Hymenobacter coalescens sp. nov., isolated from wetland freshwater

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A non-motile and rod-shaped bacterium, designated strain WW84T, was isolated from freshwater collected from the Woopo wetland (Republic of Korea). Cells were Gram-stain-negative, aerobic, catalase-positive and oxidase-negative. The major fatty acids were C16:0, C18:0, iso-C15:0 and iso-C17:1I and/or anteiso-C17:1B. The strain contained MK-7 as the major isoprenoid quinone, phosphatidylethanolamine as the major polar lipid and sym-homospermidine as the major polyamine. The DNA G+C content was 59 mol%. A phylogenetic tree based on 16S rRNA gene sequences showed that strain WW84T forms an evolutionary lineage within the radiation including the members of the genus Hymenobacter with Hymenobacter cellatus Myx 2105T (96.96 % sequence similarity) as its nearest neighbor. A number of phenotypic characteristics distinguished strain WW84T from the related members of the genus Hymenobacter. On the basis of the evidence presented in this study, a novel species, Hymenobacter coalescens sp. nov. is proposed with strain WW84T (=KCTC 32530T=JCM 19493T) as the type strain.

The genus Hymenobacter, a member of the family Cytophagaceae (Stanier, 1940), phylum Bacteroidetes, was proposed to group Gram-reaction-negative, non-spore-forming and non-motile rods producing large amounts of extracellular polymeric substances and spreading in thin pinkish layers on agar surfaces (Hirsch et al., 1999; Buczolits et al., 2006). At the time of writing, the genus comprises 40 species with Hymenobacter roseosalivarius as a type species. Species of the genus Hymenobacter have been isolated from a wide range of sources including air, soil, fresh water, estuarine water and extreme conditions such as arid land, glacier and uranium mine waste water treatment systems (Baik et al., 2006; Buczolits et al., 2002; Chang et al., 2014; Chung et al., 2010; Kang et al., 2013; Klassen & Foght, 2011). Several species are resistant to radiation (Collins et al., 2000; Dai et al., 2009; Zhang et al., 2007). In the course of our study on wetland microbial diversity, a rod-shaped bacterial strain, designated WW84T was isolated from a freshwater sample and subjected to a polyphasic taxonomy investigation.

WW84T was isolated from a freshwater sample collected from the inland wetland of Woopo (35°33’ N, 128°25’ E) located in the Republic of Korea, using the standard dilution plating technique. Isolation was achieved using Reasoner’s 2A (R2A, Becton Dickinson) agar at 25 °C for 7 days. The isolate was routinely cultured on R2A agar and preserved at −80 °C as a suspension in distilled water containing 20 % glycerol (w/v). Hymenobacter ocellatus KACC 12070T, Hymenobacter deserti KACC 14088T (purchased from KACC, Korea) and Hymenobacter koreensis GYR3077T (provided from Professor K.-Y. Jahng, Chonbuk National University, Republic of Korea) were used as reference strains for phenotypic characterization and fatty acid analysis.

Bacterial DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon/; Kim et al., 2012) and BLAST search program at the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The novel

The GenBank accession number for 16S rRNA sequence of strain WW84T (=KCTC 32530T=JCM 19493T) is KF631222.

Two supplementary figures are available with the online Supplementary Material.
sequence and related sequences were aligned by using CLUSTAL_W (Thompson et al., 1994), and the alignment was refined using BioEdit version 7.2.0 (Hall, 1999). Phylogenetic analysis was performed by using the software packages MEGA version 6.06 (Tamura et al., 2013). Phylogenetic trees were inferred using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1993) and maximum-parsimony (Fitch, 1971) algorithms. The distance matrix of the neighbour-joining method was generated according to the model of Jukes & Cantor (1969). The robustness of the topology in the neighbour-joining phylogenetic tree was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The DNA G+C content of strain WW84 was determined in triplicate by the thermal denaturation method of Marmur & Doty (1962).

The 16S rRNA gene sequence of strain WW84 was continuous stretches of 1446 nt. The closest relatives of strain WW84 were H. ocellatus Myx 2105T (96.9%, 16S rRNA gene sequence similarity), H. koreensis GYR307T (94.9%) and H. deserti ZLB-3T (94.7%). The neighbour-joining tree (Fig. 1) showed that strain WW84 formed a distinct branch with the clade comprising H. ocellatus with 99% bootstrap support. The trees based on other methods showed essentially similar topology. Thus, strain WW84 was recognized as representing a novel species of the genus Hymenobacter.

Growth was tested on nutrient (NA, Becton Dickinson), tryptic soy (TSA; Becton Dickinson), plate count (PCA, Becton Dickinson), glucose yeast extract (GYEA; Gordon & Mihm, 1962), marine (MA; Becton Dickinson) and R2A (Becton Dickinson) agars. Cells of strain WW84 were tested for growth on R2A agar supplemented with 0.5% (w/v) CMC (Sigma) and harvested at late-exponential growth phase; i.e. 3 days. Extraction of fatty acid methyl esters and separation by GC were performed by using the Instant FAME method of the Microbial Identification System (MIDI) version 6.1 and the TSBA6 database. For analyses of polar lipids, polyamine patterns and isoprenoid quinone, cells grown in R2A broth for 3 days at 30°C were purified according to the method of Minnikin et al. (1984). Fatty acid methyl esters and separation by GC were performed by using the Instant FAME method of the Microbial Identification System (MIDI) version 6.1 and the TSBA6 database. For analyses of polar lipids, polyamine patterns and isoprenoid quinone, cells grown in R2A broth for 3 days at 30°C were purified according to the method of Minnikin et al. (1984). Fatty acid methyl esters and separation by GC were performed by using the Instant FAME method of the Microbial Identification System (MIDI) version 6.1 and the TSBA6 database. For analyses of polar lipids, polyamine patterns and isoprenoid quinone, cells grown in R2A broth for 3 days at 30°C were purified according to the method of Minnikin et al. (1984). Fatty acid methyl esters and separation by GC were performed by using the Instant FAME method of the Microbial Identification System (MIDI) version 6.1 and the TSBA6 database.
The cellular fatty acid profile of strain WW84<sup>T</sup> is described in Table 2. Overall fatty acid profile of strain WW84<sup>T</sup> was similar to those of related species except for higher proportions of C<sub>16:0</sub> and C<sub>18:0</sub>. Quinone analysis revealed a spot that corresponded to menaquinone-7 (MK-7). The DNA G+C ratio of strain WW84<sup>T</sup> was 59 mol%. The major polar lipid of strain WW84<sup>T</sup> was phosphatidylethanolamine; five unidentified polar lipids, four unidentified aminolipids and an unidentified aminophospholipid were also detected (see Fig. S2). The major polyamine of strain WW84<sup>T</sup> was...
sym-homospermidine, similar to members of the genus *Hymenobacter* (Buczolits et al., 2006; Hoang et al., 2013; Kang et al., 2013; Zhang et al., 2008). A number of phenotypic characteristics and fatty acid profile clearly distinguished WW84<sup>T</sup> from strains representing other species of the genus *Hymenobacter*. Therefore, WW84<sup>T</sup> should be classified as representing a novel species within the genus *Hymenobacter*, for which the name *Hymenobacter coalescens* sp. nov., is proposed.

### Description of *Hymenobacter coalescens* sp. nov.

*Hymenobacter coalescens* [co.a.les’cens. L. part. adj. coalescens (from L. v. coalesco) coalescing, indicating that the cells coalesce].

Cells are Gram-stain-negative, aerobic, non-motile, rod-shaped and approximately 0.8 µm in diameter and 2.5–4.5 µm in length. Colonies are circular, raised, smooth, shiny, 1–2 mm in diameter and red–pink colored on R2A agar after 3 days. Growth occurs on R2A, NA, PCA and GYE but not on MA and TSA. Growth occurs with 0–0.4 % (w/v) NaCl (optimum, 0 %), at pH 6–8 (optimum, pH 7) and at 4–40 °C (optimum, 30 °C). Catalase-positive and oxidase-negative. Cells produce extracellular polymeric substances and aggregate to tight thin layers on agar surfaces. Flexirubin-type pigments are absent. Methanol extracts show the typical spectrum of carotenoid pigment with the maximum absorption at 484 nm with shoulders at 453 and 503 nm. Hydroxyflexixanthin is the major carotenoid. Nitrate is not reduced to nitrite. Indole and H<sub>2</sub>S are not produced. Aesculin, casein, gelatin, starch, Tween 20 and Tween 80 are hydrolyzed, but sodium alginate, chitin, gelatin, Tween 20 and Tween 80. All strains were negative for gliding motility and production of flexirubin-type pigments, H<sub>2</sub>S and indole, reduction of nitrate to nitrite, hydrolysis of sodium alginate, urea, CMC, chitin, pectin, xylan, hypoxanthine and xanthine and activity of lipase (C14), cystine arylamidase, Trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-fucosidase and α-mannosidase. Cells are sensitive to (µg per disc, unless otherwise indicated): ampicillin (10), erythromycin (15), vancomycin (30), nalidixic acid (30), penicillin (10 IU), tetracycline (30) and chloramphenicol (30) but resistant to amikacin (30), gentamicin (10), streptomycin (10) and kanamycin (30).

### Table 1. Phenotypic characteristics that differentiate strain WW84<sup>T</sup> from other species of the genus *Hymenobacter*

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>4 °C</td>
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<td>pH 9</td>
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<td>+</td>
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<td>Oxidase</td>
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<td>Glucose</td>
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<td>DNA G+C content (mol%)</td>
<td>59</td>
<td>65†</td>
<td>59‡</td>
<td>60‡</td>
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* Data from Buczolits et al. (2006).
† Data from Kang et al. (2013).
‡ Data from Zhang et al. (2009).
CMC, DNA, hypoxanthine, L-tyrosine, pectin, urea, xanthine and xylan are not. Acid is produced from glucose; negative for acid production from fructose, galactose, mannose, lactose, maltose, mannitol, rhamnose, sucrose, xylose and trehalose. In the API ZYM gallery, acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, N-acetyl-β-glucosaminidase, valine arylamidase and α-glucosidase are present but α-chymotrypsin, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, lipase (C14), α-mannosidase, and trypsin activities are absent. The predominant fatty acids (>10.0% of total fatty acids) were C16:0, C18:0, iso-C15:0 and summed feature 4 (iso-C17:1ω6c and anteiso-C17:1ω7c). The major respiratory quinone detected is menaquinone-7 (MK-7). The major polar lipid is phosphatidylethanolamine (PE); five unidentified polar lipids, four unidentified amnolipids and an unidentified aminophospholipid were also detected.

The type strain is WW84T (=KCTC 32530T=JCM 19493T), isolated from freshwater of Woopo wetland, Republic of Korea. The DNA G+C content of the type strain is 59.0 mol%.

Acknowledgements

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References


