Lutimonas vermicola gen. nov., sp. nov., a member of the family *Flavobacteriaceae* isolated from the marine polychaete *Periserrula leucophryna*

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A Gram-negative, yellow-coloured, non-motile, chemoheterotrophic, strictly aerobic, rod-shaped bacterium, designated IMCC1616^T, was isolated from the marine polychaete *Periserrula leucophryna* inhabiting tidal flat sediment of the Yellow Sea, and characterized by a polyphasic approach. The temperature, pH and NaCl ranges for growth were 3–37 °C, pH 5.0–11.0 and 0.5–7.5 %. Based on 16S rRNA gene sequence similarity analyses, the strain was most closely related to members of the genera *Lutibacter* (90.7 %), *Tenacibaculum* (89.2–90.4 %) and *Polaribacter* (88.4–90.2 %). Phylogenetic analysis using three treeing algorithms based on 16S rRNA gene sequences indicated that the strain formed a distinct lineage within the family *Flavobacteriaceae*. The DNA G+C content of the strain was 40.1 mol% and the predominant cellular fatty acids were iso-$C_{15:0}$ (16.5 %), anteiso-$C_{15:0}$ (10.9 %), iso-$C_{17:0}$ 3-OH (8.8 %) and iso-$C_{17:1}\omega9c$ (8.2 %). The DNA G+C content, large amount of iso-$C_{17:1}\omega9c$ and several phenotypic characteristics, including growth temperature and catalase activity, differentiated the strain from other related genera in the family. Therefore, from the taxonomic evidence collected in this study, it is proposed that strain IMCC1616^T represents a new genus and species named *Lutimonas vermicola* gen. nov., sp. nov. The type strain of *Lutimonas vermicola* is strain IMCC1616^T (=KCCM 42379^T =NBRC 102041^T).

The family *Flavobacteriaceae* (Bernardet *et al.*, 2002; Reichenbach, 1989) is one of the largest phylogenetic groups within the phylum *Bacteroidetes* (Garrity & Holt, 2001). Recent polyphasic studies, including 16S rRNA gene phylogeny, have led to a rapid increase in the description of novel members of the family *Flavobacteriaceae*. As a result, the family currently comprises more than 45 genera with validly published names, most of them originating from diverse saline environments. The present study focuses on the description of strain IMCC1616^T, isolated from a marine polychaete dwelling in tidal flat sediment. Based on the taxonomic results collected in this study, we propose the inclusion of this isolate in a new genus and species within the family *Flavobacteriaceae*.

Strain IMCC1616^T was isolated from the digestive tract of a marine polychaete, *Periserrula leucophryna*, collected from a depth of 1–2 m in tidal flat sediment on Donggum island (37° 35′ 34.1″ N 126° 31′ 7.5″ E), Korea, in July 2005. The marine polychaete was washed with sterile seawater three times and dissected under an Olympus SZH10 stereoscopic microscope. One hundred microlitres of digestive tract homogenate was spread onto R2A agar (Difco) diluted 1 : 10 (v/v) in aged seawater (1/10R2A) and the agar plates were incubated aerobically at 25 °C for 15 days. Strain IMCC1616^T, initially grown on 1/10R2A, was further purified on marine agar 2216 (MA; Difco). After its optimum growth temperature was determined, cultures of strain IMCC1616^T were maintained routinely on MA at 25 °C.

Cell morphology, including the presence of flagella and intracellular granules, was examined by transmission electron microscopy (CM200; Philips) study of a 5 day culture on MA at 25 °C. Cell size was measured using phase-contrast and epifluorescence microscopy (Nikon 80i). For electron microscopy, exponential phase cells were washed twice with sodium cacodylate buffer and negatively stained with 2 % phosphotungstic acid (pH 7.0–7.2) on Formvar-coated copper grids. Colony morphology, size and colour were examined from cultures grown aerobically on MA for 3 days. Anaerobic growth was tested using both the MGC anaerobic system and AnaeroPACK Anaero (Mitsubishi Gas Chemical Company). Flagellar and gliding motilities were examined using wet mounts and hanging drop preparations, respectively, made from exponential-phase cells grown on MA at 25 °C for 5 days. The growth temperature range and optimum were tested at 3–42 °C. The pH range and optimum for growth were examined at pH 4.0–12.0. The NaCl concentration and optimum for

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growth were determined in NaCl-free artificial seawater medium (ASW) (basic formula, l^{-1}: 19.45 g NaCl, 5.9 g $MgCl_2 . 6H_2O$, 3.24 g $MgSO_4.7H_2O$, 1.8 g $CaCl_2 . 2H_2O$, 0.55 g KCl, 0.16 g $NaHCO_3$, 0.08 g KBr, 0.034 g $SrCl_2 . 6H_2O$, 0.022 g H_3BO_3, 0.008 g $Na_2H_2PO_4$, 0.004 g Na_2SiO_3, 0.0024 g NaF, 0.0016 g KN_4NO_3), supplemented with 5.0 g peptone, 1.0 g yeast extract and various concentrations of NaCl (0–15 %, w/v). The ranges of temperature, pH and NaCl concentration for growth were determined on MA or MA adjusted to various pH values and NaCl concentrations for 2 weeks. The presence of flexirubin-type pigments was determined by the bathochromatic shift test using a 20 % (w/v) KOH solution (Bernardet *et al.*, 2002; McCammon & Bowman, 2000). Cellular pigments of the strain were extracted with acetone/methanol (1 : 1, v/v) and their absorption spectra were determined using a scanning UV/visible spectrophotometer (Optizen 2120UV; Mechasis Co.). The catalase test was performed by addition of 3.0 % hydrogen peroxide to exponential-phase colonies, and oxidase activity was determined using oxidase reagent (bioMérieux). Other biochemical tests and carbon-source utilization tests were carried out in API 20NE and API ZYM strips (bioMérieux) and in GN2 microplates (Biolog), according to the manufacturers' instructions and inoculated with bacterial suspensions in ASW. Degradation of macromolecules was tested by incubating strain IMCC1616^T on MA containing macromolecules at 25 °C for 14 days. The following macromolecules were tested: starch (0.2 % w/v), casein (10 % w/v skimmed milk), elastin (0.5 % w/v), chitin (0.5 % w/v) and CM cellulose (0.2 % w/v). Hydrolysis was determined by formation of clear zones around the colonies. For CM cellulose, the clear zones were revealed by flooding the colonies with a 0.1 % Congo red solution and rinsing with 1 M NaCl (Teather & Wood, 1982). Degradation of DNA was tested using DNase test agar (Difco) amended with 1.5 % NaCl. Susceptibility to antimicrobial agents [tetracycline (30 μg), ampicillin (10 μg), kanamycin (30 μg), chloramphenicol (25 μg), erythromycin (15 μg), gentamicin (10 μg), penicillin G (10 μg), streptomycin (10 μg), vancomycin (30 μg) and rifampicin (50 μg)] was determined using the diffusion method (Jorgensen *et al.*, 1999). The DNA G+C content of strain IMCC1616^T was analysed 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 were extracted and prepared from cultures grown on MA at 25 °C for 7 days and analysed according to the MIDI Microbial Identification System by the Korean Culture Center of Micro-organisms (KCCM).

The nearly full-length sequence of the 16S rRNA gene (1449 bp) of strain IMCC1616^T was obtained as described previously (Cho & Giovannoni, 2003). Comparative analysis of the 16S rRNA gene sequence of the strain with sequences held in GenBank showed that it belonged to the family *Flavobacteriaceae*. The 16S rRNA gene sequence of the strain was aligned against more than 100 reference sequences of members of the family *Flavobacteriaceae* using the ARB software package (Ludwig *et al.*, 2004), and 1208 unambiguously aligned nucleotide positions, generated using the *Bacteroidetes*-specific mask, were employed for phylogenetic analyses in PAUP* 4.0 beta 10 (Swofford, 2002). Phylogenetic trees were inferred by three tree-generating algorithms: neighbour-joining with the Kimura two-parameter model, maximum-parsimony and

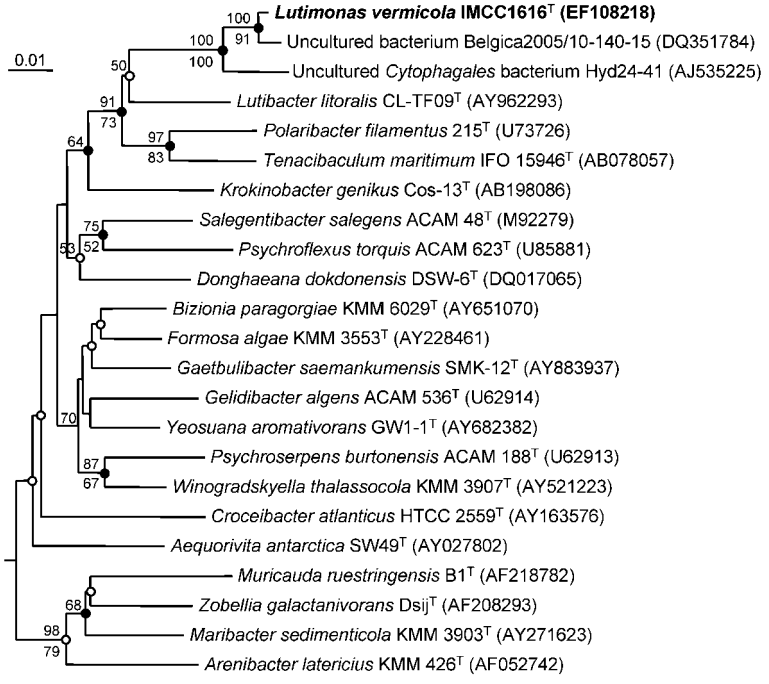

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing relationships between strain IMCC1616^T and representatives of the family *Flavobacteriaceae*. Bootstrap percentages (above 50 %) from both neighbour-joining (above nodes) and maximum-parsimony (below nodes) are shown. Filled and open circles at each node respectively indicate nodes recovered by all three treeing methods or by two treeing methods. *Bacteroides fragilis* Bfr901 (GenBank accession no. X83945) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

maximum-likelihood. The resultant neighbour-joining and parsimony trees were evaluated by bootstrap analysis based on 1000 resamplings. Sequence comparisons based on the multiple alignment in the ARB database, Ribosomal Database Project (RDP-II) and BLASTN search results revealed that strain IMCC1616^T was only distantly related to other species in the family *Flavobacteriaceae*. Strain IMCC1616^T was most closely related to members of the genera *Lutibacter* (90.7 %), *Tenacibaculum* (89.2–90.4 %) and *Polaribacter* (88.4–90.2 %). No other species with a validly published name exceeded 90.5 % 16S rRNA gene sequence similarity with strain IMCC1616^T. It formed a robust monophyletic clade with several uncultivated bacteria retrieved from marine sediments (Fig. 1). This clade formed a larger clade with *Lutibacter litoralis*; however, this relationship was not supported by bootstrap analysis. Comprehensive phylogenetic analyses revealed that the strain could not be associated with any of the known genera in the family. Therefore, strain IMCC1616^T was considered to represent a new genus in the family *Flavobacteriaceae*.

The phenotypic and biochemical characteristics of strain IMCC1616^T are listed in the genus and species descriptions and in Tables 1 and 2. Cells of strain IMCC1616^T were Gram-negative, chemoheterotrophic, non-motile, strictly aerobic and rod-shaped. The DNA G+C content (40.1 mol%) and several phenotypic characteristics, including growth temperature and catalase activity, differentiated the strain from other related genera in the family *Flavobacteriaceae* (Table 1). The predominant cellular fatty acids of the strain were iso-$C_{15:0}$ (16.5 %), anteiso-$C_{15:0}$ (10.9 %), iso-$C_{17:0}$ 3-OH (8.8 %) and iso-$C_{17:1}\omega 9c$ (8.2 %), in agreement with other related genera

Table 1. Differential phenotypic characteristics of strain IMCC1616^T and other related genera in the family *Flavobacteriaceae*

Taxa: 1, IMCC1616^T (data from this study); 2, *Lutibacter* (data from Choi & Cho, 2006); 3, *Polaribacter* (Gosink *et al.*, 1998; Nedashkovskaya *et al.*, 2005; Yoon *et al.*, 2006); 4, *Tenacibaculum* (Choi *et al.*, 2006; Frette *et al.*, 2004; Hansen *et al.*, 1992; Jung *et al.*, 2006; Suzuki *et al.*, 2001; Wakabayashi *et al.*, 1986; Yoon *et al.*, 2005); 5, *Krokinobacter* (Khan *et al.*, 2006). +, Positive ; −, negative; W, weak reaction; V, variable; ND, no data available.

Characteristic	1	2	3	4	5
Cell shape	Rod	Rod	Rod, spiral, filamentous	Rod	Rod
Colony colour	Yellow	Yellow	Yellow or orange	Yellow	Yellow
Gliding motility	−	−	−	V*	ND
Growth at 4 °C	+	+	+	V	−
Growth at 37 °C	+	−	−	V	−
Oxidase activity	−	−	V†	+	+
Catalase activity	−	+	+	+	+
Nitrate reduction	−	−	V‡	V	−
Acid production from glucose	−	−	V	V	−
β-Galactosidase activity	V§	W	V	−[a]‖	+
Hydrolysis of:					
Starch	+	+	+	V	−
Gelatin	+	+	V	+¶	+
Chitin	−	ND	−	V	−
Carbon utilization:					
Tween 80	−	−	+[b]	V	+
D-Fructose	+	+	V	−[d]	+
D-Galactose	+	−	−[c]	−[d]	ND
Glycerol	+	−	V	V	−
Sucrose	+	−	V	V	+
DNA G+C content (mol%)	40.1	33.9	30–33	30–35.2	33–39

*All species positive except *T. skagerrakense*.

†All species positive except *P. filamentus*.

‡All species negative except *P. glomeratus*.

§Positive in API 20NE strips (substrate *p*-nitrophenyl β-D-galactopyranoside) but negative in API ZYM system (substrate 2-naphthyl β-D-galactopyranoside).

‖Data available for the following species only: *a*, *T. aestuarii* and *T. litoreum*; *b*, *P. butkevichii* and *P. dokdonensis*; *c*, *P. dokdonensis*; *d*, *T. aestuarii*, *T. litoreum* and *T. lutimaris*.

¶Data not available for *T. skagerrakense*.

Table 2. Cellular fatty acid compositions (%) of strain IMCC1616^T and other related genera in the family *Flavobacteriaceae*.

Taxa: 1, IMCC1616^T (data from this study); 2, *Lutibacter* (Choi & Cho, 2006); 3, *Polaribacter* (data for *P. butkevichii*, *P. dokdonensis*, *P. filamentus*, *P. franzmannii* and *P. irgensii* from Gosink *et al.*, 1998; Nedashkovskaya *et al.*, 2005; Yoon *et al.*, 2006); 4, *Tenacibaculum* (data for *T. aestuarii*, *T. litoreum*, *T. lutimaris*, *T. maritimum*, *T. mesophilum* and *T. skagerrakense* from Choi *et al.*, 2006; Jung *et al.*, 2006; Yoon *et al.*, 2005); 5, *Krokinobacter* (Khan *et al.*, 2006). −, Not detected/not reported. Only fatty acids amounting to at least 5 % of the total cellular fatty acids of at least one of the species are shown. All species studied were grown on MA except *Polaribacter* species.

Fatty acid	1	2	3	4	5
iso-$C_{13:0}$	1.0	−	2–16.4	0.2–1.8	−
$C_{15:0}$	3.8	1.7	2.3–8.1*	2.7–8.9	−
iso-$C_{15:0}$	16.5	16.7	9–22	9.5–18.9	13–22
anteiso-$C_{15:0}$	10.9	15.1	4–6[a]†	0.7–1.8[b]	5–8
$C_{15:1}\omega6c$	1.4	1.5	3–9	1.6–4.2[b]	−
iso-$C_{15:1}$	6.7	4.0	6–15.4[c]	5.3–8.7	15–26
anteiso-$C_{15:1}$	1.0	1.6	−	−	3–6
iso-$C_{16:0}$	2.5	−	−	0.3–3.8	2–4
iso-$C_{17:1}\omega9c$	8.2	−	−	0.4–1.6[d]	−
iso-$C_{15:0}$ 3-OH	3.2	17.4	12–38	4.6–19.8	3.0
iso-$C_{16:0}$ 3-OH	3.1	13.4	5.0‡	5–12.8	5–12
iso-$C_{17:0}$ 3-OH	8.8	3.9	8.4‡	8.4–14.9	6–10
Summed feature 3§	7.1	1.0	6.6‡	11.9–24.4	−

*Detected only in *P. butkevichii* and *P. dokdonensis*.
†Not detected in the following species: *a*, *P. butkevichii* and *P. dokdonensis*; *b*, *T. skagerrakense*; *c*, *P. butkevichii*; *d*, *T. maritimum* and *T. skagerrakense*.
‡Detected only in *P. dokdonensis*.
§Comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

in the family, except for the proportion of iso-$C_{17:1}\omega9c$ (Table 2).

As shown by the low 16S rRNA gene sequence similarity (<91 %) with other taxa, the formation of an independent phyletic line in the phylogenetic tree and differential phenotypic characteristics, strain IMCC1616^T cannot be assigned to any of the known genera in the family *Flavobacteriaceae*. Therefore it should be classified as representing a novel species in a new genus, for which the name *Lutimonas vermicola* gen. nov., sp. nov. is proposed.

Description of *Lutimonas* gen. nov.

Lutimonas (Lu.ti.mo′nas. L. n. *lutum* mud; L. fem. n. *monas* a unit; N.L. fem. n. *Lutimonas* a unit from mud, pertaining to the habitat of the animal that harboured the type species, a marine tidal flat).

Cells are Gram-negative, non-motile, strictly aerobic rods. Carotenoid pigments are present, flexirubin-type pigments are absent. Chemoheterotrophic. NaCl is required for growth. Catalase- and oxidase-negative. The major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and iso-$C_{17:1}\omega9c$. The DNA G+C content of the type species is 40.1±0.5 mol%. The genus belongs to the family *Flavobacteriaceae*. The type species is *Lutimonas vermicola*.

Description of *Lutimonas vermicola* sp. nov.

Lutimonas vermicola (ver.mi′co.la. L. n. *vermis* worm; L. suff. *-cola* from L. n. *incola* inhabitant; N.L. n. *vermicola* inhabitant of worms, pertaining to the origin of the type strain, a marine polychaete).

The description of the species conforms to that of the genus. Cells are 1.6–2.5 μm long and 0.7–1.0 μm wide. Devoid of flagellar and gliding motilities. After 3 days of incubation, colonies on MA are circular, pulvinate, entire, smooth, opaque, yellow-coloured and 1.0–3.0 mm in diameter. Growth occurs at 3–37 °C (optimum, 25 °C), at pH 5–11 (optimum, pH 8) and with 0.5–7.5 % NaCl (optimum, 1.5–2.5 %). Absorption spectral peaks of the carotenoid pigments are observed at 427, 453 (major peak) and 478 nm. Degrades starch and gelatin, but not elastin, CM cellulose or chitin. Does not grow on DNase agar or MA containing casein. In API 20NE strips, positive for β-galactosidase activity (substrate, *p*-nitrophenyl β-D-galactopyranoside). Negative for urease, gelatin liquefaction and arginine dihydrolase. Indole is not produced. Acid is not produced from glucose. Nitrate is not reduced. In the API ZYM system, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase

activities are present, but lipase (C14), trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, β-galactosidase (substrate, 2-naphthyl β-D-galactopyranoside), β-glucuronidase, α-glucosidase and α-fucosidase activities are absent. Weakly positive for α-galactosidase activity. Utilizes the following carbon substrates in Biolog GN2 microplates: α-cyclodextrin, glycogen, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, D-cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, *myo*-inositol, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, methyl β-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, succinic acid monomethyl ester, acetic acid, *cis*-aconitic acid, citric acid, D-galacturonic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketoglutaric acid, DL-lactic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-ornithine, L-proline, L-threonine, γ-aminobutyric acid, inosine, uridine, thymidine, phenyl ethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, α-D-glucose 1-phosphate and D-glucose 6-phosphate. Does not utilize dextrin, Tween 40, Tween 80, adonitol, L-arabinose, D-arabitol, D-mannitol, pyruvic acid methyl ester, formic acid, D-galactonic acid, D-gluconic acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, L-leucine, L-phenylalanine, L-pyroglutamic acid, D-serine, L-serine, DL-carnitine or urocanic acid. Susceptible to rifampicin, vancomycin, erythromycin, penicillin G, tetracycline and chloramphenicol, but resistant to ampicillin, gentamicin, kanamycin and streptomycin. In addition to the major cellular fatty acids reported in Table 2, the following fatty acids are present in minor proportions: anteiso-$C_{17:1}\omega9c$ (3.0 %), unknown ECL 13.565 (2.9 %), $C_{17:1}\omega6c$ (2.7 %), $C_{17:0}$ 2-OH (2.6 %), $C_{15:0}$ 2-OH (2.5 %), $C_{15:1}$ 2-OH (2.2 %), iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B (1.2 %) and iso-$C_{14:0}$ (1.0 %). Traces (<1 %) of the following fatty acids are also present: $C_{16:0}$, $C_{17:1}\omega8c$, unknown ECL 16.582, anteiso-$C_{13:0}$, iso-$C_{17:0}$, $C_{18:1}\omega5c$, anteiso-$C_{17:0}$, $C_{17:0}$ 3-OH, iso-$C_{14:1}$ B, iso-$C_{14:0}$ 3-OH, $C_{18:0}$, unknown ECL 11.543, $C_{16:0}$ 3-OH, $C_{17:0}$, $C_{19:0}$ and iso-$C_{11:0}$.

The type strain is IMCC1616^T (=KCCM 42379^T =NBRC 102041^T), isolated from the digestive tract of a polychaete (*Periserrula leucophryna*) inhabiting a tidal flat of Donggum island, Korea.

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