 °C in marine broth (Difco) supplemented with 20 % (v/v) glycerol.

Oxidative or fermentative utilization of glucose was determined by using Hugh–Leifson medium modified for marine bacteria (Lemos *et al.*, 1985). Degradation of agar, starch, casein, gelatin, cellulose (filter paper and CM-cellulose), chitin, DNA, urea and alginic acids, flexirubin production, growth at different pH values, production of acid from carbohydrates and susceptibility to antibiotics were tested as described previously (Nedashkovskaya *et al.*, 2003). Gram-staining reaction, hydrolysis of Tweens 20, 40 and 80, nitrate reduction, production of hydrogen sulphide, indole and acetoin (Voges–Proskauer reaction) and activities of β-galactosidase, oxidase, catalase and alkaline phosphatase were tested according to the methods of Gerhardt *et al.* (1994). To examine carbon source utilization, commercial API 20NE identification strips (bioMérieux), following the instructions of the manufacturer, and a medium that contained 0·2 g $NaNO_3$, 0·2 g NH_4Cl, 0·05 g yeast extract (Difco) and 0·4 % (w/v) carbon source in 1000 ml artificial sea water were used. In order to study the temperature range for growth, bacteria were cultivated on medium A, which consisted of (l^{-1}): 5 g Bacto peptone (Difco), 2 g Bacto yeast extract (Difco), 1 g glucose, 0·02 g KH_2PO_4 and 0·05 g $MgSO_4.7H_2O$ in 50 % (v/v) natural sea water and 50 % (v/v) distilled water. Bacterial growth at different concentrations of NaCl was checked on medium A that was prepared with distilled water and contained 0, 1, 2, 3, 5, 6, 8, 10, 12, 13, 15, 16, 17 or 18 % (w/v) NaCl. Spreading growth was observed by cultivation on medium B, which contained (l^{-1}): 1 g Bacto peptone (Difco), 1 g yeast extract (Difco), 15 g agar and half-strength natural sea water under high-moisture conditions. Gliding motility was determined as described by Bowman (2000). On marine agar, colonies of strains KMM 3909^T, KMM 3936 and KMM 3937 were yellow-pigmented and those of strain KMM 3910 were whitish. Growth of strains KMM 3936 and KMM 3937 occurred in media that contained 1–12 % NaCl; strains KMM 3909^T and KMM 3910 grew in the presence of 1–15 % NaCl. Other physiological and biochemical characteristics of the strains studied are listed in the species description and Table 1. Cell morphology was observed by scanning electron microscopy (SEM) as described by Bruns *et al.* (2001). The rod-shaped cells ranged from 0·4 to 0·5 μm in width and from 1·6 to 2·3 μm in length (see Supplementary Fig. A in IJSEM Online). Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Microbial Identification system (Microbial ID). The predominant cellular fatty acids of KMM 3909^T were straight- and branched-chain unsaturated fatty acids, namely i-$C_{15:0}$, i-$C_{15:1}$, i-$C_{16:0}$, i-$C_{16:0}$ 3-OH and i-$C_{17:0}$ 3-OH (Table 2). Isoprenoid quinones were extracted from lyophilized cells and analysed as described by Akagawa-Matsushita *et al.* (1992). Menaquinones were detected by monitoring at 270 nm and were identified by comparison with known quinones from the reference strain *Salegentibacter salegens* DSM 5424^T. The main isoprenoid quinone of the strains studied was MK-6. DNA was isolated following the method of Marmur (1961) and the G+C content of the DNA was determined by the thermal denaturation method (Marmur & Doty, 1962). DNA–DNA hybridization was performed spectrophotometrically and initial renaturation rates were recorded as described by De Ley *et al.* (1970). The DNA G+C content of the strains studied ranged from 32·7 to 34·0 mol%, as determined by the thermal denaturation method. The level of DNA–DNA binding between KMM 3909^T, KMM 3910, KMM 3936 and KMM 3937 was 81–95 %.

Genomic DNA extraction, PCR and sequencing of 16S rDNA followed previous procedures (Kim *et al.*, 1998). Obtained sequence data were aligned with those of representative members of selected genera that belong to the family *Flavobacteriaceae* by using PHYDIT version 3.2 (http://plaza.snu.ac.kr/~jchun/phydit/). Phylogenetic trees were inferred by using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from the model of Jukes & Cantor (1969) and trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1993) algorithms. Bootstrap analysis was performed with 1000 resampled datasets by using the SEQBOOT and CONSENSE programs of the PHYLIP package.

Phylogenetic analysis of almost-complete 16S rDNA sequences (1385 nucleotide positions) revealed that the strains studied formed a distinct lineage within the family *Flavobacteriaceae* (Bernardet *et al.*, 2002), whilst belonging to a cluster group with [*Cytophaga*] *latercula*, *S. salegens* and *Psychroflexus* species (Fig. 1). 16S rDNA sequence similarity values of the strains studied and their close relatives,

Table 1. Differential characteristics of *Mesonia algae* and other allied genera of the family *Flavobacteriaceae*

Taxa: 1, *Mesonia algae*; 2, *Salegentibacter salegens*; 3, *Psychroflexus*; 4, *Polaribacter*; 5, *Tenacibaculum*; 6, [*C.*] *latercula*; 7, *Cellulophaga*. Data from Reichenbach (1989), Dobson *et al.* (1993), Bowman *et al.* (1998), Gosink *et al.* (1998), Johansen *et al.* (1999), McCammon & Bowman (2000), Suzuki *et al.* (2001) and this study. All strains are negative for anaerobic growth and flexirubin pigments. Abbreviations: V, variable; ND, not determined; NG, no growth.

Characteristic	1	2	3	4	5	6	7
Gliding motility	−	−	V	−	+	−	+
Oxidase/catalase	+/+	+/+	+/+	V/+	+/+	+/−	+/+
Requirement for Na$^+$ for growth	+	−	V	+	+	+	+
Acid formation from carbohydrates	−	+	+	+	V	+	+
Growth at:							
37 °C	−	−	−	−	+	−	V
15 % NaCl	+	+	V	−	−	−	−
Hydrolysis of:							
Agar	−	−	−	−	−	+	+
Casein	+	−	−	NG	+	+	V
Gelatin	+	+	+	V	V	−	+
Starch	−	+	+	+	V	+	+
DNA	−	+	+	ND	+	+	+
Urea	−	V	V	−	+	ND	V
Nitrate reduction	−	+	−	V	V	+	V
H_2S production	+	+	−	−	V	+	−
DNA G+C content (mol%)	32–34	37–38	32–36	31–33	31–33	34	33–38

[*C.*] *latercula*, *Psychroflexus torquis* and *Psychroflexus gondwanensis*, were 91·1, 90·4 and 90·6 %, respectively. The nearest neighbour, *S. salegens* DSM 5424^T, had 92·6 % 16S rDNA sequence similarity (102 nucleotide differences) with KMM 3909^T and KMM 3910 without significant bootstrap support (61 %). In addition, *S. salegens* DSM 5424^T was rather closer to [*C.*] *latercula* ATCC 23177^T (16S rDNA similarity between them was 92·8 %), whereas the latter shared around 91·2 % similarity with the strains studied.

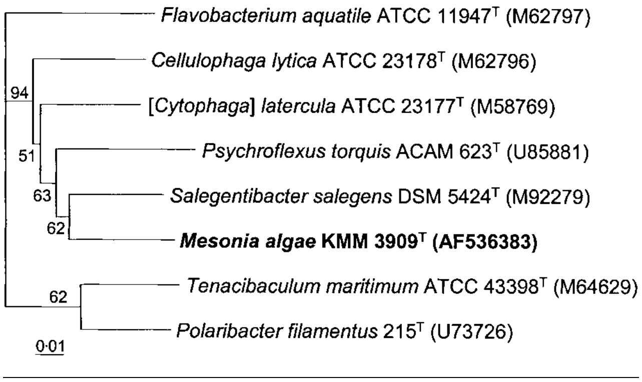

Fig. 1. Phylogenetic tree based on 16S rDNA sequences of strain KMM 3909^T and representative members of related genera in the family *Flavobacteriaceae*. The tree was generated by the neighbour-joining method (Saitou & Nei, 1987). Numbers at nodes indicate bootstrap values (%). Bar, 0·01 substitutions per nucleotide position.

The overall topology of the unrooted tree based on 16S rDNA was not changed in the trees by using other algorithms, except that KMM 3909^T clustered with *P. torquis* ACAM 623^T in the first place and then with *S. salegens* DSM 5424^T in the maximum-likelihood tree (data not shown).

It is therefore evident from phylogenetic analysis that the strains studied can be considered to form a genus separate from *Salegentibacter*. Low sequence similarities of the strains tested with other members of the CFB phylum described to date (87·2–91·1 %) demonstrate clearly that the bacteria isolated in this study represent a novel genus. Significant differences in the whole-cell fatty acid compositions of strains KMM 3909^T and *S. salegens* DSM 5424^T should be noted (Table 2). For example, fatty acid a-C$_{15:1}$ presents in significant quantity in the cell extract of *S. salegens* DSM 5424^T (3·5 %) but only in minor quantity in that of strain KMM 3909^T (0·4 %). The hydroxy fatty acid C$_{15:0}$ 3-OH is represented in *S. salegens* DSM 5424^T (3·9 %) but is absent in strain KMM 3909^T. Fatty acid a-C$_{17:1}\omega 9c$ was found in the cell extract of strain KMM 3909^T (1·9 %), but not in that of *S. salegens* DSM 5424^T. Phenotypic examination of the strains studied (including their inability to grow without Na$^+$ ions and to utilize carbohydrates, lack of DNase activity, absence of starch hydrolysis, presence of casein hydrolysis and negative nitrate reduction), in combination with phylogenetic differences and distinctiveness of cellular fatty acid composition, allows the differentiation of strains KMM 3909^T, KMM 3910, KMM 3936 and KMM 3937 from their closest relative, *S. salegens*. Phenotypic features that separate the

Table 2. Whole-cell fatty acid profiles (percentage composition) of strain KMM 3909^T and *Salegentibacter salegens* DSM 5424^T

Fatty acid	KMM 3909^T	*S. salegens* DSM 5424^T
$C_{12:1}$		0·4
i-$C_{13:0}$		0·2
i-$C_{14:0}$	0·9	
i-$C_{15:0}$	21·2	8·5
a-$C_{15:0}$	4·2	8·5
i-$C_{15:1}$	7·9	17·7
a-$C_{15:1}$	0·4	3·5
$C_{15:0}$	4·8	7·6
$C_{15:1}\omega 6c$	1·8	5·7
i-$C_{16:0}$	6·3	3·1
i-$C_{16:1}$	4·2	2·6
$C_{16:0}$	0·5	0·4
$C_{16:1}\omega 7$ + i-$C_{15:0}$ 2-OH	5·1	6·1
i-$C_{17:1}\omega 9c$	5·1	2·0
a-$C_{17:1}\omega 9c$	1·9	
$C_{17:1}\omega 8c$	0·8	
$C_{17:1}\omega 6c$	2·4	4·0
i-$C_{14:0}$ 3-OH	0·4	
$C_{15:0}$ 2-OH	1·4	2·4
i-$C_{15:0}$ 3-OH	3·2	2·5
$C_{15:0}$ 3-OH		3·9
i-$C_{16:0}$ 3-OH	6·0	5·9
$C_{16:0}$ 3-OH		0·7
i-$C_{17:0}$ 3-OH	14·6	5·9
$C_{17:0}$ 2-OH	1·8	4·7
$C_{17:0}$ 3-OH	0·6	
Unknown	3·3	2·2

strains studied from other relatives of the family *Flavobacteriaceae* are listed in Table 1.

Thus, the polyphasic data presented in this paper support the conclusion that the bacteria studied could not be affiliated to any taxa currently included in the family *Flavobacteriaceae*. Consequently, we propose that strains KMM 3909^T, KMM 3910, KMM 3936 and KMM 3937 should be placed in a novel genus as *Mesonia algae* gen. nov., sp. nov.

Description of *Mesonia* gen. nov.

Mesonia [Me.so′ni.a. N.L. fem. n. *Mesonia* arbitrary name derived from the abbreviation MES (Marine Experimental Station of the Pacific Institute of Bioorganic Chemistry, FEB RAS) near the site where the bacteria were first isolated].

Rod-shaped, non-motile, Gram-negative cells. Endospores are not formed. Na^+ ions are required for growth. Strictly aerobic. Non-diffusible yellow pigments are produced. No flexirubins are formed. Chemo-organotrophic. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. Major respiratory quinone is MK-6. Main cellular fatty acids are the straight- and branched-chain unsaturated fatty acids i-$C_{15:0}$, i-$C_{15:1}$, i-$C_{16:0}$, i-$C_{16:0}$ 3-OH and i-$C_{17:0}$ 3-OH. As determined by 16S rDNA sequence analysis, the genus *Mesonia* is a member of the family *Flavobacteriaceae* in the phylum *Cytophaga–Flavobacterium–Bacteroides*. The type species is *Mesonia algae*.

Description of *Mesonia algae* sp. nov.

Mesonia algae (al′gae. L. gen. n. *algae* of alga, seaweed; bacterium isolated from alga).

Main characteristics are otherwise the same as those given for the genus. In addition, cells range from 0·4 to 0·5 μm in width and from 1·6 to 2·3 μm in length. On marine agar, colonies are 2–4 mm in diameter, circular, shiny with entire edges and yellow in colour (whitish strains occur). Growth is observed at 4–34 °C. Optimal temperature for growth is 21–23 °C. Growth occurs at 1–15 % NaCl. Gelatin, casein and Tweens 20, 40 and 80 are decomposed. No hydrolysis of agar, alginate, starch, cellulose (CM-cellulose or filter paper) or chitin occurs. No acid is formed from arabinose, cellobiose, fucose, galactose, glucose, lactose, maltose, mannose, melibiose, raffinose, rhamnose, sucrose, xylose, citrate, adonitol, dulcitol, glycerol, inositol or mannitol. Arabinose, glucose, lactose, mannose, sucrose, mannitol, inositol, sorbitol, *N*-acetylglucosamine, gluconate, caprate, adipate, malate, malonate, phenylacetate and citrate are not utilized. Nitrate is not reduced. H_2S is produced. Indole and acetoin (Voges–Proskauer reaction) production is negative. G + C content of the DNA is 32·7–34·0 mol%.

Type strain is KMM 3909^T (= KCTC 12089^T = CCUG 47092^T). Isolated from the green alga *Acrosyphonia sonderi*, collected in Troitsa Bay of the Gulf of Peter the Great of the Sea of Japan.

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