Psychroserpens mesophilus sp. nov., a mesophilic marine bacterium belonging to the family *Flavobacteriaceae* isolated from a young biofilm

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A number of marine bacteria isolated from young biofilms were characterized as belonging to the family *Flavobacteriaceae*. The taxonomic characterization of strain KOPRI 13649^T, which was isolated from an acrylic surface at the seashore at Gangneung, Korea, is reported here. The nearly complete 16S rRNA gene sequence of strain KOPRI 13649^T was determined and was found to have a high level of similarity with that of *Psychroserpens burtonensis* (95·0–95·6 %). In addition, phylogenetic analysis and comparison with closely related strains confirmed that the strain represented a novel member of the genus *Psychroserpens*. The major respiratory quinone of strain KOPRI 13649^T was MK-6 and the DNA G+C content was 29·8 mol%. The dominant fatty acid methyl esters were i-15 : 0, a-15 : 0, i-16 : 0, i-15 : 1ω10, 16 : 1ω7 and 15 : 0. Growth was observed at 10–34 °C (optimum 30 °C), at pH 6–9 (optimum 6·5–8·0) and with 0·5–4 % NaCl (optimum 1 %). On the basis of the polyphasic taxonomic evidence presented, strain KOPRI 13649^T (=KCCM 42261^T=JCM 13413^T) should be classified as the type strain of a novel species in the genus *Psychroserpens*, for which the name *Psychroserpens mesophilus* sp. nov. is proposed.

The genus *Psychroserpens* is a member of the family *Flavobacteriaceae* in the phylum *Bacteroidetes*. *Psychroserpens burtonensis*, isolated from the Antarctic ice at Lake Burton (Bowman *et al.*, 1997), is the only currently recognized member of the genus. In the last decade, several bacterial isolates have been reported as putative members of the genus *Psychroserpens* (Brinkmeyer *et al.*, 2003; DeLong *et al.*, 1993; Li *et al.*, 1999) but have yet to be formally taxonomically characterized. We have isolated several bacteria belonging to the family *Flavobacteriaceae* from a young marine biofilm and herein characterize one of these, designated strain KOPRI 13649^T, as representing a novel member of the genus *Psychroserpens*.

Strain KOPRI 13649^T was isolated from an acrylic surface after immersion in sea water for 3 days. The acrylic surface was withdrawn from the sea water, and the biofilm that had formed on it was detached, dispersed into sterilized sea water and spread on marine agar 2216 (MA; Difco). Among the colonies subsequently formed, a yellow-coloured, morphologically distinct colony was isolated and named KOPRI 13649^T. This strain was cultivated on MA for morphological and biochemical characterization.

Unless otherwise stated, the physiological and morphological characterization was conducted according to the methods given by Sohn *et al.* (2004) and Kwon *et al.* (2005a). Requirement for NaCl was tested using modified marine broth 2216 (5 g peptone, 1 g yeast extract, 0·01 g $FePO_4$, 1·89 g $MgCl_2.6H_2O$, 0·36 g $CaCl_2.2H_2O$, per litre distilled water) supplemented with 0, 0·5, 1, 2, 3, 4, 5, 6, 7, 10, 15 or 20 % (w/v) NaCl. Degradation of starch and casein and production of hydrogen sulfide were tested by using the procedures of Smibert & Krieg (1994). The physiological, biochemical and morphological characteristics of strain KOPRI 13649^T are given in the species description (see later) and in Table 1.

Cells of strain KOPRI 13649^T were Gram-negative, non-motile rods, 0·57–0·63 μm in length and 0·41–0·51 μm in diameter. During growth, the length of the cells increased up to 1·7 μm. Colonies were yellow to yellowish orange when grown on MA plates at 30 °C. Growth of strain KOPRI

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KOPRI 13649^T is DQ001321.

A table detailing the cellular fatty acid content of strain KOPRI 13649^T and closely related members of the *Flavobacteriaceae* is available as supplementary material in IJSEM Online.

Table 1. Phenotypic characteristics differentiating strain KOPRI 13649^T from closely related members of the family *Flavobacteriaceae*

Taxa: 1, KOPRI 13649^T; 2, *Psychroserpens burtonensis*; 3, *Formosa algae* KMM 3553^T; 4, *Algibacter lectus* KMM 3914^T; 5, *Gelidibacter*. Data were taken from Bowman *et al.* (1997), Macián *et al.* (2002), Ivanova *et al.* (2004), Nedashkovskaya *et al.* (2004) and Bowman & Nichols (2005). All strains have menaquinone MK-6 as the major respiratory quinone, produce catalase and do not produce H_2S or indole. All are able to liquefy gelatin. +, Positive; −, negative; ND, not determined; V, variable.

Characteristic	1	2*	3	4	5*
Cell size (μm)	0·6–1·7 × 0·4–0·5	2–6 × 0·5–0·6	0·8–1·8 × 0·4–0·9	2–3 × 0·4–0·5	1–4 × 0·5
Colony size (mm)	2–3	4–5	1–3	3–4	2–3
Gliding motility	−	−	+	+	+
Growth range:					
Temperature (°C)†	10–34 (30)	0–<20 (10–12)	5–35 (23)	4–35 (21–23)	0–37 (15–18)
pH†	6–9 (6·5–8)	ND	5·0–10·0 (8·0–8·5)	ND	ND
NaCl concentration (%)†	0·5–4 (1)	1·4–5·2	0–6	1–6	1–8
Sea water requirement‡	+	+	−	+	+
Growth requirements	−	YE§/vitamin	YE	YE	YE/organic N
O_2 requirement	+	+	−	−	+
Oxidase activity	+	−	+	+	−
Nitrate reduction	+	−	+	−	−
Production of urease	−	−	+	−	−
Acid from carbohydrate	−	−	+	+	+
Hydrolysis of:					
Agar	−	−	−	+	−
Casein	+	+	−	−	V
Starch	−	−	+	V	+
DNA G+C content (mol%)	29·8	27–29	34–35	31–33	36–40

*Percentage range for all species in the genus or for all strains in the species.

†Values in parentheses are the optimum range.

‡Requirement of sea water indicates that Na^+ alone does not support growth; instead, the strain requires additional cations for growth, such as Mg^{2+} and Ca^{2+}, present in sea water.

§YE, yeast extract.

13649^T was observed at 10–34 °C; growth was poor at temperatures below 20 °C or above 34 °C, and no growth was observed at 8 or 37 °C.

Phylogenetic analysis using the 16S rRNA gene sequence of strain KOPRI 13649^T was conducted according to the methods of Sohn *et al.* (2004). The sequence was compared against the 16S rRNA gene sequences of strains belonging to *P. burtonensis* and of members of the genera *Winogradskyella* (Lau *et al.*, 2005; Nedashkovskaya *et al.*, 2005a), *Gelidibacter* (Bowman *et al.*, 1997; Bowman & Nichols, 2005; Macián *et al.*, 2002) and *Subsaximicrobium* (Bowman & Nichols, 2005), *Subsaxibacter broadyi* (Bowman & Nichols, 2005), *Yeosuana aromativorans* (Kwon *et al.*, 2005b), *Bizionia paragorgiae* (Nedashkovskaya *et al.*, 2005b), *Formosa algae* (Ivanova *et al.*, 2004), *Algibacter lectus* (Nedashkovskaya *et al.*, 2004) and *Lacinutrix copepodicola* (Bowman & Nichols, 2005). Closest sequence similarity was to *P. burtonensis* (95·0–95·6 %). Phylogenetic analysis based on 16S rRNA gene sequences placed strain KOPRI 13649^T within the outer area of strains belonging to *P. burtonensis*, but in the same phylogenetic line with the genus *Psychroserpens* (Fig. 1).

The cellular fatty acid methyl ester profile of strain KOPRI 13649^T was determined according to the methods given by Sohn *et al.* (2004). The dominant fatty acid methyl esters were i-15 : 0 (31·4 %), a-15 : 0 (10·9 %), i-16 : 0 (7·3 %), i-15 : 1ω10 (7·1 %), 16 : 1ω7 (5·6 %) and 15 : 0 (5·6 %). The isolate also contained small amounts of i-14 : 0 (4·1 %), 17 : 0 cyclic (3·5 %), 10-methyl 16 : 0 (3·2 %), a-17 : 0 (3·0 %), i-17 : 0 (2·7 %), 16 : 0 (2·2 %), 15 : 1 (1·6 %) and 14 : 0 (1·4 %). This profile differed from that of *P. burtonensis* (Bowman *et al.*, 1997) as follows: relatively large amounts of i-15 : 0, presence of i-14 : 0, i-16 : 0, a-17 : 0, 10-methyl-16 : 0 and 17 : 0 cyclic, and insignificant amounts of 15 : 1, a-15 : 1 and br-16 : 1 (for more complete details see Supplementary Table S1 available in IJSEM Online). The difference in optimal growth temperature might explain these differences.

The DNA G+C content of strain KOPRI 13649^T, as determined using the thermal denaturation method of Kim *et al.* (2000), was 29·8 mol%, a value similar to that of *P. burtonensis*. The major respiratory quinone was determined to be menaquinone according to the reversed-phase TLC method described by Kim *et al.* (2000) and was

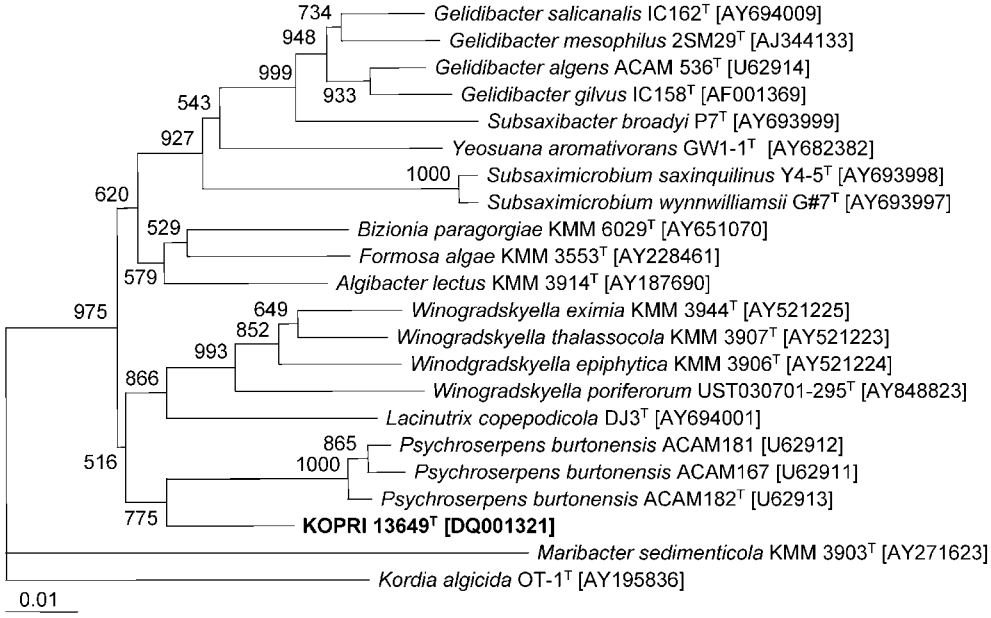

Fig. 1. Phylogenetic tree based on nearly complete 16S rRNA gene sequences (1326 unambiguously aligned base pairs) showing the relationship between strain KOPRI 13649^T and other members of the family *Flavobacteriaceae*. The tree is based on the maximum-likelihood distances model and the neighbour-joining method. Bootstrap values of >50 % (from 1000 resampled datasets) are shown. Bar, 0·01 nucleotide substitutions per nucleotide position.

confirmed to be MK-6 following the HPLC method of Collins (1985).

The results of our phylogenetic analysis combined with the DNA G+C determination provide strong support that strain KOPRI 13649^T should be classified as a member of the genus *Psychroserpens*. However, the isolate showed strict mesophilic growth characteristics and had a cellular fatty acid profile distinct from that of *P. burtonensis*. Thus, on the basis of the polyphasic taxonomic evidence presented, strain KOPRI 13649^T is considered to represent a novel species in the genus *Psychroserpens*, for which the name *Psychroserpens mesophilus* sp. nov. is proposed.

Description of *Psychroserpens mesophilus* sp. nov.

Psychroserpens mesophilus [me.so.phi′lus. Gr. adj. *mesos* middle; Gr. adj. *philus* loving; N.L. adj. *mesophilus* middle (temperature)-loving, mesophilic].

Cells are Gram-negative, non-motile rods, 0·41–0·51 × 0·57–1·67 μm. Gliding motility is absent. Colonies on solid MA are yellow to yellowish orange. Oxidase and catalase activities are positive. Positive for lysine decarboxylase, tryptophan deaminase, nitrate reduction, Voges–Proskauer reaction, and assimilation of glucose, mannose, mannitol, sorbitol, rhamnose, melibiose, amylose and arabinose (according to the API 20E kit). Oxidizes α-cyclodextrin, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, L-arabinose, D-fructose, α-D-glucose, maltose, D-trehalose, sucrose, methyl pyruvate, monomethyl succinate, α- and β-hydroxybutyric acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, propionic acid, D-alanine, L-leucine, L-ornithine, L-proline, L-serine, L-threonine, uridine, inosine and DL-α-glycerol phosphate (Microlog GN2 MicroPlate). Growth is observed at 10–34 °C (optimum 30 °C), at pH 6–9 (optimum pH 6·5–8·0) and with 0·5–4 % NaCl (optimum 1 %). Requires either 0·18 % (w/v) $CaCl_2$ or 0·59 % (w/v) $MgCl_2$ for growth in addition to NaCl. The major respiratory quinone is MK-6. The DNA G+C content is 29·8 mol%. The dominant fatty acids are i-15 : 0, a-15 : 0, i-16 : 0, i-15 : 1ω10, 16 : 1ω7 and 15 : 0.

The type strain, KOPRI 13649^T (=KCCM 42261^T=JCM 13413^T), was isolated from a young biofilm formed on an acrylic surface in Gangneung, Korea.

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