Allohumibacter endophyticus gen. nov., sp. nov., isolated from the root of wild Artemisia princeps (mugwort)

Yu Ri Kim,1 Tae-Su Kim,1 Ji-Hye Han,1,2 Yochan Joung,1,3 Jisun Park1 and Seung Bum Kim1

Correspondence
Seung Bum Kim
sbk01@cnu.ac.kr

A novel actinobacterium designated strain MWE-A11T was isolated from the root of wild Artemisia princeps (mugwort). The isolate was aerobic, Gram-stain-positive and short rod-shaped, and the colonies were yellow and circular with entire margin. Strain MWE-A11T grew at 15–37 °C and pH 6.0–8.0. The predominant isoprenoid quinones were MK-11 and MK-10. The predominant fatty acids were anteiso-C15:0 and iso-C16:0, and the DNA G+C content was 68.8 mol%. The main polar lipids were diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. The peptidoglycan contained 2,4-diaminobutyric acid as the diagnostic diamino acid, and the acyl type was glycolyl. Phylogenetic analyses based on 16S rRNA gene sequence comparisons indicated that strain MWE-A11T was affiliated with the family Microbacteriaceae, and was most closely related to the type strains of Humibacter antri (96.4 % 16S rRNA gene sequence similarity), Herbiconiux moechotypica (96.3 %), Leifsonia soli (96.2 %), Leifsonia xylla subsp. cynodontis (96.1 %), Microbacterium testaceum (96.0 %) and Humibacter albus (96.0 %). However, the combination of chemotaxonomic properties clearly distinguished strain MWE-A11T from the related taxa at genus level. Accordingly, Allohumibacter endophyticus gen. nov., sp. nov. is proposed to accommodate a new member of the family Microbacteriaceae. The type strain of the type species is MWE-A11T (=JCM 19371T =KCTC 29232T).

The family Microbacteriaceae was first proposed by Park et al. (1993) and later emended by Stackebrandt et al. (1997) and Zhi et al. (2009). At the time of writing, 51 genera are classified as members of the family Microbacteriaceae (http://www.bacterio.net), including Conyziola, Frigoribacterium, Labedella and Okibacterium isolated from plant roots (Kim et al., 2014; Wang et al., 2015a, b, c). Members of the family Microbacteriaceae have ornithine, lysine or 2,4-diaminobutyric acid as diagnostic diamino acids in their cell-wall peptidoglycan, menaquinones with 7–9 isoprene units as the major respiratory quinones, anteiso-C15:0, anteiso-C17:0 and iso-C16:0 as the main fatty acids, and DNA G+C contents in the range 59–76 mol% (Evtushenko, 2012; Table 1).

Strain MWE-A11T was isolated from the root of natively growing Artemisia princeps (mugwort) sampled in the Daejeon area (geographical location: 36°41 ′ 61.04N, 127°53′ 09.66E), by using a standard dilution-plating technique on R2A (BD Difco) plates incubated at 30 °C under aerobic conditions. The isolate was sub-cultured several times to obtain a pure culture, and was stored at −80 °C in 20 % aqueous glycerol solution. The growth of strain MWE-A11T was observed on other media such as tryptone soy agar (TSA; BD) or nutrient agar (NA; BD) for 2 days at 30 °C.

Cellular morphology was observed using transmission electron microscopy (JEOL-1010; JEOL) of cells grown on TSA for 2 days at 30 °C. A transmission electron micrograph of
Table 1. Differential characteristics between strain MWE-A11\textsuperscript{T} and related genera of the family Microbacteriaceae

| Characteristic | Taxa: 1, MWE-A11\textsuperscript{T}; 2, \textit{Humibacter} (data from Kim et al., 2015; Lee, 2013; Vaz-Mareira et al., 2008); 3, \textit{Herbiconiux} (Behrendt et al., 2011; Hamada, 2012; Kim et al., 2012a); 4, \textit{Leifsonia} (Evtushenko, 2012); 5, \textit{Agromyces} (Evtushenko, 2012); 6, \textit{Schumannella} (An, 2008); 7, \textit{Lysinimonas} (Jang et al., 2013); 8, \textit{Rudaibacter} (Kim et al., 2013). DAB, 2,4-diaminobytyric acid; Orn, ornithine; Lys, lysine; +, positive; −, negative; ND, not determined.
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characteristic</strong></td>
<td>1</td>
</tr>
<tr>
<td>Morphology</td>
<td>Rods</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
</tr>
<tr>
<td>Major quinones</td>
<td>MK-13, 12</td>
</tr>
<tr>
<td>Diamino acids</td>
<td>DAB</td>
</tr>
<tr>
<td>Murein Acyl type</td>
<td>Acetyl</td>
</tr>
<tr>
<td>Major fatty acids</td>
<td>ai-C15 : 0, ai-C17 : 0, C18 : 1v7c</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>68.8</td>
</tr>
</tbody>
</table>

* Determined from this study.

Colonies of strain MWE-A11\textsuperscript{T} on TSA were pale yellow, round and convex. The cells were aerobic, non-motile and short rod-shaped. Strain MWE-A11\textsuperscript{T} grew at 15–37 °C (optimum 30 °C) and also at pH 6.0–8.0, and could tolerate up to 5 % (w/v) NaCl (optimum 0 %). The biochemical and physiological properties are listed in the species description.

Extraction of genomic DNA, and PCR amplification and sequence analysis of the 16S rRNA gene sequence followed previously described procedures (Kim et al., 2014). The 16S rRNA gene sequence of the isolate was compared with other sequences in the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net; Kim et al., 2012b) based on the pairwise alignment method. The 16S rRNA gene sequences of the isolate and other closely related species were aligned, and phylogenetic trees were reconstructed using MEGA software version 6 (Tamura et al., 2013). Phylogenetic trees were inferred with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms. Tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 replicates.

The nearly complete 16S rRNA gene sequence (1450 bp) of strain MWE-A11\textsuperscript{T} was obtained, and the comparative analysis based on the EzTaxon-e database indicated that strain MWE-A11\textsuperscript{T} displayed the highest 16S rRNA gene sequence similarity with \textit{Humibacter antri} D7-27\textsuperscript{T} (96.4 %) (Lee, 2013), \textit{Herbiconiux moechotypica} RB-62\textsuperscript{T} (96.3 %), \textit{Leifsonia soli} TG-S248\textsuperscript{T} (96.3 %), \textit{Leifsonia lichenia} 2Sb\textsuperscript{T} (96.2 %), \textit{Leifsonia xyi} subsp. \textit{cynodontis} JCM 9733\textsuperscript{T} (96.1 %), \textit{Microbacterium testaceum} DSM 10429\textsuperscript{T} (96.0 %) and \textit{Humibacter albuss} DSM 18994\textsuperscript{T} (96.0 %) were also found as neighbouring species.
taxa. However, strain MWE-A11\(^T\) formed an independent lineage in the maximum-likelihood, neighbour-joining and maximum-parsimony trees (Fig. 1). The 16S rRNA gene sequence of strain MWE-A11\(^T\) contained the signature nucleotides defined for the family *Microbacteriaceae* (Stackebrandt *et al.*, 1997; Zhi *et al.*, 2009), which supported the affiliation of the isolate to the family.

The genomic DNA G+C content was determined by the \(T_m\) method of Gonzalez & Saiz-Jimenez (2002).
The isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified using Sep-Pak vac silica cartridges (Waters) and analysed by HPLC as described by Collins et al. (1982). For the analysis of cell-wall amino acids and sugars, the cell wall was prepared as described by Schleifer & Kandler (1972). The amino acid composition of the peptidoglycan was determined by one-dimensional TLC as described by Harper & Davis (1979). The sugars of the peptidoglycan were also separated and identified by TLC using cellulose plates as described by Staneck & Roberts (1974). The murein acyl type of the cell wall was analysed according to the method of Uchida et al. (1999). Polar lipids were extracted from dried cell biomass and analysed using two-dimensional TLC as described by Minnikin et al. (1984). Fatty acid methyl esters were analysed by gas chromatography with Sherlock MIDI software (version 6.0) and a TSBA database (version 6.0).

The genomic DNA G+C content of strain MWE-A11T was 68.8 ± 0.5 mol%. The isoprenoid quinones consisted of MK-10 (30.4 %), MK-11 (62.2 %) and MK-12 (7.4 %). The diagnostic diamino acid in the cell wall peptidoglycan was 2,4-diaminobutyric acid. The diagnostic cell-wall sugars were rhamnose and xylose. The murein was of glycolyl type. The major polar lipids of strain MWE-A11T were diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid, and minor amounts of unidentified lipids were also present (Fig. S2). The major fatty acid of strain MWE-A11T was anteiso-C15 : 0 (52.8 %), and iso-C15 : 0 (6.3 %) were also present. The detailed fatty acid profiles of strain MWE-A11T and related taxa are listed in Table S1.

The chemotaxonomic properties clearly distinguished strain MWE-A11T from other related genera, as the major isoprenoid quinones of strain MWE-A11T differed from those of the genera Humibacter and Herbiconius, and the diamino acids and cell-wall sugars also differed from those of the genus Humibacter (Table 1). In addition, species of the genera Humibacter and Herbiconius are known to contain acetyl type murein, but that of strain MWE-A11T was glycolyl type. Moreover, fatty acid profiles also differed substantially (Tables 1 and S1). Hence, based on phylogenetic and chemotaxonomic analysis, strain MWE-A11T evidently represents a novel species of a new genus in the family Microbacteriaceae, for which the name Allohumibacter endophyticus gen. nov., sp. nov. is proposed.

Description of Allohumibacter endophyticus sp. nov.

Allohumibacter endophyticus (Gr. pref. endo within; Gr. n. phuton plant; L. masc. suff. -icus adjetival suffix used with the sense of belonging to; N.L. masc. adj. endophyticus within plant, endophytic, pertaining to the original isolation from plant tissues).

The species shows the following properties in addition to those given for the genus. Cells are short rods, approximately 0.5–1 μm long and 0.5–0.7 μm wide. Colonies are glistening and soft yellow after incubation for 2 days at 30 °C. Growth occurs at 15–37 °C, at pH 6.0–8.0, and in the presence of 0–5 % (w/v) NaCl; optimum growth is observed at 30 °C, pH 7 and with 0 % NaCl on TSA. Tweens 20 and 80, starch, casein, cellulose and DNA are hydrolysed. According to API ZYM tests, gelatin and aesculin are hydrolysed. β-Glucosidase and β-galactosidase activities are present, but arminine dihydrolase and urease activities are absent. According to API 20NE tests, nitrate is reduced, but indole is not produced. D-Glucose is not utilized. The following substrates are assimilated: D-glucose, D-mannitol, D-maltose, potassium gluconate and trisodium citrate. According to the API 50CH test, acids are produced from glycerol, L-arabinose, D-galactose, D-glucose, D-fucose, D-mannose, D-mannitol, amygdalin, arbutin, aesculin, salicin, cellobiose and maltose. The main fatty acids (> 5 %) are anteiso-C15 : 0, iso-C15 : 0, anteiso-C17 : 0 and a summed feature consisting of C16 : 0 2,07c and/or C16 : 0 1,06c.

The type strain is MWE-A11T (=JCM 19371T=KCTC 29232T), isolated from the root of Artemisia princeps (mugwort). The genomic DNA G+C content of the type strain is 68.8 mol%.

Acknowledgements

The authors acknowledge support from the Project on Survey and Excavation of Korean Indigenous Species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Korean Government, and also from the CNU Research Grant (grant no. 2015-1417-01) of Chungnam National University.

References


