Litoricolaceae fam. nov., to include *Litoricola lipolytica* gen. nov., sp. nov., a marine bacterium belonging to the order *Oceanospirillales*

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A Gram-negative, non-motile, chemoheterotrophic, facultatively aerobic, short-rod-shaped bacterium, designated IMCC1097^T, was isolated from coastal seawater (10 m depth) of the East Sea, Korea. The temperature, pH and NaCl ranges for growth were 15–30 °C, pH 5.0–10.0 and 1.5–10 % NaCl. The colonies of the strain were very small, having a mean diameter of 0.05 mm. 16S rRNA gene sequence data indicated that the strain was most closely related to genera within the class *Gammaproteobacteria*. Members of the most closely related genera showed less than 90 % sequence similarity and included *Saccharospirillum* (89.3 %), *Oleiphilus* (88.7 %), *Reinekea* (88.2 %), *Alcanivorax* (86.4–87.6 %) and *Zooshikella* (87.6 %), which represent five different families of the order *Oceanospirillales*. Phylogenetic analyses showed that this marine strain represented a distinct phylogenetic lineage in the order *Oceanospirillales* and could not be assigned to any of the defined families in the order. The predominant fatty acids were $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, $C_{18:1}\omega7c$ and $C_{10:0}$ 3-OH, and the DNA G+C content was 57.9 mol%. These chemotaxonomic properties, together with phenotypic characteristics, served to differentiate the strain from phylogenetically closely related genera. The very low sequence similarities (<90 %) and distant relationships between IMCC1097^T and members of the order *Oceanospirillales* suggested that the strain merited classification within a novel genus within a novel family in the order. On the basis of taxonomic evidence collected in this study, a novel genus and species are proposed, *Litoricola lipolytica* gen. nov., sp. nov., within a new family *Litoricolaceae* fam. nov. Strain IMCC1097^T (=KCCM 42360^T =NBRC 102074^T) is the type strain of *Litoricola lipolytica*.

The order *Oceanospirillales* within the class *Gammaproteobacteria* (Garrity *et al.*, 2005a) is largely based on 16S rRNA gene sequence phylogeny. The order encompasses a diverse range of Gram-negative bacteria that are generally halophilic or halotolerant, rod-shaped, motile (except for the members of the genus *Alcanivorax*) and chemoheterotrophic. The order currently contains five families with validly published names and one that remains to be validly published: *Oceanospirillaceae* (Garrity *et al.*, 2005b), *Alcanivoracaceae* (Golyshin *et al.*, 2005), *Halomonadaceae* (Franzmann *et al.*, 1988), *Hahellaceae* (Garrity *et al.*, 2005c), *Oleiphilaceae* (Golyshin *et al.*, 2002) and '*Saccharospirillaceae*' (Labrenz *et al.*, 2003). In this study, we report on the isolation and taxonomy of a single strain that could not be assigned to any of the defined families in the order *Oceanospirillales*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMCC1097^T is EF176580.

Transmission electron micrographs of cells of strain IMCC1097^T are available as a supplementary figure with the online version of this paper.

A sample of coastal seawater was collected, at a depth of 10 m, near Goseong, East Sea, Korea (38° 20′ N 128° 33′ E), in June 2005. An aliquot (100 µl) of the seawater sample was spread onto an oligotrophic medium, R2A agar (Difco) diluted 1 : 10 (v/v) with aged seawater (referred to as 1/10R2A), and the agar plates were incubated aerobically at 20 °C for 1 month. Strain IMCC1097^T, initially grown on 1/10R2A, was further purified on marine agar 2216 (MA; Difco) after growth of the strain at 20 °C for 2 weeks. After the optimum growth temperature for the strain had been determined, cultures were maintained routinely on MA at 25 °C.

The almost-complete sequence of the 16S rRNA gene (1483 bp) for strain IMCC1097^T was obtained as described previously (Cho & Giovannoni, 2003). Phylogenetic analyses, including multiple alignment of 16S rRNA gene sequences and generation of phylogenetic trees, were performed using the ARB package (Ludwig *et al.*, 2004) and PAUP* (Swofford, 2002) as described by Cho & Giovannoni (2006). Preliminary sequence comparisons against the 16S rRNA gene sequences deposited in

GenBank and the Ribosomal Database Project showed that the strain belonged to the class *Gammaproteobacteria*. The sequence similarity of strain IMCC1097^T with respect to recognized species within the *Gammaproteobacteria* was very low: no species with validly published names showed more than 90 % sequence similarity. Comparative analyses of 16S rRNA gene sequence similarity based on manually aligned sequences in the ARB database showed that the most closely related type strains of species with validly published names included *Saccharospirillum impatiens* DSM 12546^T (89.3 %), *Oleiphilus messinensis* DSM 13489^T (88.7 %), *Reinekea marinisedimentorum* DSM 15388^T (88.2 %), *Alcanivorax borkumensis* ATCC 700651^T (87.6 %) and *Zooshikella ganghwensis* DSM 15267^T (87.6 %). The above species all belong to the order *Oceanospirillales*; however, they represent five different families in the order, which indicates a unique phylogeny for strain IMCC1097^T. In all of the phylogenetic trees, generated using three different algorithms (Fig. 1), strain IMCC1097^T formed a robust monophyletic clade with uncultured bacteria F3C13 (99.5 % sequence similarity; Prabagaran *et al.*, 2007) and CHAB-V-35 (97.6 % sequence similarity; Schäfer *et al.*, 2000). This monophyletic clade containing the novel isolate was clearly distinguishable from other families within the order *Oceanospirillales*. In the maximum-likelihood and neighbour-joining trees, the clade containing strain IMCC1097^T formed a larger clade with *Z. ganghwensis* DSM 15267^T (Fig. 1). However, this relationship between IMCC1097^T and *Z. ganghwensis* was not found in the maximum-parsimony tree, and, moreover, the bootstrap percentages obtained did not support monophyletic relationships for the clade. According to our phylogenetic analyses, the order *Oceanospirillales* and the families *Oceanospirillaceae* and *Hahellaceae* had polyphyletic properties. In spite of incomplete phylogenetic resolution of the order *Oceanospirillales* and the class *Gammaproteobacteria*, the very low sequence similarities (<90 %) and the distant relationships between strain IMCC1097^T and other families within the order *Oceanospirillales* suggested that the strain represented a novel genus within a novel family in the order *Oceanospirillales*.

Phenotypic and chemotaxonomic characteristics were determined using MA at 25 °C, unless otherwise indicated. Cellular morphology was examined with transmission electron microscopy (CM200; Philips). Exponentially grown bacterial cultures were washed with sodium cacodylate buffer twice and negatively stained with 2 % phosphotungstic acid (pH 7.0–7.2) on Formvar-coated copper grids. Cell size and morphology were also determined using phase-contrast microscopy and epifluorescence microscopy (Nikon 80i) with 4′,6-diamidino-2-phenylindole (DAPI) staining. Motility was tested from wet mounts of exponential-phase cells. The presence of poly-β-hydroxybutyrate granules was checked using epifluorescence microscopy after staining of the cells with Nile blue A (Ostle & Holt, 1982). Colony morphology, size and colour were examined using cultures grown aerobically on MA for 1 week. The capacity of strain IMCC1097^T for anaerobic growth was tested using the MGC anaerobic system with AnaeroPACK Anaero (Mitsubishi Gas Chemical) with cells incubated for up to 3 weeks. A catalase test was performed by adding 3.0 % hydrogen peroxide to fresh colonies, and oxidase activity was determined using Kovács solution (Kovács, 1956). The temperature range and optimum were tested from 4 to 42 °C. The pH range and optimum were examined from pH 4.0 to 12.0. The NaCl concentrations and optimum for growth were determined in NaCl-free artificial seawater medium (ASW; basic formula, with NaCl, containing the following, 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 KNO_3), supplemented with 5.0 g peptone, 1.0 g yeast extract and 0–15 % (w/v) NaCl. Other biochemical tests were performed using API 20NE and API ZYM (bioMérieux) according to the manufacturer's instructions, by inoculating the cells into ASW medium. The utilization of various compounds as sole carbon sources was tested as described in a previous study (Cho & Giovannoni, 2003), using custom-made 48-well microtitre plates containing 47 different carbon compounds (listed in the species description) at a final concentration of 0.02 % (w/v or v/v) in ASW medium. The microtitre plates were incubated aerobically at 25 °C for 1 week, and cellular growth in each well was screened using epifluorescence microscopy with DAPI staining. Susceptibility to the following antimicrobial agents was determined (using the diffusion plate method): 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. The DNA G+C content of strain IMCC1097^T was analysed by using HPLC according to Mesbah *et al.* (1989). Cellular fatty acid methyl esters were prepared from cultures grown on MA at 25 °C for 1 week, and analysed according to the instructions of the Microbial Identification System (MIDI) by the Korean Culture Center of Microorganisms.

Cells of strain IMCC1097^T were Gram-negative, non-pigmented, chemoheterotrophic, non-motile, facultatively aerobic, short rods that required NaCl for growth. The colonies were 0.05 mm in diameter, increasing to 1 mm after a prolonged incubation period of 3 weeks. The taxonomic characteristics of the strain are described in detail in the genus and species descriptions. Several phenotypic and genomic characteristics clearly differentiated strain IMCC1097^T from phylogenetically distantly related genera in the families *Hahellaceae*, *Oceanospirillaceae*, *Oleiphilaceae*, '*Saccharospirillaceae*' and *Alcanivoracaceae* (Table 1). The strain was differentiated from the genera *Zooshikella* and *Hahella* on the basis of several characteristics, including pigmentation, flagellar motility, catalase activity and DNA G+C content. The major cellular fatty acids detected in strain IMCC1097^T were

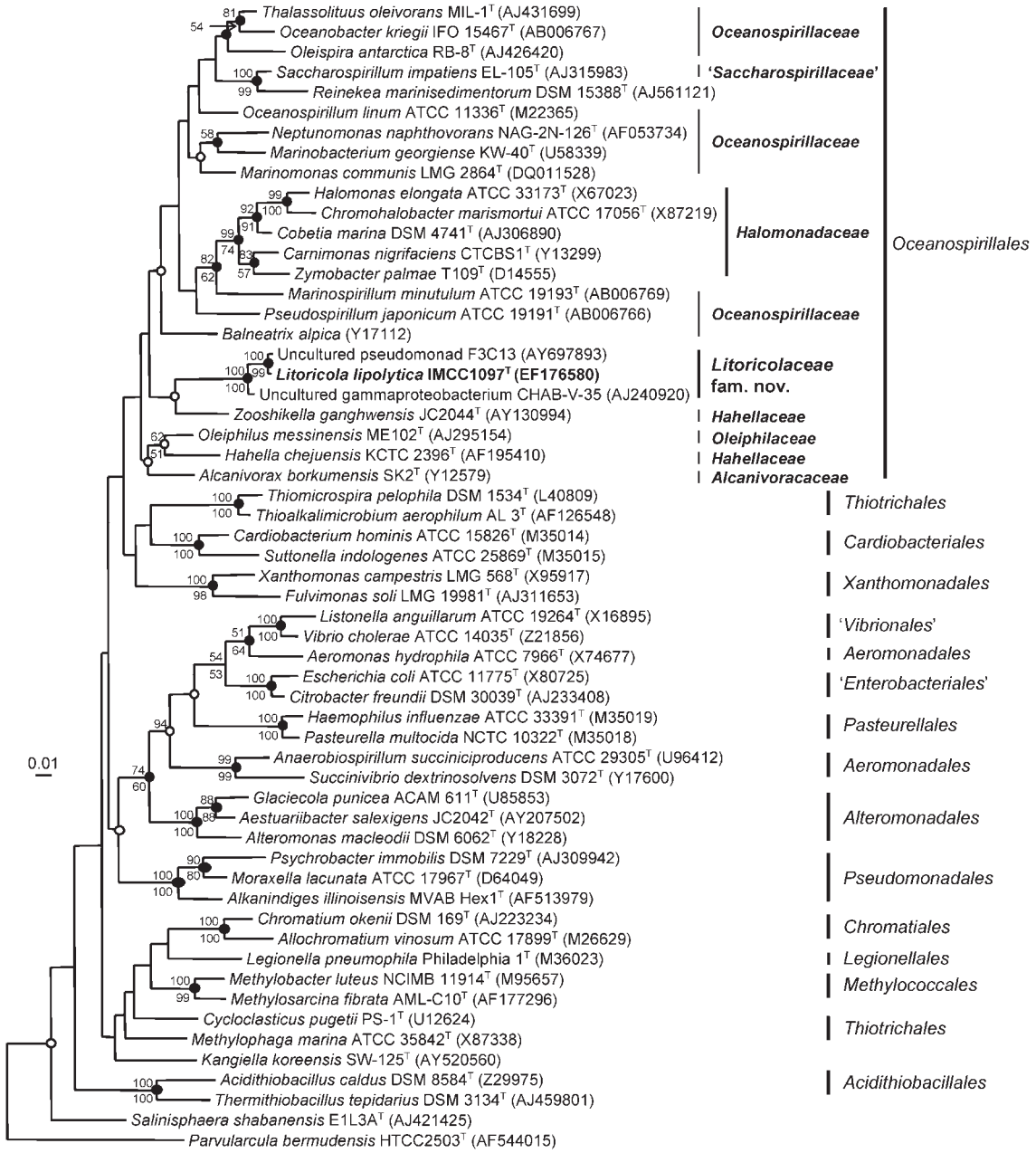

Fig. 1. Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the distant relationships between strain IMCC1097^T and representatives of the class *Gammaproteobacteria*. Bootstrap percentages (above 50 %) from both neighbour-joining (above nodes) and maximum-parsimony (below nodes) approaches are shown. Filled and open circles at each node respectively indicate nodes recovered reproducibly by all treeing methods or by two treeing methods. Each family and order in the *Gammaproteobacteria* is indicated. Bar, 0.01 substitutions per nucleotide position.

$C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (42.8 %), $C_{18:1}\omega7c$ (20.6 %), $C_{10:0}$ 3-OH (14.1 %) and $C_{16:0}$ (6.5 %), and the overall fatty acid composition was different from that of other genera of the order *Oceanospirillales* (Table 2). The presence of $C_{10:0}$ 3-OH and $C_{12:1}$ 3-OH and the absence of $C_{18:1}\omega9c$ in IMCC1097^T could serve as a fatty acid signature for differentiating the strain from the members of the genera *Zooshikella*, *Hahella* and *Reinekea* (the fatty acid profiles of which were obtained from biomass grown on MA). On the basis of the above results, strain IMCC1097^T cannot be characterized as a member of any of the known genera within the order *Oceanospirillales.*

It is evident from the low levels of 16S rRNA gene sequence similarity (<90 %), the unique branching patterns in the phylogenetic analyses and the phenotypic characteristics

Table 1. Differential characteristics of strain IMCC1097^T and other marine bacteria related to the order *Oceanospirillales*

Taxa: 1, IMCC1097^T; 2, *Zooshikella* (data from Yi *et al.*, 2003); 3, *Hahella* (Lee *et al.*, 2001; Baik *et al.*, 2005); 4, *Reinekea* (Romanenko *et al.*, 2004); 5, *Oleiphilus* (Golyshin *et al.*, 2002); 6, *Saccharospirillum* (Labrenz *et al.*, 2003); 7, *Alcanivorax* (Bruns & Berthe-Corti, 1999; Fernández-Martínez *et al.*, 2003; Liu & Shao, 2005; Yakimov *et al.*, 1998). +, Positive ; −, negative ; V, variable among species; ND, no data available. All organisms are positive for oxidase activity.

Characteristic	1	2	3	4	5	6	7
Cell shape*	SR	CR	LR	R	TR	S	R
Pigmentation	−	+	+	−	−	−	−
Growth at/with:							
4 °C	−	−	−	+	−	+	V
40 °C	−	+	+	−	−	+	V
8 % NaCl	+	−	+	−	+	+	+
Catalase activity	−	+	+	+	+	+	+
Flagella	−	+	+	+	+	+	V
Poly-β-hydroxybutyrate accumulation	−	−	ND	+	+	−	ND or V
Anaerobic growth	+	−	V†	+	−	−	V
Nitrate reduction	−	+	V†	+	+	+	V
Acid from glucose	−	+	V†	+	−	−	−‡
Hydrolysis of:							
Gelatin	−	+	+	−	−	+	−§
Aesculin	+	−	+	−	−	+	−‖
DNA G+C content (mol%)	57.8	40–42	44–53	51.1	49	55	53–66

*CR, Curved rod; LR, long rod; R, rod; S, spirillum; SR, short rod; TR, thick rod.
†*H. chejuensis* is positive and *H. ganghwensis* is negative.
‡Data for *A. venustensis*.
§Data not available for *A. jadensis*.
‖Data for *A. borkumensis*.

that the coastal marine, oligotrophic isolate cannot be assigned to any previously recognized bacterial family or genus and therefore should be characterized as a novel species within a novel genus, *Litoricola lipolytica* gen. nov., sp. nov., belonging to a novel family, *Litoricolaceae* fam. nov.

Description of *Litoricola* gen. nov.

Litoricola (Li.to.ri′co.la. L. n. *litus -oris* seashore; L. suff. *-cola* from L. masc. or fem. n. *incola* inhabitant; N.L. fem. n. *Litoricola* inhabitant of the seashore).

Cells are short rods. Gram-negative. Oxidase-positive and catalase-negative. Chemoheterotrophic and facultatively aerobic. Non-motile. Nitrate is not reduced. Acid is not produced from glucose fermentation. NaCl is required for growth. The predominant fatty acids are $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, $C_{18:1}\omega7c$ and $C_{10:0}$ 3-OH . The DNA G+C content of the type strain of the type species is 57.9 mol%. Phylogenetically, the genus belongs to the family *Litoricolaceae* within the order *Oceanospirillales*. The type species of the genus is *Litoricola lipolytica*.

Description of *Litoricola lipolytica* sp. nov.

Litoricola lipolytica [li.po.ly′ti.ca. Gr. n. *lipos* fat; Gr. adj. *lytikos* dissolving; N.L. fem. adj. *lipolytica* fat-dissolving, pertaining to esterase lipase (C8) activity of the species].

In addition to having the properties given in the genus description, the species is characterized as follows. Cells are 0.5–0.7 μm wide and 0.8–1.3 μm long, dividing by binary fission (see Supplementary Fig. S1, available in IJSEM Online). Colonies on MA are circular, smooth, convex, opaque, cream-coloured and 0.05 mm in diameter, after 1 week of incubation. Colonies are approximately 1 mm in diameter after 3 weeks incubation. Growth occurs at 15–30 °C (optimum, 25 °C), pH 5–10 (optimum, pH 7.0) and 1.5–10.0 % NaCl (optimum, 3.0–3.5 % NaCl). No growth is observed at 10 or 35 °C, at pH 4 or 10 or at 1.0 or 15 % NaCl. Aesculin is hydrolysed. β-Galactosidase activity is present. Negative for indole production, arginine dihydrolase, gelatinase and urease. Only esterase lipase (C8) activity is detected in API ZYM tests; alkaline phosphatase, esterase (C4), acid phosphatase, naphthol-AS-BI-phosphohydrolase, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin,

Table 2. Cellular fatty acid compositions (%) of strain IMCC1097^T and members of related genera in the order *Oceanospirillales*

Taxa: 1, IMCC1097^T; 2, *Zooshikella* (data from Yi *et al.*, 2003); 3, *Hahella* (Baik *et al.*, 2005); 4, *Reinekea* (Romanenko *et al.*, 2004); 5, *Oleiphilus* (Golyshin *et al.*, 2002); 6, *Saccharospirillum* (recalculated from Labrenz *et al.*, 2003); 7, *Alcanivorax* (data for *A. dieselolei*/*A. borkumensis*/*A. venustensis*/*A. jadensis* from Bruns & Berthe-Corti, 1999; Fernández-Martínez *et al.*, 2003; Liu & Shao, 2005; Yakimov *et al.*, 1998). For cellular fatty acid analyses, strain IMCC1097^T and members of the genera *Zooshikella*, *Hahella* and *Reinekea* were grown on MA. *O. messinensis* was grown on ONR7a or SM1 agar medium supplemented with n-hexadecane. *S. impatiens* was grown on PYGV agar. *A. dieselolei* was grown on SM1 agar supplemented with alkanes and sodium citrate, *A. borkumensis* and *A. venustensis* were grown on SM1 medium supplemented with pyruvate and *A. jadensis* was grown in an ASW medium (liquid). Only those fatty acids representing at least 5 % of the total cellular fatty acids of at least one of the genera are shown. –, Trace amount or not detected.

Fatty acid	1	2	3	4	5	6	7
$C_{12:0}$	–	–	1.3–2.4	–	–	–	8.9/–/5.1/5.2
$C_{14:0}$	–	6.0	2.4–2.8	2.0	5.5	–	–/1.1/1.4/1.7
$C_{16:0}$	6.5	31.9	12.5–18.1	31.6	38.6	18.9	32.1/31.5/20.2/23.4
$C_{17:0}$	–	–	–	5.9	1.4	–	–/–/2.8/–
$C_{18:0}$	–	–	1.1–2.8	–	7.7	–	–/2.0/–/–
$C_{16:1}\omega7c$*	42.8†	37.1	3.3–11.9‡	26.7	16.6	21.8	11.3†/17.1§/15.4/13.5
$C_{16:1}\omega9c$	–	–	3.2–8.8	–	10.9	–	–
$C_{18:1}\omega7c$‖	20.6	14.5	<1–9.4	19.0	–	51.2	22.4/47.1¶/19.9/20.7
$C_{18:1}\omega9c$	–	–	19.8–39.0	–	1.4	–	–/–/1.2/–
$C_{18:3}\omega6c$	–	–	9.0–10.7	–	–	–	–
$C_{19:0}$ cyclo $\omega8c$	–	–	–	–	–	–	14.3/–/10.1/–
$C_{10:0}$ 3-OH	14.1	2.1	–	–	–	–	–
$C_{12:0}$ 3-OH	–	5.1	2.5–3.8	–	–	–	2.29/–/10.7/4.9
$C_{12:1}$ 3-OH	5.6	–	–	–	–	–	–
$C_{16:0}$ N alcohol	–	–	6.5–7.2	1.2	–	–	–
$C_{17:0}$ 10-methyl	–	–	0–14.4	–	–	–	–

*$C_{16:1}\omega7c$ or $C_{16:1}\omega7c$-containing fatty acid mixtures.
†$C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.
‡$C_{16:1}\omega7c$ and/or iso-$C_{16:0}$ 2-OH.
§$C_{16:1}\omega7c$ and/or $C_{16:1}\omega9t$.
‖$C_{18:1}\omega7c$ or $C_{18:1}\omega7c$-containing fatty acid mixtures.
¶One or more of $C_{18:1}\omega7c$, $C_{18:1}\omega7t$, $C_{18:1}\omega9t$ and $C_{18:1}\omega12c$.

α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. In tests for the utilization of sole carbon sources, positive results are obtained for the following carbon substrates: glycerol, DL-glyceraldehyde, D-ribose, L-arabinose, L-rhamnose, D-cellobiose, sucrose, trehalose, D-raffinose, adonitol, *myo*-inositol, D-xylitol, citric acid, D-glucuronic acid, pyruvic acid, L-alanine, L-histidine, L-lysine, L-ornithine and L-serine. The following carbon sources are utilized weakly: methylamine, ethanol, D-galactose, D-mannose, melibiose, D-melezitose, D-mannitol, gluconic acid, itaconic acid, propionic acid and DL-proline. Methanol, D-xylose, D-fructose, *N*-acetyl-D-glucosamine, D-glucosamine hydrochloride, D-glucose, α-D-lactose, maltose, L-arabitol, D-sorbitol, malonic acid, succinic acid, L-arginine, L-glutamic acid, L-glycine and L-leucine are not utilized as sole carbon sources. Susceptible to chloramphenicol, erythromycin, gentamicin, kanamycin, rifampicin, streptomycin, tetracycline and vancomycin, but resistant to ampicillin and penicillin G. The cellular fatty acids are composed of $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (42.8 %), $C_{18:1}\omega7c$ (20.6 %), $C_{10:0}$ 3-OH (14.1 %), an unknown fatty acid (equivalent chain-length 11.799) (6.8 %), $C_{16:0}$ (6.5 %), $C_{12:0}$ 3-OH (5.6 %), $C_{10:0}$ (0.9 %), $C_{14:0}$ (0.7 %), $C_{19:1}\omega6c$ (0.8 %), $C_{18:1}\omega7c$ 11-methyl (0.6 %), $C_{16:1}\omega5c$ (0.4 %), $C_{12:0}$ (0.1 %) and $C_{12:0}$ 3-OH (0.1 %).

The type strain, IMCC1097^T (=KCCM 42360^T =NBRC 102074^T), was isolated from surface seawater off the coast at Goseong, East Sea, Korea.

Description of *Litoricolaceae* fam. nov.

Litoricolaceae (Li.to.ri.co.la′ce.ae. N.L. fem. n. *Litoricola* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Litoricolaceae* the family of the genus *Litoricola*).

The family *Litoricolaceae* is within the order *Oceanospirillales* and encompasses Gram-negative bacteria retrieved from marine environments. Currently, the family comprises the genus *Litoricola* and several uncultured

marine bacteria. The delineation of the family is primarily determined from the phylogenetic position of the 16S rRNA gene sequence. The detailed description is the same as that given for the genus *Litoricola*. The type genus of the family is *Litoricola*.

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References

Baik, K. S., Seong, C. N., Kim, E. M., Yi, H., Bae, K. S. & Chun, J. (2005). *Hahella ganghwensis* sp. nov., isolated from tidal flat sediment. *Int J Syst Evol Microbiol* **55**, 681–684.

Bruns, A. & Berthe-Corti, L. (1999). *Fundibacter jadensis* gen. nov., sp. nov., a new slightly halophilic bacterium, isolated from intertidal sediment. *Int J Syst Bacteriol* **49**, 441–448.

Cho, J.-C. & Giovannoni, S. J. (2003). *Parvularcula bermudensis* gen. nov., sp. nov., a marine bacterium that forms a deep branch in the α-*Proteobacteria*. *Int J Syst Evol Microbiol* **53**, 1031–1036.

Cho, J. C. & Giovannoni, S. J. (2006). *Pelagibaca bermudensis* gen. nov., sp. nov., a novel marine bacterium within the *Roseobacter* clade in the order *Rhodobacterales*. *Int J Syst Evol Microbiol* **56**, 855–859.

Fernández-Martínez, J., Pujalte, M. J., García-Martínez, J., Mata, M., Garay, E. & Rodríguez-Valeral, F. (2003). Description of *Alcanivorax venustensis* sp. nov. and reclassification of *Fundibacter jadensis* DSM 12178^T (Bruns and Berthe-Corti 1999) as *Alcanivorax jadensis* comb. nov., members of the emended genus *Alcanivorax*. *Int J Syst Evol Microbiol* **53**, 331–338.

Franzmann, P. D., Wehmeyer, U. & Stackebrandt, E. (1988). *Halomonadaceae* fam. nov., a new family of the class *Proteobacteria* to accommodate the genera *Halomonas* and *Deleya*. *Syst Appl Microbiol* **11**, 16–19.

Garrity, G. M., Bell, J. A. & Lilburn, T. (2005a). Order VIII. *Oceanospirillales* ord. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, p. 270. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Garrity, G. M., Bell, J. A. & Lilburn, T. (2005b). Family I. *Oceanospirillaceae* fam. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, p. 271. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Garrity, G. M., Bell, J. A. & Lilburn, T. (2005c). Family III. *Hahellaceae* fam. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, p. 299. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Golyshin, P. N., Chernikova, T. N., Abraham, W. R., Lunsdorf, H., Timmis, K. N. & Yakimov, M. M. (2002). *Oleiphilaceae* fam. nov., to include *Oleiphilus messinensis* gen. nov., sp. nov., a novel marine bacterium that obligately utilizes hydrocarbons. *Int J Syst Evol Microbiol* **52**, 901–911.

Golyshin, P. N., Harayama, S., Timmis, K. N. & Yakimov, M. M. (2005). Family II. *Alcanivoraceae* fam. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, p. 295. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Kovács, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* **178**, 703.

Labrenz, M., Lawson, P. A., Tindall, B. J., Collins, M. D. & Hirsch, P. (2003). *Saccharospirillum impatiens* gen. nov., sp. nov., a novel γ-*Proteobacterium* isolated from hypersaline Ekho Lake (East Antarctica). *Int J Syst Evol Microbiol* **53**, 653–660.

Lee, H. K., Chun, J., Moon, E. Y., Ko, S. H., Lee, D. S., Lee, H. S. & Bae, K. S. (2001). *Hahella chejuensis* gen. nov., sp. nov., an extracellular-polysaccharide-producing marine bacterium. *Int J Syst Evol Microbiol* **51**, 661–666.

Liu, C. & Shao, Z. (2005). *Alcanivorax dieselolei* sp. nov., a novel alkane-degrading bacterium isolated from sea water and deep-sea sediment. *Int J Syst Evol Microbiol* **55**, 1181–1186.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Ostle, A. G. & Holt, J. G. (1982). Nile blue A as a fluorescent stain for poly-β-hydroxybutyrate. *Appl Environ Microbiol* **44**, 238–241.

Prabagaran, S. R., Manorama, R., Delille, D. & Shivaji, S. (2007). Predominance of *Roseobacter*, *Sulfitobacter*, *Glaciecola* and *Psychrobacter* in seawater collected off Ushuaia, Argentina, Sub-Antarctica. *FEMS Microbiol Ecol* **59**, 342–355.

Romanenko, L. A., Schumann, P., Rohde, M., Mikhailov, V. V. & Stackebrandt, E. (2004). *Reinekea marinisedimentorum* gen. nov., sp. nov., a novel gammaproteobacterium from marine coastal sediments. *Int J Syst Evol Microbiol* **54**, 669–673.

Schäfer, H., Servais, P. & Muyzer, G. (2000). Successional changes in the genetic diversity of a marine bacterial assemblage during confinement. *Arch Microbiol* **173**, 138–145.

Swofford, D. (2002). PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4. Sunderland, MA: Sinauer Associates.

Yakimov, M. M., Golyshin, P. N., Lang, S., Moore, E. R., Abraham, W. R., Lunsdorf, H. & Timmis, K. N. (1998). *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *Int J Syst Bacteriol* **48**, 339–348.

Yi, H., Chang, Y. H., Oh, H. W., Bae, K. S. & Chun, J. (2003). *Zooshikella ganghwensis* gen. nov., sp. nov., isolated from tidal flat sediments. *Int J Syst Evol Microbiol* **53**, 1013–1018.