Lacinutrix undariae sp. nov., isolated from a brown algae reservoir

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A Gram-stain-negative, aerobic, non-flagellated, non-gliding and ovoid or rod-shaped bacterium, designated strain W-BA8T, was isolated from a brown algae reservoir on the South Sea, South Korea, and subjected to a polyphasic taxonomic approach. Strain W-BA8T grew optimally at 25 °C, at pH 7.0–7.5 and in the presence of 1.0–2.0 % (w/v) NaCl. Neighbour-joining and maximum-parsimony phylogenetic trees based on 16S rRNA gene sequences showed that strain W-BA8T clustered with the type strains of species of the genus Lacinutrix. Strain W-BA8T exhibited 16S rRNA gene sequence similarity values of 94.9–96.5 % to the type strains of Lacinutrix species and of less than 95.8 % to the type strains of other recognized species. Strain W-BA8T contained MK-6 as the predominant menaquinone and iso-C15 : 0, iso-C15 : 1G, iso-C15 : 03-OH and iso-C17 : 03-OH as major fatty acids. The polar lipid profile of strain W-BA8T contained phosphatidylethanolamine, two unidentified lipids and one unidentified glycolipid as major components. The DNA G+C content of strain W-BA8T was 35 mol%. Differential phenotypic properties, together with phylogenetic distinctiveness, revealed that strain W-BA8T is separated from other species of the genus Lacinutrix. On the basis of the data presented, strain W-BA8T is considered to represent a novel species of the genus Lacinutrix, for which the name Lacinutrix undariae sp. nov. is proposed. The type strain is W-BA8T (=KCTC 42176T=CECT 8671T).

Members of the phylum Bacteroidetes, which are common in nature, are known to play an important role in the degradation of organic matter, including polysaccharides, in marine environments (Cottrell & Kirchman, 2000; Kirchman, 2002). From our studies to screen novel bacteria from a brown algae reservoir on the South Sea of the Korean peninsula, many novel bacterial taxa belonging to the phylum Bacteroidetes have been described (Park et al., 2013a, b, c, 2014; Park & Yoon, 2013). One of these bacterial strains, designated W-BA83, which showed degradative activities against some of the polysaccharides tested, is described in this study. Comparative 16S rRNA gene sequence analysis indicated that the novel strain is phylogenetically most closely associated with members of the genera Lacinutrix and Olleya of the family Flavobacteriaceae of the phylum Bacteroidetes. The genus Lacinutrix, which was proposed by Bowman & Nichols (2005), comprises, at the time of writing, five species with validly published names (Euzéby, 1997; Lee et al., 2014; Oren & Garrity, 2015), and one additional Lacinutrix species, 'Lacinutrix venerupis' (Lasa et al., 2015), has recently been described. The genus Olleya, which was proposed by Nichols et al. (2005), comprises three species with validly published names (Euzéby, 1997). Members of the two genera have been isolated from a variety of marine environments (Bowman & Nichols, 2005; Nichols et al., 2005; Nedashkovskaya et al., 2008; Lee et al., 2010, 2013; Srinivas et al., 2013). The aim of the present work was to determine the exact taxonomic position of strain W-BA8T by using a polyphasic characterization that included the determination of chemotaxonomic and other phenotypic properties and detailed phylogenetic investigations based on 16S rRNA gene sequences.

Leachate from a brown algae reservoir was collected from Wando, an island located on the South Sea in South Korea, and used as the source for the isolation of bacterial strains. Strain W-BA8T was isolated by the standard dilution plating technique at 25 °C on marine agar 2126 (MA; BD Difco) and cultivated routinely under the same culture conditions.
Lacinutrix algicola KCCM 42313T and Lacinutrix mariniflava KCCM 42306T and Lacinutrix himadriensis KCTC 23612T and Lacinutrix jangbogonensis KCTC 32573T, which were used as reference strains for phenotypic characterization and fatty acid and polar lipid analyses, were obtained from the Korean Culture Center of Microorganisms (KCCM; South Korea) and the Korean Collection for type Cultures (KCTC; South Korea), respectively. Unfortunately, the type strain of Lacinutrix copepodica (the type species of the genus) was not available from these two culture collections and thus could not be used in this study. Cell morphology, Gram reaction, pH range for growth and anaerobic growth were determined as described by Park et al. (2014). Growth at 4, 10, 20, 25, 28, 30, 35 and 37 °C was measured on MA to determine the optimal temperature and temperature range for growth. Gliding motility was investigated as described by Bowman (2000). Growth at various concentrations of NaCl (0, 0.5 and 1.0–10.0 %, w/v, at increments of 1.0 %) was investigated by supplementing with appropriate concentrations of NaCl in marine broth from which CaCl₂ was excluded to avoid the formation of precipitates.

Cell biomass of strain W-BA8T for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown for 2 days in MB at 25 °C, and cell biomass of L. algicola KCCM 42313T, L. mariniflava KCCM 42306T and L. himadriensis KCTC 23612T for polar lipid analysis was obtained from cultures grown under the same culture conditions. Chromosomal DNA was extracted and purified according to Yoon et al. (1996), with the modification that RNase T1 was used in combination with RNase A to minimize contamination of RNA. The 16S rRNA gene was amplified by PCR as described previously (Yoon et al., 1998) using two universal primers, 9F (5'-GAGTATTGATC-CTGGCTCAG-3') and 1512R (5'-ACGGTTACCTTGTTA-CGACTT-3'). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon et al. (2003).

Isoprenoid quinones were extracted and analysed as described by Komagata & Suzuki (1987), using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. The isoprenoid quinones were eluted by a mixture of methanol/2-propanol (2 : 1, v/v) using a flow rate of 1 ml min⁻¹ at room temperature and detected by UV absorbance at 270 nm. For cellular fatty acid analysis, cell mass of strain W-BA8T was harvested from MA plates after cultivation for 2, 3 and 5 days at 25 °C, cell mass of L. algicola KCCM 42313T, L. mariniflava KCCM 42306T and L. himadriensis KCTC 23612T was harvested from MA plates after cultivation for 3 days at 25 °C, and cell mass of L. jangbogonensis KCTC 32573T was harvested from MA plates after cultivation for 21 days at 10 °C. Fatty acids were saponified, methylated and extracted using the standard MIDI protocol (Sherlock Microbial Identification System, version 6.2B). The fatty acids were analysed by GC (Hewlett Packard 6890) and identified using the TSBA6 database of the Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin et al. (1984), and separated by two-dimensional TLC using chloroform/methanol/water (65 : 25 : 3.8, by vol.) for the first dimension and chloroform/methanol/acetic acid/water (40 : 7.5 : 6 : 1.8, by vol.) for the second dimension as described by Embley & Wait (1994). Individual polar lipids were identified by spraying the plates with 10 % ethanolic molybdophosphoric acid, molybdenum blue, ninhydrin and x-napthol reagents (Minnikin et al., 1984; Komagata & Suzuki, 1987) and with Dragendorff’s reagent (Sigma). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed using a reversed-phase HPLC system equipped with a YMC ODS-A (250 × 4.6 mm) column. The nucleotides were eluted by a mixture of 0.55 M NH₄H₂PO₄ (pH 4.0) and acetonitrile (40 : 1, v/v), using a flow rate of 1 ml min⁻¹ at room temperature and detected by UV absorbance at 270 nm.

Morphological, cultural, physiological and biochemical characteristics of strain W-BA8T are given in the species description and Table 1 and Fig. S1 (available in the online
Table 1. Differential characteristics between strain W-BA8T and the type strains of four recognized species of the genus Lacinutrix.

<table>
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<td>–</td>
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</table>

| DNA G+C content (mol%)†         | 35  | 37.0| 34.7| 29.0| 32.1|

†Data for the reference strains were taken from Nedashkovskaya et al. (2008), Srinivas et al. (2013) and Lee et al. (2014).

In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain W-BA8T joined the cluster comprising the type strains of Lacinutrix species with a bootstrap resampling value of 58.7 %, and this cluster joined the cluster comprising the type strains of Olleeya species (Fig. 1). The cluster of strain W-BA8T and the type strains of species of the genus Lacinutrix was also found in the tree reconstructed using the maximum-parsimony algorithm, but included the type strain of Corallibacter vietnamensis in the maximum-likelihood phylogenetic tree (Figs S2 and S3). In the maximum-likelihood and maximum-parsimony trees, the type strains of species of the genus Olleeya formed phylogenetic lineages independent of the genus Lacinutrix and other genera (Figs S2 and S3).

The predominant isoprenoid quinone detected in strain W-BA8T was menaquinone-6 (MK-6), in line with all other members of the family Flavobacteriaceae (Bernardet, 2011). The major fatty acids (>10 % of the total) detected in strain W-BA8T were iso-C15 : 0 3-OH, iso-C16 : 0 3-OH and iso-C17 : 0 3-OH (Table 1). The fatty acid profile of strain W-BA8T was similar to those of the type strains of species of the genera Lacinutrix and Olleeya, although there were differences in the proportions of some fatty acids, particularly iso-C15 : 0 anteiso-C15 : 0 3-OH and iso-C17 : 0 3-OH (Table 2; Lee et al., 2013; Srinivas et al., 2013). The major polar lipids detected in strain W-BA8T were phosphatidylethanolamine, two unidentified lipids and one unidentified glycolipid; minor amounts of one unidentified aminolipid, one unidentified aminophospholipid, two unidentified phospholipids and five other unidentified lipids were also present (Fig. S4). The polar lipid profile of strain W-BA8T was clearly distinguished from those of the type strains of Lacinutrix species based on the presence or absence of some polar lipids, particularly one unidentified lipid (L1) and one unidentified glycolipid as major components (Fig. S4). The polar lipid profile of strain W-BA8T was clearly distinguished from those of the type strains of Olleeya species based on the presence or absence of some major polar lipids (Lee et al., 2013). The DNA G+C content of strain W-BA8T was 35 mol% (SD 0.1 mol%), a value in the range reported for members of the genus Lacinutrix (Table 1; Srinivas et al., 2013).

Strain W-BA8T should be classified as a member of the genus Lacinutrix as revealed by phylogenetic data and the absence of distinct differential chemotaxonomic properties from the type strains of Lacinutrix species (Fig. 1; Table 2; Figs S2–S4). Strain W-BA8T could be distinguished from the type strains of recognized species of the genus Lacinutrix based on differences in several phenotypic characteristics, including catalase activity, hydrolysis of some substrates, acid production from some substrates, activity of cysteine arylamidase and susceptibility to some antibiotics (Table 1). These differences, in combination with

Supplementary Material). The almost-complete 16S rRNA gene sequence of strain W-BA8T determined in this study comprised 1442 nt. Strain W-BA8T exhibited highest 16S rRNA gene sequence similarity (96.5 %) to the type strain of L. jangbogonensis. It exhibited 16S rRNA gene sequence similarity values of 94.9–95.7 % to the type strains of other recognized species of the genus Lacinutrix and of 95.1–95.8 % to the type strains of species of the genus Olleeya.
phylogenetic distinctiveness, suggest that strain W-BA8$^T$ is separated from other species of the genus Lacinutrix (Stackebrandt and Goebel, 1994). On the basis of the data presented, strain W-BA8$^T$ is considered to represent a novel species of the genus Lacinutrix, for which the name Lacinutrix undariae sp. nov. is proposed.

**Description of Lacinutrix undariae sp. nov.**

Lacinutrix undariae (un.da’ri.ae. N.L. gen. n. undariae of Undaria, named after the generic name of the brown algae Undaria pinnatifida, from the reservoir of which the type strain was isolated).

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**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain W-BA8$^T$, the type strains of species of the genus Lacinutrix and representatives of some other related taxa. Only bootstrap values (expressed as percentages of 1000 replications) greater than 50 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms, while open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. *Capnocytophaga ochracea* ATCC 27872$^T$ (GenBank accession number U41350) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.
Cells are Gram-stain-negative, non-flagellated, non-gliding and ovoid or rod-shaped, approximately 0.2–0.6 μm in width and 0.3–3.0 μm in length. Colonies on MA are circular, slightly convex, smooth, glistening, light yellow and 0.5–1.0 mm in diameter after incubation for 3 days at 25 °C. Optimal temperature for growth is 25 °C; growth occurs at 4 and 30 °C, but not at 35 °C. Optimal pH for growth is 7.0–7.5; growth occurs at pH 5.0, but not at pH 4.5. Optimal growth occurs in the presence of 1.0–2.0 % (w/v) NaCl; growth occurs in the presence of 1.0–7.0 % (w/v) NaCl. Mg^{2+} ions are not required for growth. Growth does not occur under anaerobic conditions on MA or on MA supplemented with nitrate. Catalase- and oxidase-positive. Flexirubin-type pigments are not produced. Nitrate is not reduced to nitrite. Aesculin, alginate, carrageenan, casein, gelatin, pectin, starch, Tween 80, L-tyrosine and xylan are hydrolysed, but agar, carboxymethylcellulose, curdlan, hypoxanthine, urea and xanthine are not. Acid is produced from D-glucose, maltose and D-mannose, but not from L-arabinose, cellobiose, D-fructose, D-galactose, lactose, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, sucrose, trehalose, D-xyllose, myo-inositol, D-mannitol or D-sorbitol. In assays with the API ZYM system, activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase is present, but activity of lipase (C14), cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase is absent. Susceptible to carbenicillin, cephalotin, chloramphenicol, lincomycin, novobiocin, oleandomycin, penicillin G and tetracycline, but not to ampicillin, gentamicin, kanamycin, neomycin, polymyxin B or streptomycin. The predominant menaquinone is MK-6. The major fatty acids (>10 % of the total) are iso-C_{15:0} 3-OH-iso-C_{15:0} 3-OH and iso-C_{17:0} 3-OH. The major polar lipids are phosphatidylethanolamine, two unidentified lipids and one unidentified glycolipid.

The type strain, W-BA8^T (=KCTC 42176^T=CECT 8671^T), was isolated from leachate from a brown algae reservoir in Wando, an island located on the South Sea in South Korea. The DNA G+C content of the type strain is 35 mol%.

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### References


