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

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The taxonomic position of two spore-forming strains 6T19<sup>T</sup> and 6T29, isolated from cotton composts for the cultivation of oyster mushroom (*Pleurotus ostreatus*), was investigated by a polyphasic approach. Cells of strains 6T19<sup>T</sup> 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 and 97–5 %, respectively. The isoprenoid quinones of isolate 6T19<sup>T</sup> 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-C<sub>16 : 0</sub>. DNA–DNA hybridization experiments resulted in relatedness values of 37 % between 6T19<sup>T</sup> and *U. thermosphaericus* DSM 10633<sup>T</sup> and 41 % between 6T19<sup>T</sup> and *U. terrenus* DSM 12654<sup>T</sup>. Based on the polyphasic data, strains 6T19<sup>T</sup> 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 6T19<sup>T</sup> (= KACC 11287<sup>T</sup> = DSM 16752<sup>T</sup>).

*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 MnSO<sub>4</sub> l<sup>−1</sup>. 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,
temperature range for growth (5–70 °C, increments of 5 °C), egg-yolk reaction, resistance to lysozyme, growth in the presence of NaCl (0, 2, 5, 7 and 10%), growth at pH 5–7, acids from carbohydrates (D-glucose, L-arabinose, D-xylose and D-mannitol), formation of gas from glucose, hydrolysis of starch, utilization of citrate and propionate, nitrate reduction, production of indole, deamination of phenylalanine, decomposition of casein and tyrosine and liquefaction of gelatin. Oxidase test and hydrolysis of aesculin were conducted according to Smibert & Krieg (1994). Motility test was performed on 1/10 CESP agar (1·5 g casitone, 0·5 g yeast extract, 0·3 g soytone, 0·2 g peptone, 0·015 g MgSO$_4$, 0·007 g FeCl$_3$, 0·002 g MnCl$_2$, made up to 1 l with distilled water, pH 7·2) (Fortina et al., 2001).

The 16S rRNA gene sequences were determined by PCR amplification (Kwon et al., 2003) and direct sequencing (Hiraishi, 1992). The 16S rRNA gene sequences were aligned by using the MEGALIGN program of DNASTAR. An evolutionary distance matrix was generated as described by Jukes & Cantor (1969). The evolutionary tree for the datasets was inferred from the neighbour-joining method of Saitou & Nei (1985). Probe labelling was conducted by using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using the DIG-High prime system (Roche) and hybridized by using the DIG hybridization method described by Seldin & Dubnau (1985). DNA–DNA hybridization was carried out as a filter-hybridization method described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG–High prime system (Roche) and hybridized DNA was visualized by using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio–Rad). The DNA G + C content (mol%) was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah et al. (1989) using a reverse-phase column (Supelcosil LC-18-S; Supelco).

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 6T19$^T$ 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 6T19$^T$ and 6T29 fell within the radiation of cluster comprising the two Ureibacillus species and Bacillus thermocloacae DSM 5250$^T$ (Fig. 1). The 16S rRNA gene sequence similarity between strains 6T19$^T$ and 6T29 was 99·8%. The closest relatives of

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

Species: 1, U. suwonensis sp. nov.; 2, U. thermosphaericus; 3, U. terrenus. Data from Fortina et al. (2001) and this study. All strains were negative for urease according to our results. +, Positive; −, negative; v, variable among strains.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Spore shape*</td>
<td>S/O</td>
<td>S</td>
<td>S</td>
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<td>Growth at/in:</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>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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*S, Spherical; O, oval.
Ureibacillus suwonensis sp. nov.

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, 0.5-5-0.7×1.5-2.0 μm 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 smears. Growth occurs at temperatures ranging from 35 to 60°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_{16.0}. The major isoprenoid quinones are MK-9, MK-8 and MK-7. The DNA G+C content of the type strain 6T19^T is 41.5 mol%.

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

References


