Olleya marilimosa gen. nov., sp. nov., an exopolysaccharide-producing marine bacterium from the family *Flavobacteriaceae*, isolated from the Southern Ocean

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A Gram-negative, aerobic, gliding, orange–yellow marine bacterium was isolated from particulate material sampled from the Southern Ocean. This strain produced an exopolysaccharide in liquid culture. 16S rRNA gene sequence analysis showed that this isolate was a member of the family *Flavobacteriaceae*, but represented a separate lineage. Major whole-cell fatty acids included i15 : 1ω10*c*, i15 : 0, β-OH i15 : 0, a15 : 1ω10*c*, 15 : 0 and α-OH i15 : 0. The G+C content of the DNA was 49 mol%. Based on phylogenetic, phenotypic, chemotaxonomic and genotypic analyses, this bacterium was placed in a novel taxon as *Olleya marilimosa* gen. nov., sp. nov. with type strain CAM030^T (=ACAM 1065^T=CIP 108537^T).

The family *Flavobacteriaceae* (Bernardet *et al.*, 2002) is one of the major branches of the Gram-negative phylum '*Bacteriodetes*' that has been known until recently as the *Cytophaga–Flexibacter–Bacteroides* (CFB) group (Garrity & Holt, 2001). Within this family, 16S rRNA gene sequence phylogenetic analyses have shown that many marine species cluster into a well-defined 'marine clade', which dominates marine and marine-derived surface waters (Bowman & Nichols, 2005). In the world's oceans, members of the 'marine clade' of the *Flavobacteriaceae* make a significant contribution to the remineralization of organic matter (Kirchman, 2002). Community structure studies of microbial assemblages in the Southern Ocean have shown that this group forms a substantial proportion of the heterotrophic microbial biomass (Simon *et al.*, 1999).

Marine aggregates are ubiquitous and abundant in the world's oceans (Fowler & Knauer, 1986) and consist of complex assemblages of zooplankton faecal pellets, phytoplankton and other material enriched in bacterial communities (Logan & Hunt, 1987; Mueller-Niklas *et al.*, 1994) dominated by members of the family *Flavobacteriaceae* (Kirchman, 2002). As centres of high bacterial activity, marine aggregates are believed to have a major role in the downward transport of carbon (Kiorboe, 2001). In the Ross Sea near Antarctica, concentrations of aggregates were found to be greater than at most other locations in the oceans (Asper & Smith, 2003) and aggregate sinking accounted for a significant proportion of transport of organic material to bottom waters and sediments. Exopolysaccharides (EPS) secreted by bacteria are among the polymeric substances that provide a network to hold these structures together (Flemming & Wingender, 2001).

The availability of iron (Fe^{3+}) as a trace metal is of critical importance in the Southern Ocean where it is known to limit primary production (Scharek *et al.*, 1997). As much as 99 % of dissolved iron in the ocean is bound to organic ligands (Rue & Bruland, 1995). Results from a recent study indicated that the EPS produced by one Antarctic bacterial isolate, designated CAM030^T, derived from Southern Ocean particulate material included uronic acids (Mancuso Nichols *et al.*, 2005). These monosaccharide components are negatively charged at seawater pH, give the EPS a 'sticky' quality (Decho, 1990; Sutherland, 2001) and may influence the availability of trace metals such as iron. EPS similar to those produced by CAM030^T may be acting as ligands for cations such as iron and other trace metals in the Southern Ocean environment.

Phylogenetic analysis of strain CAM030^T showed that this bacterium belongs to the family *Flavobacteriaceae*, but

Abbreviation: EPS, exopolysaccharides.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAM030^T is AY586527.

The whole-cell fatty acid profile of CAM030^T compared with those of related genera is available as a supplementary table in IJSEM Online.

represents a separate lineage (Mancuso Nichols *et al.*, 2005). In the current study, we provide results of chemotaxonomic, genomic and phenotypic studies that support the placement of this strain in a novel taxon, *Olleya marilimosa* gen. nov., sp. nov., in the family *Flavobacteriaceae*.

Samples for isolation of bacteria were obtained during the November/December 2001 voyage of RSV *Aurora Australis*. CAM030^T was isolated from material sampled from the cod end of a plankton net (20 μm) trawled through the Southern Ocean at approximately 65° 32′ 06″ S 143° 10′ 16″ E, where the sea temperature was 4 °C and salinity was 3·5 %. Isolations were carried out according to methods described in Mancuso Nichols *et al.* (2005).

Phenotypic methods used to characterize strain CAM030^T have been described by Bowman *et al.* (1996, 1997). Unless otherwise specified, marine agar [1 g yeast extract (Oxoid L21); 5 g bacteriological peptone (Oxoid L37); 32 g artificial sea salts (Sigma S9883); 15 g agar; 1000 ml distilled water] was used as a basal medium and incubations were carried out at 20 °C. Motility was tested using the hanging drop method and gliding motility was examined after growing the strain for 1–2 days at 12 °C on 0·1 × marine agar (solidified with 1 % agar). After incubation, growth margins were observed by using phase-contrast microscopy (Bowman *et al.*, 2003). Media used in testing for hydrolysis of starch, tyrosine, xanthine, crystalline cellulose, aesculin and elastin and for utilization of uric acid were supplemented with 3·2 % (w/v) artificial sea salts (Atlas, 1993). DNA hydrolysis was tested by using DNase test agar (Oxoid CM321). Lipase activity and Tween 80 and casein hydrolysis were tested as described by Smibert & Krieg (1994). Acid production from glucose was determined according to the method described by Leifson (1963). Additional biochemical tests were carried out using API 20E, API 20NE and Rapid ID 32A strips (bioMérieux) according to the manufacturer's instructions and as described by Bowman *et al.* (1996). For these tests, inoculating or suspension media contained 3·2 % (w/v) artificial sea salts. API 20E and API 20NE strips were incubated at 20 °C for 3 days, whereas Rapid ID 32A strips were incubated for 24 h at 20 °C. The results of these phenotypic tests are given in the species description.

16S rRNA gene sequence analysis of CAM030^T was carried out according to procedures described by Bowman *et al.* (1996) and Mancuso Nichols *et al.* (2005). The phylogenetic tree constructed (see Fig. 1) included 16S rRNA gene sequences from *Flexibacter flexilis* ATCC 23079^T (GenBank accession no. M62794) and *Chlorobium limicola* UdG-6037 (AJ299414) as outgroups. Bootstrap analysis was performed with 500 resampled datasets by using the SEQBOOT and CONSENSE programs within the PHYLIP package (Felsenstein, 1993). High molecular mass DNA for determination of the G+C content was extracted using the technique of Marmur & Doty (1962). The G+C content was determined by the thermal denaturation procedure using spectrophotometry (Bowman *et al.*, 1998; Sly *et al.*, 1986).

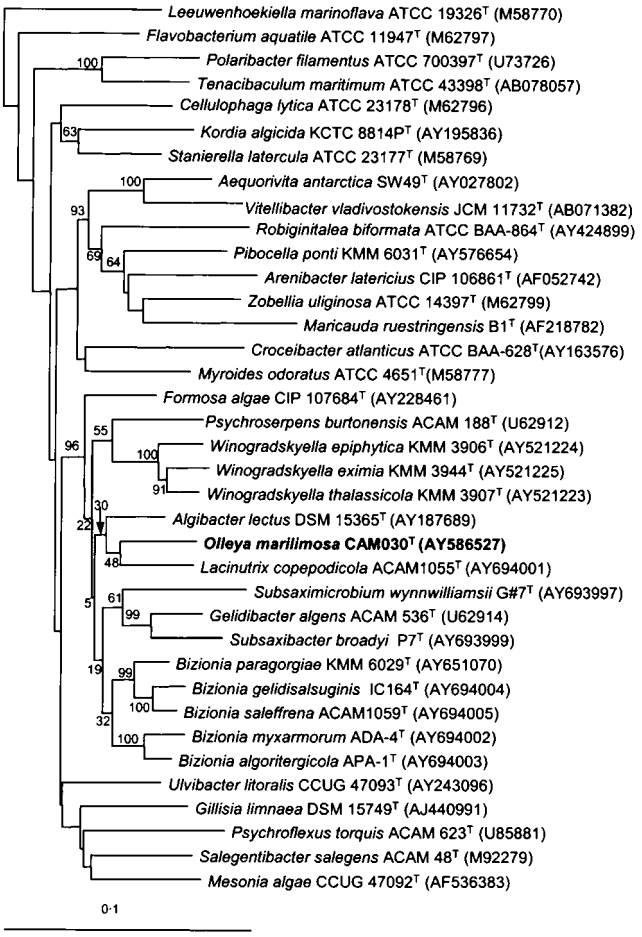

Fig. 1. Phylogenetic relationship of Antarctic marine bacterial isolate CAM030^T within the family *Flavobacteriaceae*. The tree is based on 16S rRNA gene sequences (positions 31–1470, *Escherichia coli* numbering scheme) and was created using maximum-likelihood distances clustered by the neighbour-joining method. Numbers in parentheses are GenBank nucleotide accession numbers. 16S rRNA gene sequences from *Flexibacter flexilis* ATCC 23079^T (M62794) and *Chlorobium limicola* UdG-6037 (AJ299414) were used as outgroups (not shown). Bar, 0·1 changes per mean nucleotide position.

16S rRNA gene sequence analysis indicated that CAM030^T was distinct from all recognized members of the family *Flavobacteriaceae*. *Lacinutrix copepodicola* ACAM 1055^T, *Bizionia saleffrena* ACAM 1059^T, *Bizionia paragorgiae* KMM 6029^T and *Algibacter lectus* DSM 15365^T were the most closely related bacteria with sequence similarities of 94·0, 94·1, 94·2 and 94·5 %, respectively (Fig. 1). The low bootstrap support (< 50 %) for the 16S rRNA gene sequence of CAM030^T with other related members of the family *Flavobacteriaceae* further suggests that CAM030^T represents a discrete taxon. Characteristics used to differentiate CAM030^T from other closely related genera within this family are shown in Table 1. The G+C content of the DNA

Table 1. Differential characteristics of *Olleya marilimosa* gen. nov., sp. nov. CAM030^T and related genera belonging to the family *Flavobacteriaceae*

Data are from Bowman *et al.* (1997), Ivanova *et al.* (2004), Nedashkovskaya *et al.* (2004, 2005a, b), Bowman & Nichols (2005) and this study. YL, Yellow; OR, orange; −, negative; +, positive; A, aerobic; F, facultatively anaerobic; V, characteristics vary among species within this genus; ND, not determined. All genera were positive for catalase and negative for flexirubin pigments, growth at 37 °C, production of indole and urease and degradation of crystalline cellulose.

Characteristic	CAM030^T	*Psychroserpens*	*Gelidibacter*	*Lacinutrix*	*Algibacter*	*Formosa*	*Winogradskyella*	*Bizionia*
Cell morphology	Rods with tapered ends	Ring shaped, helical or coiled cells	Rods	Straight or slightly curved rods	Rods	Slightly pointed rods	Rods	Rods
Pigment production	OR/YL	YL	YL/OR	YL	OR	YL	YL	YL
Gliding motility	+	−	+	−	+	+	+	−
Requirement of Na^+	+	+	V	+	+	−	+	+
Growth at 25 °C	+	−	V	V	+	+	+	V
Growth at 30 °C	+	−	−	−	+	+	+	V
Metabolism	A	A	A	A	F	A	A	A
Acid production from carbohydrates	+	−	V	−	+	+	V	−
Acid production from glucose	+	−	+	−	+	+	V	−
Production of:								
DNase	−	−	V	−	−	ND	V	V
Oxidase	+	−	−	−	+	−	+	+
β-Galactosidase	−	ND	V	−	+	ND	−	−
Nitrate reduction	−	−	V	−	−	+	−	−
Carbohydrate utilization	+	−	+	+	+	+	V	−
Degradation of:								
Agar	−	−	−	−	+	−	+	−
Starch	−	−	V	−	+	+	V	−
Aesculin	−	−	V	−	ND	ND	ND	−
Casein	−	+	V	−	−	−	V	+
Gelatin	+	V	V	+	+	+	+	+
H_2S production	−	−	−	−	−	−	V	+
G+C content (mol%)	49	27–29	37–42	37	31–33	34–35	35	38–45

of CAM030^T was 49 mol%, which also suggests that CAM030^T is distinct from other related species (Table 1).

Whole-cell fatty acid analysis was performed on cells of CAM030^T grown for 4 weeks at 12 °C on marine agar. Extraction and analysis of whole-cell fatty acids was carried out according to procedures described by Mancuso Nichols *et al.* (2005). Fatty acids are designated by the total number of carbon atoms : number of double bonds, followed by the position of the double bond from the terminal (ω) end of the molecule. The suffixes *c* and *t* indicate *cis* and *trans* geometry and the prefixes i and a indicate iso and anteiso branching. The position of the hydroxyl group (OH) may occur on the second (α) or third (β) carbon from the carboxyl end of the molecule.

The major whole-cell fatty acids present in CAM030^T were i15 : 1ω10*c* (22 %), i15 : 0 (19 %), β-OH i15 : 0 (10 %), a15 : 1ω10*c* (8 %), 15 : 0 (7 %) and α-OH i15 : 0 (7 %). The whole-cell fatty acid profile of CAM030^T compared with those of related genera is given in Supplementary Table S1 in IJSEM Online. Major fatty acids found in CAM030^T and also found in other closely related genera, as well as in other members of the family *Flavobacteriaceae*, are also listed in Supplementary Table S1. The predominance of branched saturated, branched monounsaturated and branched hydroxy fatty acids is a common characteristic in the *Flavobacteriaceae* (Bowman *et al.*, 1998, 2003; Nedashkovskaya *et al.*, 2005b). It is interesting to note that for CAM030^T as well as for two closely related genera, *Algibacter* and *Lacinutrix*, there were few minor fatty acids with a chain length of other than 15 carbons, with the exception of br16 : 1 (5 %) and β-OH i17 : 0 (9 %) found in *Lacinutrix* and *Algibacter*, respectively. Variations in culture conditions can have a significant impact on the type and abundance of whole-cell fatty acids. At present, it is difficult to draw further conclusions from discrepancies in fatty acid profiles obtained from strains grown under dissimilar laboratory conditions.

Based upon the above data, we consider that strain CAM030^T represents a novel taxon in the family *Flavobacteriaceae*, for which the name *Olleya marilimosa* gen. nov., sp. nov. is proposed.

Description of *Olleya* gen. nov.

Olleya (Ol.ley′a. N.L. fem. n. *Olleya* named in honour of June Olley, who has made significant contributions to the area of predictive microbiology).

Cells are Gram-negative rods, approximately 0·3–0·5 μm in width and 2·0–2·5 μm in length. Motile by gliding. Endospores are not formed. Cell mass is orange/yellow. Flexirubin pigments are absent. Strictly aerobic chemoheterotrophs. Produce catalase. Produce acid from carbohydrates. Major fatty acids include i15 : 1ω10*c*, i15 : 0, β-OH i15 : 0, a15 : 1ω10*c*, 15 : 0 and α-OH i15 : 0. Phylogenetically, the genus is a member of the family *Flavobacteriaceae*, class *Flavobacteria*, phylum '*Bacteriodetes*'. The type species is *Olleya marilimosa*.

Description of *Olleya marilimosa* sp. nov.

Olleya marilimosa (mar.i.lim.o′sa. L. gen. neut. n. *maris* of the sea; L. adj. *limosus -a -um* full of slime, slimy; N.L. fem. adj. *marilimosa* of the sea and slimy).

Description is as for the genus with the following additions. When incubated on marine agar for 1 week at 20 °C, CAM030^T forms orange/yellow, translucent colonies 1–2 mm in diameter, circular, convex, with an entire edge and a butyrous consistency. Colonies exhibit spreading margin on dilute agar and enhanced mucoid morphology when grown on marine agar supplemented with 3 % glucose. Growth occurs in the pH range 5–9 and in the temperature range 4–30 °C. No growth occurs at 37 °C. Requires Na^+ or sea salts for growth. Growth occurs between 0·2 and 0·9 M NaCl with optimal growth occurring at approximately 0·2–0·5 M NaCl. Requires yeast extract or peptone for growth. Produces acid from glucose, assimilates a range of carbohydrates, but does not reduce nitrate to nitrite or produce H_2S. Indole, DNase, β-galactosidase, lipase, urease and acetoin (Vogues-Proskauer reaction) are not produced, but oxidase and catalase are formed. Tween 80, elastin, gelatin and tyrosine are degraded, but agar, starch, aesculin, casein, cellulose and xanthine are not. Citrate is utilized as a sole carbon source, but uric acid is not. Glucose, maltose and mannose are assimilated; arabinose, mannitol, D-gluconate, capric acid, adipic acid, malate and trisodium citrate are not. Tests for β-*N*-acetyl-glucosaminidase, alkaline phosphatase, arginine arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase, glutamyl glutamic acid arylamidase and serine arylamidase are positive. Tests for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, α-galactosidase, β-galactosidase, β-galactosidase-6-phosphate, α-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, glutamic acid decarboxylase, α-fucosidase, proline arylamidase and pyroglutamic acid arylamidase are negative. The G+C content of the DNA is 49 mol%.

The type strain, CAM030^T (=ACAM 1065^T=CIP 108537^T), was isolated from Southern Ocean particulate material.

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