Ureibacillus suwonensis sp. nov., isolated from cotton waste composts

Byung-Yong Kim,1 Seon-Young Lee,1,2 Hang-Yeon Weon,3 Soon-Wo Kwon,1 Seung-Joo Go,1 Yong-Keun Park,2 Peter Schumann4 and Dagmar Fritze4

1Korean Agricultural Culture Collection (KACC), Genetic Resources Division, National Institute of Agricultural Biotechnology, Rural Development Administration (RDA), Suwon 441-707, Korea
2School of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Korea
3Applied Microbiology Division, National Institute of Agricultural Science and Technology, RDA, Suwon 441-707, Korea
4Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

The taxonomic position of two spore-forming strains 6T19T and 6T29, isolated from cotton composts for the cultivation of oyster mushroom (Pleurotus ostreatus), was investigated by a polyphasic approach. Cells of strains 6T19T and 6T29 were rod-shaped, Gram-negative and strictly aerobic. Sequencing and comparative analyses for the 16S rRNA genes of these strains clearly showed their phylogenetic affiliation to the genus Ureibacillus. Their closest relatives Ureibacillus thermosphaericus and Ureibacillus terrenus have sequence similarity of 96–97.5%, respectively. The isoprenoid quinones of isolate 6T19T were MK-9, MK-8, MK-7, MK-10 and MK-6 (45:27:18:5:4%), the peptidoglycan type was L-Lys-D-Asp and the main cellular fatty acid was i-C16:0. DNA–DNA hybridization experiments resulted in relatedness values of 37% between 6T19T and U. thermosphaericus DSM 10633T and 41% between 6T19T and U. terrenus DSM 12654T. Based on the polyphasic data, strains 6T19T and 6T29 can be described as members of a novel species of the genus Ureibacillus, for which the name Ureibacillus suwonensis sp. nov. is proposed. The type strain is 6T19T (= KACC 11287T = DSM 16752T).

Ureibacillus was first described by Fortina et al. (2001) and it comprises two ureolytic thermophilic bacilli species, Ureibacillus thermosphaericus and Ureibacillus terrenus.

In Korea, composted cotton wastes are frequently used as medium for cultivation of oyster mushroom, Pleurotus ostreatus. During the sterilization and composting of cotton wastes, the temperature of composts is raised gradually from room temperature to 65°C. Two bacterial strains were obtained from the cotton wastes during the composting process. These strains were isolated from trypticase soy agar (TSA) at pH 7.0 incubated at 50°C and maintained in TSA medium. Colonies were not detected as single entities because of the smeared growth on both TSA and nutrient agar (Oxoid). Gram staining was done by using a Gram stain kit (Difco) according to the manufacturer’s recommended protocol. KOH test and L-alanine aminopeptidase assay were also used (Gregersen, 1978). The presence of flagella was sought according to Heimbrook et al. (1989). Cell morphology was observed in a phase-contrast microscope after incubation for 2 days on CASO agar (DSMZ medium no. 220; http://www.dsmz.de/media/media.htm) supplemented with 10 mg MnSO4 l−1. Cells grown on TSA for 24 h were examined by scanning electron microscopy. An agar block was cut from the plate, fixed in 1% osmium tetroxide and observed under a scanning electron microscope (Hitachi S-2460N).

The following physiological tests were carried out according to Gordon et al. (1973) and Claus & Berkeley (1986): catalase test, anaerobic growth, Voges–Proskauer (VP) test,
Peptidoglycan structure, menaquinones and polar lipids were analysed according to Fortina et al. (2001). After growth of cells on TSA for 24 h at 30 °C, fatty acid methyl esters were extracted and prepared by the standard protocol of the Microbial Identification system (MIDI; Microbial ID).

Supplementary Fig. S1 showing the morphological shapes of strain 6T19T is available in IJSEM Online. Comparisons of phenotypic properties among Ureibacillus species are presented in Table 1. In the report of the novel genus Ureibacillus, Fortina et al. (2001) characterized the genus as ureolytic, aerobic bacilli. For the urease test of the Ureibacillus species, Fortina et al. (2001) used the method by Atlas (1993), which relies on the demonstration of alkalinity. However, alkalinity can be produced from the use of peptone or other proteins in the medium, showing false-positive results (MacFaddin, 2000). In our tests, based on two different media, the medium used by Fortina et al. (2001) changed from yellow to pink-red colour, indicating a positive reaction. However, both control media inoculated with and without urea also showed a colour change to pink, indicating it to be an inappropriate test for urease of Ureibacillus strains. On the other hand, the cultures which were inoculated in Rustigian and Stuart’s urea broth showed no colour change, indicating a negative reaction in all the strains tested. The phenotypic comparison among Ureibacillus species is shown in Table 1.

For the phylogenetic analysis of the 16S rRNA gene sequences, representative sequences retrieved from GenBank were aligned from position 37 (5′) to 1431 (3′) (Escherichia coli numbering; Brosius et al., 1978). Strains 6T19T and 6T29 fell within the radiation of cluster comprising the two Ureibacillus species and Bacillus thermocloacae DSM 5250T (Fig. 1). The 16S rRNA gene sequence similarity between strains 6T19T and 6T29 was 99-8%. The closest relatives of

### Table 1. Differentiating phenotypic characteristics of Ureibacillus species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Spore shape*</td>
<td>S/O</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Growth at/in:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>35 °C</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>65 °C</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>5% NaCl</td>
<td>+</td>
<td>+</td>
<td>v</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Major menaquinones</td>
<td>MK-9, MK-8, MK-7, MK-10, MK-6</td>
<td>MK-7</td>
<td>MK-9, MK-8, MK-10, MK-7, MK-11</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>41-5</td>
<td>35-7–39.2</td>
<td>39.6–41.5</td>
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</table>

*S, Spherical; O, oval.
strain 6T19\textsuperscript{T} were \textit{U. terrenus} (97.5\%) and \textit{U. thermo-sphaericus} (96.9\%). On the other hand, strain 6T19\textsuperscript{T} was very distinct from species classified in other genera (\(<92\%\) sequence similarity).

DNA–DNA hybridization was performed to determine the genetic relatedness between strains 6T19\textsuperscript{T} and 6T29 and between strain 6T19\textsuperscript{T} and the type strains of the two \textit{Ureibacillus} species. The reassocation value between 6T19\textsuperscript{T} and 6T29 was shown to be 92\%, indicating that the two strains belong to the same species (Wayne et al., 1987). The DNA–DNA reassocation values of 6T19\textsuperscript{T} with \textit{U. terrenus} DSM 10633\textsuperscript{T} and \textit{U. terrenus} DSM 12654\textsuperscript{T} proved to be 37 and 41\%, respectively. The DNA G+C content of strain 6T19\textsuperscript{T} was 41.5 mol\%.

Strains 6T19\textsuperscript{T} and 6T29 contained L-Lys→D-Asp type (variation A4x) (A11.31) as the diagnostic diamino acid in the cell-wall peptidoglycan. The isoprenoid quinone composition of 6T19\textsuperscript{T} was MK-9, MK-8, MK-7, MK-10 and MK-6 (45:27:18:5:4 \%), and 6T29 contained MK-9, MK-8, MK-7, MK-6 and MK-10 (36:30:27:5:2 \%). Strains 6T19\textsuperscript{T} and 6T29 contained straight-chain and terminally saturated fatty acids with a composition of 5–6 and 76–85 \%, respectively (Supplementary Table S1 available in IJSEM Online). No significant differences in fatty-acid profiles were found with other \textit{Ureibacillus} species, except that strains 6T19\textsuperscript{T} and 6T29 produced a higher proportion of ai-C\textsubscript{17}:0 and lower proportions of C\textsubscript{16}:0, i-C\textsubscript{15}:0 and i-C\textsubscript{17}:0 than the type strains of the two \textit{Ureibacillus} species. Polar lipids consisted of phosphatidylglycerol, diphosphatidylglycerol, phospholipids and glycolipids of unknown composition.

On the basis of the polyphasic data, strains 6T19\textsuperscript{T} and 6T29 can be described as members of a novel species of the genus \textit{Ureibacillus}, for which the name \textit{Ureibacillus suwonensis} sp. nov. is proposed.

**Description of \textit{Ureibacillus suwonensis} sp. nov.**

\textit{Ureibacillus suwonensis} (su.won.en’sis. N.L. masc. adj. suwonensis referring to Suwon Region in Korea, where the bacteria were first found).

Cells are Gram-negative, spore-forming rods, 0.5–0.7×1.5–2.0 \(\mu\)m in size, single or in chains. Cells form spherical or oval spores, occurring subterminally or terminally in swollen sporangia. Cells are peritrichously flagellated and motile. Clear colonies are not formed. Colonies grow smudged. Growth occurs at temperatures ranging from 35 to 60 \(^\circ\)C and in the presence of 5 \% NaCl. Positive for catalase, oxidase and arginine dihydrolase. Weak reaction for phenylalanine deamination. Negative for anaerobic growth, formation of indole and dihydroxyacetone, VP test, nitrate reduction, acid production from D-glucose, L-arabinose, D-xylose and D-mannitol and hydrolysis of aesculin, starch, gelatin, casein and urea. The cross-linkage of peptidoglycan is L-Lys→D-Asp type (variation A4z). The major cellular fatty acid is i-C\textsubscript{16}:0. The major isoprenoid quinones are MK-9, MK-8 and MK-7. The DNA G+C content of the type strain 6T19\textsuperscript{T} is 41.5 mol\%.

The type strain, 6T19\textsuperscript{T} (=KACC 11287\textsuperscript{T}=DSM 16752\textsuperscript{T}), was isolated from cotton composts in Suwon, Korea.

**References**


