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 i15:1ω10c, i15:0, β-OH i15:0, a15:1ω10c, 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 1065T = CIP 108537T).

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 (Fe³⁺) 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
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, Ollela 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. CAM030T was isolated from material sampled from the cod end of a plankton net (20 mm) 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 CAM030T 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 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 Mancuso Nichols (1962). The G+C content of the DNA was determined 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).

16S rRNA gene sequence analysis of CAM030T 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 23079T (GenBank accession no. M62794) and Chlorobium limicola Udg-6037 (A1299414) 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).

16S rRNA gene sequence analysis indicated that CAM030T was distinct from all recognized members of the family Flavobacteriaceae. Lacinutrix copepodica ACAM 1055T, Bizionia saleffrena ACAM 1059T, Bizonia paragorgiae KMM 6029T and Algibacter lectus DSM 15365T were the most closely related bacteria with sequence similarities of 94.0-94.1, 94.2 and 94.5-94.6%, respectively (Fig. 1). The low bootstrap support (< 50%) for the 16S rRNA gene sequence of CAM030T with other related members of the family Flavobacteriaceae further suggests that CAM030T represents a discrete taxon. Characteristics used to differentiate CAM030T 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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CAM030(^T)</th>
<th>Psychroserpens</th>
<th>Gelidibacter</th>
<th>Lacinutrix</th>
<th>Algibacter</th>
<th>Formosa</th>
<th>Winogradskyella</th>
<th>Bizonia</th>
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<tr>
<td>Cell morphology</td>
<td>Rods with</td>
<td>Ring shaped,</td>
<td>Rods</td>
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<td></td>
<td>tapered ends</td>
<td>helical or coiled cells</td>
<td>straight or slightly curved rods</td>
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<td>Pigment production</td>
<td>OR/YL</td>
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<td>YL</td>
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<td>Gliding motility</td>
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<td>−</td>
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<td>−</td>
<td>+</td>
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<td>+</td>
<td>−</td>
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<td>Requirement of Na(^+)</td>
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<td>+</td>
<td>V</td>
<td>+</td>
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<td>−</td>
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<td>Growth at 25 °C</td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Growth at 30 °C</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>V</td>
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<td>A</td>
<td>A</td>
<td>A</td>
<td>F</td>
<td>A</td>
<td>A</td>
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<td>Acid production from carbohydrates</td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>−</td>
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<td>Acid production from glucose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>V</td>
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<td>Production of:</td>
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<td>DNase</td>
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<td>Oxidase</td>
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<td>β-Galactosidase</td>
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<td>Nitrate reduction</td>
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<td>−</td>
<td>V</td>
<td>−</td>
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<td>−</td>
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<td>Carbohydrate utilization</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>+</td>
<td>V</td>
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<td>Degradation of:</td>
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<td>Agar</td>
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<td>+</td>
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<td>+</td>
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<td>Starch</td>
<td>−</td>
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<td>Aesculin</td>
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<td>−</td>
<td>V</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>Casein</td>
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<td>+</td>
<td>V</td>
<td>−</td>
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<td>−</td>
<td>V</td>
<td>+</td>
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<tr>
<td>Gelatin</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>+</td>
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<td>H(_2)S production</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
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<td>G + C content (mol%)</td>
<td>49</td>
<td>27–29</td>
<td>37–42</td>
<td>37</td>
<td>31–33</td>
<td>34–35</td>
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<td>38–45</td>
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of CAM030<sup>T</sup> was 49 mol%, which also suggests that CAM030<sup>T</sup> is distinct from other related species (Table 1).

Whole-cell fatty acid analysis was performed on cells of CAM030<sup>T</sup> 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<sup>T</sup> were i15 : 0(22%), i15 : 0(19%), β-OH i15 : 0(10%), a15 : 0(8%), 16 : 0(7%) and α-OH i15 : 0(7%). The whole-cell fatty acid profile of CAM030<sup>T</sup> compared with those of related genera is given in Supplementary Table S1 in IJSEM Online. Major fatty acids found in CAM030<sup>T</sup> 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 Flavobacteriaceae (Bowman et al., 1998, 2003; Nedashkovskaya et al., 2005b). It is interesting to note that for CAM030<sup>T</sup> 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<sup>T</sup> 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 chemo-heterotrophs. Produce catalase. Produce acid from carboxylates. Major fatty acids include i15 : 0, i15 : 0, β-OH i15 : 0, a15 : 0, 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<sup>T</sup> 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<sup>+</sup> 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<sub>2</sub>S. 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-glucosone, 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, β-galactosidase, β-galactosidase-β-glucosidase, 6-1-phosphatase, α-glucosidase, β-glucosidase, α-arabinosidase, β-glucuronidase, glutamic acid decarboxylase, α-fucosidase, proline arylamidase and pyrogulatamic acid arylamidase are negative. The G+C content of the DNA is 49 mol%.

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

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References


