Leeia oryzae gen. nov., sp. nov., isolated from a rice field in Korea

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A strictly aerobic, non-spore-forming, Gram-negative bacterium, designated strainHW7T, was isolated from a rice field in Korea. Cells of strainHW7were short rod-shaped and motile with single polar flagella. The major cellular fatty acids of strainHW7were C16:0 and summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH). The genomic DNA G+C content was 56 mol% and the major isoprenoid quinone was Q-8. Phylogenetic analysis based on 16S rRNA gene sequences showed that strainHW7forms a distinct lineage with respect to closely related genera within the class Betaproteobacteria and that the levels of 16S rRNA gene sequence similarity with respect to the type species of related genera are less than 93%. On the basis of the physiological and phylogenetic data, strainHW7represents a novel genus and species of the Betaproteobacteria, for which the nameLeeia oryzaegen. nov., sp. nov. is proposed. The type strain isHW7 (=KCTC 12585T =DSM 17879T).

Rice serves as the principal food for nearly half of the world’s population; about 90% of the total area of rice fields in the world is found in Asia. After flooding, rice fields generally become anoxic and the methanogenic microbial community develops, with active degradation of organic matter (Chin & Janssen, 2002; Eller et al., 2005). Thus there have been many studies on anaerobic microbial communities in anoxic rice-field soils, and many anaerobic and methane-producing bacteria have been isolated from rice paddies (Akasaka et al., 2003; Dianou et al., 2001; Ueki et al., 2006). However, research has been relatively scant in relation to aerobic bacterial communities in rice fields. Therefore, in the course of screening aerobic bacteria from rice fields, we isolated a novel aerobic Gram-negative bacterium and investigated its physiological and molecular characteristics. Here we describe its taxonomic characteristics, which identify it as representing a novel genus and species within the Betaproteobacteria.

StrainHW7was isolated from rice-paddy soil associated with the roots ofOryzae sativa growing in the Milyang area of Korea. We sampled rice roots from a rice paddy in June and removed soil debris from the roots by tapping. The roots were washed and the washings then diluted serially using 0.9% (w/v) saline. The diluted solutions were spread on nutrient agar (NA; Difco) and incubated for 3 days at 30°C. The isolated strain (HW7T) was routinely grown aerobically on NA for 2 days at 35°C, except where indicated otherwise. Gram staining was determined using the bioMérieux Gram stain kit according to the manufacturer’s instructions. Cell morphology and motility were studied using phase-contrast microscopy and transmission electron microscopy as described by Jeon et al. (2005). Growth was tested at different temperatures (4–45°C) and at different pH values (5.0–10.0). NA media with different pH values were prepared as described previously (Gomori, 1955). Oxidase activity was tested by assessing the oxidation of 1% (w/v) tetramethyl-p-phenylenediamine (Merck), and catalase activity was determined by assessing the production of oxygen bubbles in a 3% (v/v) aqueous hydrogen peroxide solution. The hydrolysis of L-tyrosine, aesculin, gelatin, starch, Tween 80 and urea and the reduction of nitrate were tested on NA according to previously described methods (Lanyi, 1987; Smibert & Krieg, 1994). Acid production from carbohydrates was tested as described by Leifson (1963).

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strainHW7T is DQ280369.

Figures showing the general morphology and the polar lipids of cells of strainHW7T are available as supplementary material with the online version of this paper.
Table 1. Cellular fatty acid compositions of strain HW7T and related strains

| Fatty acid | Strain HW7T | SGLY2T | SGLY2T 
|------------|-------------|--------|--------
| C10:0      | 1.62        | ND     | ND     |
| C10:0-3-OH | 0.99        | ND     | 5.2    |
| C12:0      | 2.5         | 7.0    | 5.6    |
| C12:0-2-OH | ND          | 9.6    | 3.0    |
| C12:0-3-OH | ND          | ND     | 5.0    |
| C14:0      | 0.54        | ND     | ND     |
| C14:0      | 1.29        | ND     | 1.7    |
| iso-C15:0  | 0.72        | ND     | ND     |
| C15:0      | 1.12        | ND     | ND     |
| iso-C15:0  | 2.13        | 1.1    | ND     |
| C16:0      | 42.66       | 21.6   | 23.9   |
| C17:0 cyclo| 7.86        | ND     | 1.7    |
| C17:0      | 1.03        | ND     | ND     |
| C17:0-6c   | ND          | 1.9    | ND     |
| C18:0      | 1.18        | 17.4   | 15.0   |
| C18:0      | 0.59        | ND     | ND     |
| C16:0-7c + iso-C16:0-7c-2-OH | ND | ND | 35.8 |
| Summed feature 3 | 33.49 | 38.3 | ND |

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contains C16:0-7c and/or iso-C15:0-2-OH.

Additional enzyme activities were determined by using the API ZYM kit at 30 °C, as recommended by the manufacturer (bioMérieux). On NA, strain HW7T formed cream, circular/slightly irregular and slightly convex colonies when grown at 35 °C for 2 days. Growth was observed at temperatures between 10 and 40 °C (optimum, 32–35 °C) and at pH 5.0–8.5 (optimum, pH 6.0). The cells were rods (0.5–0.8 μm wide and 1.1–1.8 μm long) and were motile by means of single polar flagella (see Supplementary Fig. S1, available in IJSEM Online). Anaerobic growth was not observed over 5 days at 35 °C on NA. Other phenotypic features of strain HW7T are presented in the descriptions of the novel genus and species.

Analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI; Microbial ID). Isoprenoid quinones and polar lipids were determined using the methods described by Komagata & Suzuki (1987). The DNA G+C content was determined using HPLC apparatus fitted with a reversed-phase column (GROM-SIL 100 ODS-2PE; Grom), according to the method of Tamaoka & Komagata (1984). The major cellular fatty acids of strain HW7T were C16:0 (42.66 %) and summed feature 3 (C16:1-97c and/or iso-C15:0-2-OH; 33.49 %) (Table 1). Strain HW7T contained phosphatidylethanolamine, phosphatidylglycerol and diphostatidylglycerol (see Supplementary Fig. S2, available in IJSEM Online). Unfortunately, polar lipids have not been reported for the genera Microvirga and Laribacter and hence it is not clear whether strain HW7T can be distinguished from these two genera on the basis of polar lipid profiles. The genomic DNA G+C content of strain HW7T was 56 mol% and the predominant isoprenoid quinone was Q-8. The typical phenotypic characteristics of strain HW7T are summarized, and compared with those of the type strains of closely related taxa, in Table 2. Some of them are in accordance with characteristics of members of the Betaproteobacteria, whereas some others facilitate the differentiation of strain HW7T from closely related members (Bazylinski et al., 1986; Grimes et al., 1997; Patureau et al., 1998; Coenye et al., 2000; Yuen et al., 2001).

The sequencing and assembly of the 16S rRNA gene was carried out as described previously (Lane, 1991). The 16S rRNA gene sequence (1416 nt) of strain HW7T was compared with 16S rRNA gene sequences available from GenBank, using the BLAST program (http://www.ncbi.nlm.nih.gov/blast/) to determine the approximate phylogenetic affiliation and using CLUSTAL W software (Thompson et al., 1994) to align the sequences of closely related organisms. Phylogenetic trees were constructed using three different methods, namely the neighbour-joining, maximum-likelihood and maximum-parsimony algorithms available in PHYLIP software, version 3.6 (Felsenstein, 2002). Similarity calculations were obtained by using the FASTA3 program of the European Bioinformatics Institute (Hinxton, Cambridge, UK; http://www.ebi.ac.uk) to compare the 16S rRNA gene sequence of the novel strain with those of related organisms. A bootstrap analysis was performed according to the algorithm of the Kimura two-parameter model (Kimura, 1980) of the neighbour-joining method in the PHYLIP package. The phylogenetic analysis based on 16S rRNA gene sequences showed that strain HW7T forms a distinct phylogenetic lineage with respect to the closely related genera Microvirga and Laribacter within the Betaproteobacteria (Fig. 1). The topologies of the phylogenetic trees constructed using the maximum-likelihood and maximum-parsimony algorithms also supported the notion that the isolate could be a distinct phylogenetic genus (data not shown). A comparative analysis of 16S rRNA gene sequences showed that the levels of sequence similarity between strain HW7T and the closely related strains Microvirga aerodenitrificans SGLY2T and Laribacter hongkongensis HKU1T were 92.8% and 91.0%, respectively. Therefore, on the basis of the physiological, biochemical and phylogenetic data, strain HW7T represents a novel genus and species within the Betaproteobacteria, for which the name Leeia oryzae gen. nov., sp. nov. is proposed.

**Description of Leeia gen. nov.**

Leeia (Lee”i.a. N.L. fem. n. Leeia named after Keho Lee, a Korean microbiologist who devoted his life to the study of food micro-organisms).
Cells are Gram-negative, non-spore-forming short rods. Oxidase- and catalase-positive. Cells are aerobic and are motile by means of single polar flagella. Nitrate is reduced to nitrite. The major isoprenoid quinone is Q-8. Polar lipids are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The predominant cellular fatty

Table 2. Differential characteristics of strain HW7<sup>T</sup> and related genera

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Rod</td>
<td>Rod</td>
<td>Seagull-shape or spiral rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Curved rod</td>
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<tr>
<td>Size (μm)</td>
<td>0.5–0.8 × 1.1–1.8</td>
<td>ND</td>
<td>ND</td>
<td>0.5 × 3.5</td>
<td>ND</td>
<td>0.7 × 3.0–3.5</td>
<td>0.5–0.6 × 1.1–2.5</td>
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<tr>
<td>Motility</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>–</td>
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<tr>
<td>Growth at 45°C</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
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<td>Major fatty acids</td>
<td>C&lt;sub&gt;16:0&lt;/sub&gt; summed feature 3*</td>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>C&lt;sub&gt;18:1ω7c&lt;/sub&gt;, summed feature 3*</td>
<td>ND</td>
<td>ND</td>
<td>C&lt;sub&gt;16:1ω7c&lt;/sub&gt; + iso-&lt;sub&gt;C&lt;sub&gt;16:1ω7c&lt;/sub&gt; 2-OH&lt;/sub&gt;,&lt;br&gt;C&lt;sub&gt;16:0&lt;/sub&gt;</td>
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<td>Hydroxy fatty acids:</td>
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<tr>
<td>C&lt;sub&gt;10:0&lt;/sub&gt; 3-OH</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
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<tr>
<td>C&lt;sub&gt;12:0&lt;/sub&gt; 2-OH</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>ND</td>
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<td>ND</td>
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<td>C&lt;sub&gt;12:0&lt;/sub&gt; 3-OH</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Anaerobic growth</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>DNA G + C content (mol%)</td>
<td>56</td>
<td>65</td>
<td>68 ± 2.43</td>
<td>65.4</td>
<td>65–68</td>
<td>50–52</td>
<td>59–61</td>
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</table>

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contains C<sub>16:1ω7c</sub> and/or iso-C<sub>15:0 2-OH</sub>.

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships of strain HW7<sup>T</sup> and related taxa. Bootstrap percentages (based on 1000 replicates) greater than 45% are shown at branch points. Escherichia coli ATCC 11775<sup>T</sup> was used as an outgroup. Bar, 0.1 changes per nucleotide position.
acids are C₁₆:0 and summed feature 3 (C₁₆:1ω7c and/or iso-C₁₅:0 2-OH). Phylogenetically, the genus belongs to the class Betaproteobacteria. The type species is Leeia oryzae.

Description of Leeia oryzae sp. nov.

Leeia oryzae (o.r’y’zæ. L. gen. n. oryzae of rice, referring to the rice-paddy fields where the strain was isolated).

Exhibits the following properties in addition to those given in the genus description. Colonies are cream, circular/ slightly irregular and slightly convex on NA. Cells are approximately 0.5–0.8 μm wide and 1.1–1.8 μm long. Growth occurs at 10–40°C (optimum, 35–35°C) and pH 5.0–8.5 (optimum, pH 6.0). L-Tyrosine is hydrolysed. Hydrolysis of ascuclen, gelatin, starch, Tween 80 and urea is not observed. Acid is produced from L-arabinose, D-glucose, D-mannose and D-ribose, but not from maltose, D-trehalose, D-xyllose, glycerol, sucrose, cellobiose, D-fructose, x-D-lactose or adonitol. API ZYM kit gives positive results for esterase (C4), leucine arylamidase and acid phosphatase but negative results for lipase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-norimbergensis and/or iso-C₁₅:0 2-OH). Phylogenetically, the genus belongs to the class Betaproteobacteria.

Acknowledgements

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References


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