Salegentibacter agarivorans sp. nov., a novel marine bacterium of the family *Flavobacteriaceae* isolated from the sponge *Artemisina* sp.

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A sponge-associated strain, KMM 7019^T, was investigated in a polyphasic taxonomic study. The bacterium was strictly aerobic, heterotrophic, Gram-negative, yellow-pigmented, motile by gliding and oxidase-, catalase-, β-galactosidase- and alkaline phosphatase-positive. A phylogenetic analysis based on 16S rRNA gene sequences revealed that strain KMM 7019^T is closely related to members of the genus *Salegentibacter*, namely *Salegentibacter holothuriorum*, *Salegentibacter mishustinae* and *Salegentibacter salegens* (97·7–98 % sequence similarities). The DNA–DNA relatedness between the strain studied and *Salegentibacter* species ranged from 27 to 31 %, clearly demonstrating that KMM 7019^T belongs to a novel species of the genus *Salegentibacter*, for which the name *Salegentibacter agarivorans* sp. nov. is proposed. The type strain is KMM 7019^T (=KCTC 12560^T=LMG 23205^T).

Bacteria belonging to the genus *Salegentibacter*, a member of the family *Flavobacteriaceae*, are aerobic, halotolerant or halophilic, pigmented yellow or yellow–orange and motile by gliding (McCammon & Bowman, 2000). At present, there are three *Salegentibacter* species with validly published names: *Salegentibacter salegens*, formerly *Flavobacterium salegens* (Dobson *et al.*, 1993), isolated from a meromictic lake in Antarctica; *Salegentibacter holothuriorum*, recovered from the edible holothurian *Apostichopus japonicus* in the Sea of Japan; and *Salegentibacter mishustinae*, isolated from the sea urchin *Strongylocentrotus intermedius* (McCammon & Bowman, 2000; Nedashkovskaya *et al.*, 2004, 2005). The genus *Mesonia* is the nearest phylogenetic neighbour of *Salegentibacter* species.

During the 29th cruise of the *R/V Akademician Oparin*, a novel agar-decomposing bacterium, strain KMM 7019^T, was isolated from a sponge (*Artemisina* sp.) collected in July 2003 near Onecotan Island, Kuril Islands, Sea of Okhotsk, Pacific Ocean, from a depth of 150 m. For strain isolation, 0·1 ml tissue homogenate was transferred to marine agar (Difco) plates. After primary isolation and purification the strain was cultivated at 28 °C on the same medium and stored at −80 °C in marine broth (Difco) supplemented with 20 % (v/v) glycerol.

Genomic DNA extraction, PCRs and sequencing of the 16S rRNA gene were performed according to published procedures (Kim *et al.*, 1998). The sequence obtained was aligned, using PHYDIT, version 3.2 (http://plaza.snu.ac.kr/~jchun/phydit/), with those of representative members of selected genera belonging to the family *Flavobacteriaceae*. Phylogenetic trees were inferred by using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from the models of Kimura (1980), and trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein,

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KMM 7019^T is DQ191176.

1993) algorithms. Bootstrap analysis was performed with 1000 resampled datasets by using the SEQBOOT and CONSENSE programs of the PHYLIP package.

Analysis of 16S rRNA gene sequences indicated that strain KMM 7019^T is a member of the family *Flavobacteriaceae* and that its nearest neighbours are *Salegentibacter holothuriorum* KMM 3524^T, *Salegentibacter mishustinae* KMM 6049^T and *Salegentibacter salegens* DSM 5424^T, with sequence similarities of 97·7–98·0 % (Fig. 1).

DNA was isolated by following the method of Marmur (1961), and the G+C content was determined by using the thermal denaturation method (Marmur & Doty, 1962). The DNA G+C content of the strain studied was 39·2 mol%. DNA–DNA hybridization experiments were performed using the method described by De Ley *et al.* (1970). DNA–DNA relatedness levels between strain KMM 7019^T and *Salegentibacter* species were in the range 27–31 %. These values are significantly below 70 %, and, consequently, the strain studied represents a novel species of the genus *Salegentibacter* (Wayne *et al.*, 1987).

Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Microbial Identification System (Microbial ID). All strains tested were grown on marine agar at 25 °C for 48 h. The predominant cellular fatty acids were $C_{15:1}$ iso (12·1 %), $C_{15:0}$ iso (12·3 %), $C_{15:0}$ anteiso (7·1 %), $C_{15:0}$ (5·6 %), $C_{17:0}$ iso 3-OH (9·8 %) and summed feature 3 (12·2 %), comprising $C_{16:1}\omega7c$ and/or $C_{15:0}$ iso 2-OH. The complete fatty acid content of strain KMM 7019^T is given in Table 1 and compared with that of other *Salegentibacter* species.

A phenotypic analysis was performed using methods described previously (Nedashkovskaya *et al.*, 2003a, b). API ZYM strips (bioMérieux) and Microlog GN2 plates (Biolog) were also used to assess physiological and biochemical features; these were employed according to the manufacturers' instructions, except that the solution used for the bacterial suspension consisted of 1·5 % NaCl saline, and the strips and the microplates were incubated at 25 °C. Motility was determined as described by Bowman (2000).

The physiological, biochemical and morphological characteristics of strain KMM 7019^T are listed in the species description and in Table 2. The phenotypic features of the strain tested are consistent with those of the *Salegentibacter* species (Table 2). However, strain KMM 7019^T differs from all *Salegentibacter* species with validly published names by its ability to grow at 41 °C, to hydrolyse agar, to form acid from L-arabinose, D-cellobiose and DL-xylose, to utilize L-arabinose, to move by means of gliding, and by its susceptibility to kanamycin and neomycin. Additional phenotypic traits also distinguish the strain studied from some of the *Salegentibacter* species (Table 2).

Thus, genomic divergences supported by phenotypic and chemotaxonomic data allow affiliation of strain KMM 7019^T to the genus *Salegentibacter*, in which it forms a distinct lineage, as *Salegentibacter agarivorans* sp. nov.

Description of *Salegentibacter agarivorans* sp. nov.

Salegentibacter agarivorans (a.ga.r.i.vo′rans. N.L. n. *agarum* agar, algal polysaccharide; L. v. *vorare* to devour, to digest; N.L. part. adj. *agarivorans* agar-digesting).

Cells are Gram-negative, strictly aerobic, chemo-organotrophic, asporogenic rods 0·5–0·7 μm wide and 2·0–4·7 μm long and motile by gliding. Oxidase-, catalase-, β-galactosidase- and alkaline phosphatase-positive. Colonies are circular, convex, slimy, shiny with entire edges, sunken into the agar and 1–3 mm in diameter on marine agar 2216. Produces yellow, carotenoid, non-diffusible pigments. Flexirubin-type pigments are absent. Grows in the presence of 1–18 % NaCl; growth optimum observed at 2–4 % NaCl. Growth

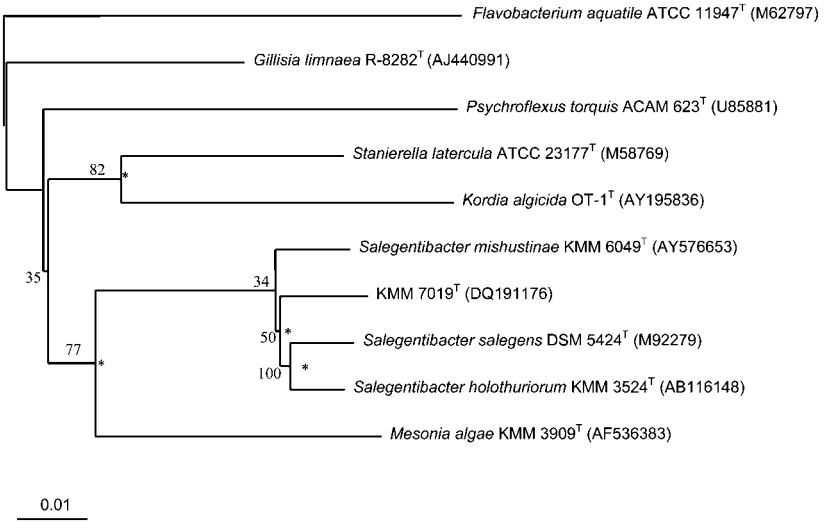

Fig. 1. Phylogenetic tree based on the 16S rRNA gene sequences of KMM 7019^T and related members of the family *Flavobacteriaceae*. Asterisks indicate branches that were also recovered in the maximum-likelihood algorithm. Numbers at nodes indicate levels of bootstrap support (%) from 1000 resamplings. Bar, 0·01 substitutions per nucleotide position.

Table 1. Cellular fatty acid content (%) of the *Salegentibacter* species

Fatty acids amounting to less than 1 % of the total in all strains studied are not listed. The fatty acid content of *Salegentibacter holothuriorum* KMM 3524^T was not included in the table because it was determined for bacteria grown under conditions different from those used for the other species. Data are from Nedashkovskaya *et al.* (2003c, 2005) and this study. ND, Not detected.

Fatty acid	*Salegentibacter agarivorans* KMM 7019^T	*Salegentibacter mishustinae* KMM 6049^T	*Salegentibacter salegens* DSM 5424^T
$C_{15:1}$ iso	**12·1**	**12·3**	**17·7**
$C_{15:1}$ anteiso	1·9	1·3	3·5
$C_{15:0}$ iso	**12·3**	**12·1**	**8·5**
$C_{15:0}$ anteiso	**7·1**	**7·9**	**8·5**
$C_{15:0}$	**5·6**	**6·7**	**7·6**
$C_{15:1}\omega6c$	2·3	2·4	5·7
$C_{16:1}$ iso	1·5	1·9	2·6
$C_{16:0}$ iso	2·8	4·6	3·1
$C_{16:0}$	ND	1·8	ND
$C_{15:0}$ 3-OH iso	3·6	2·9	2·5
$C_{15:0}$ 3-OH	2·3	2·1	3·9
$C_{15:0}$ 2-OH	3·5	2·6	2·4
$C_{17:1}\omega9c$ iso	2·5	2·2	2·0
$C_{17:1}\omega6c$	3·8	3·5	4·1
$C_{16:0}$ 3-OH iso	**3·9**	**4·7**	**5·9**
$C_{16:0}$ 3-OH	1·3	0·5	ND
$C_{17:0}$ 3-OH iso	**9·8**	**8·6**	**5·9**
$C_{17:0}$ 2-OH	4·5	3·1	4·7
$C_{18:0}$	ND	1·3	ND
Summed feature 3*	**12·2**	**7·9**	**6·1**

*Summed features consist of one or more fatty acids that could not be separated by the Microbial Identification System. Summed feature 3: $C_{15:0}$ 2-OH iso and/or $C_{16:1}\omega7c$.

detected at 4–41 °C, with an optimum at 28–32 °C. The pH range for growth is 5·7–10·0, with optimum growth occurring between pH 7·5 and 8·3. Hydrolyses agar, gelatin, starch, alginic acids, DNA and Tweens 20, 40 and 80, but not casein, cellulose (CM-cellulose and filter paper), chitin or urea. Forms acid from L-arabinose, D-cellobiose, D-fructose, L-fucose, D-galactose, D-glucose, DL-xylose, D-lactose, D-maltose, L-raffinose, D-sucrose and *N*-acetylglucosamine, but not from L-rhamnose, L-sorbose, adonitol, dulcitol, glycerol, inositol, sorbitol or mannitol. Utilizes D-mannose, but not inositol, sorbitol, mannitol, citrate or malonate. In the Microlog GN2 plate, KMM 7019^T utilizes α-D-glucose, α-lactose, sucrose, methyl pyruvate, monomethyl succinate, D-galactonic acid, D-gluconic acid, β-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketoglutaric acid, DL-lactic acid, propionic acid, succinic acid, succinamic acid, alaninamide, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, L-phenylalanine, L-proline, L-pyroglutamic acid, L-threonine and urocanic acid. Does not utilize α-cyclodextrin, dextrin, glycogen, *N*-acetyl-D-galactosamine, adonitol, L-arabitol, i-erythritol, gentiobiose, *myo*-inositol, lactulose, D-mannitol, D-melibiose, methyl β-D-glucoside, psicose, D-sorbitol, D-trehalose, turanose, xylitol, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketobutyric acid, α-ketovaleric acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, glucuronamide, D-alanine, L-alanine, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, D-serine, L-serine, DL-carnitine, γ-aminobutyric acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, glucose 1-phosphate or glucose 6-phosphate. In the API ZYM gallery, KMM 7019^T produces α-galactosidase, β-galactosidase, alkaline phosphatase, acid phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase, but not esterase (C4), lipase (C14), cystine arylamidase, β-glucuronidase, α-mannosidase or α-fucosidase. H_2S is produced. Nitrates are reduced to nitrites under oxic conditions. Does not produce indole or acetoin (Voges–Proskauer reaction). Susceptible to chloramphenicol, doxycycline, erythromycin, kanamycin, neomycin, oleandomycin and streptomycin. Resistant to ampicillin,

Table 2. Phenotypic characteristics of *Salegentibacter agarivorans* sp. nov. KMM 7019^T and other *Salegentibacter* species

Taxa: 1, *Salegentibacter agarivorans* sp. nov. (KMM 7019^T; this study); 2, *Salegentibacter holothuriorum* (KMM 3524^T; data from Nedashkovskaya *et al.*, 2004); 3, *Salegentibacter mishustinae* (KMM 6049^T; data from Nedashkovskaya *et al.*, 2005); 4, *Salegentibacter salegens* (DSM 5424^T; data from Dobson *et al.*, 1993; McCammon & Bowman, 2000; and this study). All strains were positive for the following characteristics: respiratory metabolism; oxidase, catalase, β-galactosidase and alkaline phosphatase activities; hydrolysis of Tweens 20, 40 and 80, gelatin, elastin, starch and alginic acids; growth at 34 °C and with 8 % NaCl; acid production from D-maltose; utilization of D-glucose and D-mannose; production of H_2S; susceptibility to oleandomycin; and resistance to gentamicin and polymyxin B. All strains were negative for the following characteristics: hydrolysis of cellulose (CM-cellulose and filter paper), urea and chitin; acid production from L-rhamnose, L-sorbose, succinate, citrate, glycerol, adonitol, dulcitol, sorbitol, inositol and mannitol; utilization of inositol, mannitol, sorbitol, malonate and citrate; and production of indole and acetoin (Voges–Proskauer reaction).

Characteristic	1	2	3	4
Gliding motility	+	−	−	−
NaCl requirement for growth	+	+	+	−
Nitrate reduction	+	−	−	+
Growth at 41 °C	+	−	−	−
Growth with 18 % NaCl	+	−	+	+
Hydrolysis of:				
Agar	+	−	−	−
Casein	−	−	+	−
DNA	+	+	−	+
Acid production from:				
L-Arabinose, DL-xylose, D-cellobiose	+	−	−	−
D-Sucrose, L-raffinose	+	−	+	−
D-Galactose, D-glucose	+	+	−	+
D-Lactose, L-fucose, *N*-acetylglucosamine	+	+	−	−
Utilization of:				
L-Arabinose	+	−	−	−
D-Sucrose	+	−	+	+
D-Lactose	+	+	+	−
Susceptibility to:				
Ampicillin, benzylpenicillin, lincomycin, tetracycline	−	+	+	+
Carbenicillin	−	+	−	+
Kanamycin, neomycin	+	−	−	−
Streptomycin	+	−	−	+
DNA G+C content (mol%)	39·2	37·5	37·5	37·8

benzylpenicillin, carbenicillin, lincomycin, gentamicin, tetracycline and polymyxin B. The predominant cellular fatty acids are $C_{15:1}$ iso (12·1 %), $C_{15:0}$ iso (12·3 %), $C_{15:0}$ anteiso (7·1 %), $C_{15:0}$ (5·6 %), $C_{17:0}$ iso 3-OH (9·8 %) and summed feature 3 (12·2 %), comprising $C_{16:1}\omega7c$ and/or $C_{15:0}$ iso 2-OH. The DNA G+C content is 39·2 mol%.

The type strain, KMM 7019^T (=KCTC 12560^T=LMG 23205^T), was isolated from a sponge (*Artemisina* sp.) collected near Onecotan Island, Kuril Islands, Sea of Okhotsk, Pacific Ocean.

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