

## *Ulvibacter antarcticus* sp. nov., isolated from Antarctic coastal seawater

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A seawater bacterium, designated IMCC3101<sup>T</sup>, was isolated from Antarctic coastal seawater. The strain was Gram-negative, chemoheterotrophic, obligately aerobic, pigmented dark yellow (flexirubin-type pigments) and devoid of gliding and flagellar motility. On the basis of 16S rRNA gene sequence comparisons, the most closely related species was *Ulvibacter litoralis* (96.6%). Phylogenetic trees generated by using 16S rRNA gene sequences confirmed that the strain belonged to the genus *Ulvibacter* in the family *Flavobacteriaceae*. The DNA G+C content was 37.0 mol% and the major respiratory quinone was MK-6. Several phenotypic characteristics, including cell and colony morphology, the absence of gliding motility and the temperature range for growth, serve to differentiate the strain from the only species in the genus *Ulvibacter* with a validly published name (*U. litoralis*). Therefore, strain IMCC3101<sup>T</sup> represents a novel species of the genus *Ulvibacter*, for which the name *Ulvibacter antarcticus* sp. nov. is proposed. The type strain is IMCC3101<sup>T</sup> (=KCCM 42686<sup>T</sup>=NBRC 102682<sup>T</sup>).

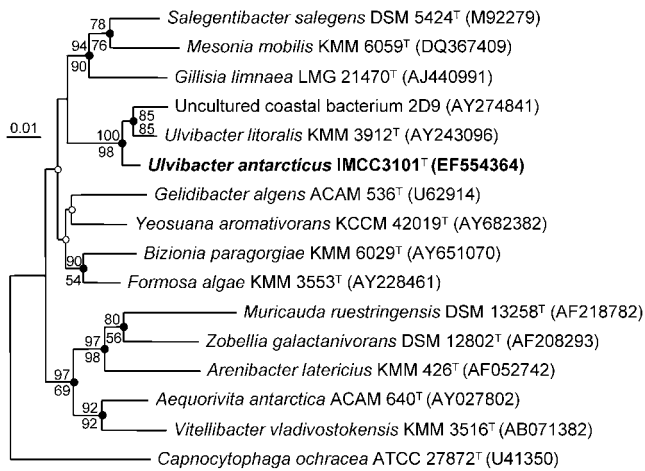
The genus *Ulvibacter* (Nedashkovskaya *et al.*, 2004), a member the family *Flavobacteriaceae* (Bernardet *et al.*, 2002; Bernardet & Nakagawa, 2006; Reichenbach, 1989), currently contains one species, *Ulvibacter litoralis*, isolated from the green alga *Ulva fenestrata*. In this study, a dark yellow-pigmented bacterial strain, designated IMCC3101<sup>T</sup>, was isolated from Antarctic coastal seawater and subjected to a taxonomic study according to the minimal standards for the description of new taxa in the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). On the basis of phenotypic characteristics and phylogenetic data, strain IMCC3101<sup>T</sup> represents a novel species of the genus *Ulvibacter*.

Strain IMCC3101<sup>T</sup> was isolated from a seawater sample collected from the coast of King George Island, Weaver Peninsula, Antarctica (62° 14' S 58° 47' E), using a standard dilution-plating method on marine agar 2216 (MA; Difco) at 20 °C. After the optimum growth temperature of strain IMCC3101<sup>T</sup> had been determined, cultures were maintained routinely on MA at 25 °C and preserved as a suspension in marine broth (MB; Difco)/glycerol (9:1, v/v) at –75 °C.

The methods used for DNA extraction, PCR amplification and sequencing of the 16S rRNA gene were as described in a previous study (Cho & Giovannoni, 2003). The resulting

almost-complete 16S rRNA gene sequence (1461 bp) of strain IMCC3101<sup>T</sup> was aligned with those of its nearest neighbours by using the ARB software package (Ludwig *et al.*, 2004), and levels of 16S rRNA gene sequence similarity were calculated by using the ARB software on the basis of this alignment. An unambiguous segment of 1121 nt, determined from the alignment of the 16S rRNA gene sequences of 16 members of the phylum *Bacteroidetes*, was used for phylogenetic analyses in PAUP\* 4.0 beta 10 (Swofford, 2002). Phylogenetic trees were generated by using the neighbour-joining algorithm (Saitou & Nei, 1987) with the Jukes–Cantor distance model (Jukes & Cantor, 1969) and using the maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) algorithms. The robustness of the neighbour-joining and maximum-likelihood phylogenetic trees was confirmed with bootstrap analyses based on 1000 and 100 resamplings of the sequences, respectively. Preliminary sequence comparisons with 16S rRNA gene sequences deposited in GenBank (Altschul *et al.*, 1997), at the Ribosomal Database Project (RDP-II; Cole *et al.*, 2005) and on the EzTaxon server (<http://www.eztaxon.org>) indicated that strain IMCC3101<sup>T</sup> was related closely to the genus *Ulvibacter* in the family *Flavobacteriaceae*. The strain showed the highest 16S rRNA gene sequence similarity to *U. litoralis* KMM 3912<sup>T</sup> (96.6%), but showed <93.5% sequence similarity to members of other genera in the family. In all of the phylogenetic trees generated in this study (Fig. 1), strain IMCC3101<sup>T</sup>, *U. litoralis* KMM 3912<sup>T</sup> and an uncultured

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMCC3101<sup>T</sup> is EF554364.



**Fig. 1.** Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain IMCC3101<sup>T</sup> and representatives of the family *Flavobacteriaceae*. Bootstrap percentages (above 50 %) from both neighbour-joining (above nodes) and maximum-likelihood (below nodes) analyses are shown. Filled and open circles at each node indicate nodes recovered by all three treeing methods or by two treeing methods, respectively. *Capnocytophaga ochracea* ATCC 27872<sup>T</sup> was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

coastal bacterium, 2D9 (GenBank accession no. AY274841; Kirchman *et al.*, 2003), formed a robust monophyletic clade. This monophyletic clade was clearly separated from other genera of the family *Flavobacteriaceae* with high levels of bootstrap support, indicating that strain IMCC3101<sup>T</sup> represents a novel species within the genus *Ulvibacter*.

Cell morphology and size were examined by transmission electron microscopy (CM200; Philips) and phase-contrast microscopy (80i; Nikon) using a 4 day culture in MB at 25 °C. Colony morphology, size and colour were examined from cultures grown aerobically on MA for 4 days and for 3 weeks. Flagellar and gliding motility were investigated by using wet mounts made from fresh cultures grown in MB at 25 °C for 3 days. Gliding motility was investigated further by using phase-contrast microscopy on 17 h cultures of strain IMCC3101<sup>T</sup> on microscopic slides coated with MA (0.7 % agar), according to Bowman (2000). Cellular pigments were extracted with acetone/methanol (1 : 1, v/v) from a 4 day culture on MA at 25 °C and their absorption spectra were determined by using a scanning UV/visible spectrophotometer (Optizen 2120UV; Mechasis). The presence of flexirubin-type pigments was investigated by using the bathochromic shift test with a 20 % (w/v) KOH solution (Bernardet *et al.*, 2002; McCammon & Bowman, 2000). The growth-temperature range and optimum were tested at 3–42 °C on MA. The pH range and optimum for growth were tested on MA adjusted to pH values from 4.0 to 12.0. The NaCl concentrations and optimum for growth were determined in NaCl-free artificial seawater medium (Choo *et al.*, 2007)

supplemented with 5.0 g peptone, 1.0 g yeast extract and various concentrations of NaCl (0–15 %, w/v). Catalase and oxidase tests were performed according to standard methods (Smibert & Krieg, 1994). Other biochemical tests and carbon-source oxidation tests were carried out using API 20NE and API ZYM strips (bioMérieux) and in GN2 microplates (Biolog), according to the manufacturers' instructions except that the strips were inoculated with bacterial suspensions in artificial seawater medium and incubated at 25 °C for 3 days. Degradation of macromolecules was tested by incubating strain IMCC3101<sup>T</sup> at 25 °C for 3 weeks on MA containing macromolecules. The following macromolecules were tested: starch (0.2 %, w/v), casein (10 % skimmed milk, w/v), elastin (0.5 %, w/v), chitin (0.5 %, w/v), agar (1.5 %, w/v) and CM-cellulose (0.2 %, w/v). Hydrolysis was revealed by the formation of clear zones around the colonies either immediately or after flooding of the plate with the appropriate staining solution (Teather & Wood, 1982). Susceptibility to 10 different antimicrobial agents (Choo *et al.*, 2007) was determined by using the disc diffusion method (Jorgensen *et al.*, 1999) on MA incubated for 4 days at 25 °C. The DNA G + C content of strain IMCC3101<sup>T</sup> was determined by using HPLC according to Mesbah *et al.* (1989), with a Discovery C18 column (5 µm, 15 cm × 4.6 mm; Supelco). Cellular fatty acid methyl esters, extracted and prepared from cultures grown on MA at 25 °C for 4 days, were analysed by the Korean Culture Center of Microorganisms (KCCM, Seoul, Republic of Korea), using the MIDI system. The quinone content was also analysed by the KCCM, using reversed-phase HPLC analysis (Komagata & Suzuki, 1987).

Phenotypic characteristics of strain IMCC3101<sup>T</sup> are listed in the species description and in Table 1. In summary, cells of strain IMCC3101<sup>T</sup> were Gram-negative, chemoheterotrophic, obligately aerobic, non-motile, straight rods that produced flexirubin-type pigments. As shown in Table 1, strain IMCC3101<sup>T</sup> shared a number of characteristics with *U. litoralis*, including the production of flexirubin-type pigments, a similar DNA G + C content and a similar fatty acid profile. However, there were several phenotypic differences with respect to *U. litoralis*, e.g. cell and colony morphology, temperature range for growth, gliding motility and the proportions of iso-C<sub>15:0</sub>, iso-C<sub>15:1</sub> and iso-C<sub>16:0</sub> (Table 1). These differences, together with the level of 16S rRNA gene sequence similarity (<97 %), support the description of strain IMCC3101<sup>T</sup> as representing a novel species of the genus *Ulvibacter*, for which the name *Ulvibacter antarcticus* sp. nov. is proposed.

**Description of *Ulvibacter antarcticus* sp. nov.**

*Ulvibacter antarcticus* (an.tarc'ti.cus. L. masc. adj. *antarcticus* of the Antarctic environment, referring to the place where the organism was isolated).

Cells are Gram-negative, chemoheterotrophic, obligately aerobic, straight rods devoid of flagellar and gliding motility. Cells are 0.5–2.3 µm in length and 0.4–0.7 µm

**Table 1.** Characteristics that serve to differentiate strain IMCC3101<sup>T</sup> from *U. litoralis* KMM 3912<sup>T</sup>

Strains: 1, IMCC3101<sup>T</sup>; 2, *U. litoralis* KMM 3912<sup>T</sup>. Data are from this study and that of Nedashkovskaya *et al.* (2004). +, Positive; -, negative. Both species are Gram-negative, obligately aerobic, chemoheterotrophic bacteria that are positive for the following characteristics: production of flexirubin-type pigments; catalase, oxidase and alkaline phosphatase activities; gelatin hydrolysis; susceptibility to tetracycline. Both species are negative for the following characteristics: flagellar motility;  $\beta$ -galactosidase activity; indole production; acid production from glucose; hydrolysis of urea, casein, agar, CM-cellulose and chitin; utilization of glucose, arabinose, *N*-acetylglucosamine, lactose, maltose, mannose, sucrose, inositol, sorbitol, citric acid, gluconic acid and malonic acid; susceptibility to ampicillin, kanamycin, gentamicin and streptomycin.

Characteristic	1	2
Gliding motility	-	+
Growth at 30 °C	-	+
Growth with NaCl (%)	1-3	1-6
Hydrolysis of starch	+	-
Nitrate reduction	-	+
Utilization of:		
Tween 40	-	+
Mannitol	+	-
Susceptibility to penicillin G	+	-
Major fatty acids (%)*		
iso-C <sub>15:0</sub>	15.3	21.3
iso-C <sub>15:1</sub>	3.9	12.5
iso-C <sub>16:0</sub>	11.0	2.8
DNA G+C content (mol%)	37.0	36.7-38.0

\*Both strains were grown on MA for fatty acid analysis. However, the culture conditions used to grow the strains for other analyses were different.

in diameter. Colonies grown on MA for 4 days are 0.2-0.4 mm in diameter, circular, shiny with entire edges, viscous and dark yellow in colour. After 3 weeks incubation, colonies reach up to 2 mm in diameter. Growth occurs at 3-25 °C (optimum, 25 °C), at pH 6-10 (optimum, pH 7) and at 1-3% NaCl (optimum, 2%). Catalase- and oxidase-positive. Peaks in absorption spectra for the cellular pigments are observed at 397, 450 (major peak) and 470 nm. Flexirubin-type pigments are produced. Starch is degraded, but casein, agar, elastin, CM-cellulose and chitin are not. In API 20NE strips, gelatin hydrolysis is positive, but nitrate reduction, hydrolysis of aesculin and urea,  $\beta$ -galactosidase and arginine dihydrolase activities, indole production and acid production from glucose are negative. In the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present, but lipase (C14), trypsin,  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase, *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase

and  $\alpha$ -fucosidase activities are absent. The following carbon substrates are assimilated (Biolog GN2 microplates):  $\alpha$ -cyclodextrin, glycogen, *N*-acetyl-D-galactosamine, D-arabitol, D-mannitol, pyruvic acid methyl ester, succinic acid monomethyl ester, D-galacturonic acid,  $\alpha$ -ketoglutaric acid,  $\alpha$ -ketovaleric acid, bromosuccinic acid, L-alaninamide, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-leucine, L-ornithine, L-proline, L-serine, L-threonine and uronic acid. None of the other carbon substrates in Biolog GN2 microplates is assimilated. Susceptible to chloramphenicol (25  $\mu$ g), erythromycin (15  $\mu$ g), penicillin G (10  $\mu$ g), rifampicin (50  $\mu$ g) and tetracycline (30  $\mu$ g), but resistant to ampicillin (10  $\mu$ g), gentamicin (10  $\mu$ g), kanamycin (30  $\mu$ g), streptomycin (10  $\mu$ g) and vancomycin (30  $\mu$ g). Cellular fatty acids amounting to  $\geq 1\%$  of the total fatty acid content are as follows: iso-C<sub>17:0</sub> 3-OH (19.4%), iso-C<sub>15:0</sub> (15.3%), iso-C<sub>16:0</sub> (11.0%), iso-C<sub>16:0</sub> 3-OH (9.2%), summed feature 3 (comprising C<sub>16:1</sub> $\omega$ 7c and/or iso-C<sub>15:0</sub> 2-OH; 5.9%), iso-C<sub>16:1</sub> (5.4%), C<sub>17:0</sub> 2-OH (4.7%), iso-C<sub>15:0</sub> 3-OH (4.1%), iso-C<sub>15:1</sub> (3.9%), C<sub>17:1</sub> $\omega$ 6c (3.4%), iso-C<sub>17:1</sub> $\omega$ 9c (2.2%), C<sub>15:0</sub> 2-OH (2.1%), iso-C<sub>14:0</sub> 3-OH (1.8%), iso-C<sub>14:0</sub> (1.7%), an unidentified fatty acid with an equivalent chain length of 13.565 (1.6%), anteiso-C<sub>15:0</sub> (1.3%) and C<sub>15:0</sub> (1.2%). Traces (<1%) of the following fatty acids are also present: anteiso-C<sub>17:1</sub> $\omega$ 9c, C<sub>17:0</sub> 3-OH, C<sub>19:0</sub>, C<sub>17:1</sub> $\omega$ 8c, C<sub>16:0</sub>, iso-C<sub>13:0</sub>, iso-C<sub>12:0</sub>, C<sub>10:0</sub> 3-OH, iso-C<sub>17:0</sub>, anteiso-C<sub>15:1</sub>, C<sub>15:1</sub> $\omega$ 6c, C<sub>12:0</sub> 3-OH and anteiso-C<sub>17:0</sub>. The major respiratory quinone is MK-6. The DNA G+C content is 37.0 mol%.

The type strain, IMCC3101<sup>T</sup> (=KCCM 42686<sup>T</sup>=NBRC 102682<sup>T</sup>), was isolated from surface seawater from Maxwell Bay, King George Island, west Antarctica.

## Acknowledgements

We are grateful to Drs Soon-Gyu Hong and Il-Chan Kim for providing Antarctic seawater samples. This research was supported by a research grant (PE07050) from the Korea Polar Research Institute.

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