Oryzihumus leptocrescens gen. nov., sp. nov.

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Three novel strains were isolated from a soil sample collected in Japan using GPM agar plates supplemented with superoxide dismutase and/or catalase. The strains were Gram-positive, catalase-positive, irregular rod-shaped bacteria with meso-diaminopimelic acid as a peptidoglycan diagnostic diamino acid, and the acyl type of the peptidoglycan was acetyl. The major menaquinone was MK-8(H2). Mycolic acids were not detected. The G+C content of the DNA was 72–73 mol%. On the basis of the morphological and chemotaxonomic properties and a phylogenetic analysis using 16S rRNA gene sequences, these strains were classified as a novel genus and species, Oryzihumus leptocrescens gen. nov., sp. nov., in the family Intrasporangiaceae of the order Actinomycetales. The type strain is KV-628T (= NRRL B-24347T = JCM 12835T = NBRC 100762T).

Various techniques for the isolation of novel microbial strains have been reported in order to facilitate the discovery of novel bioactive compounds from micro-organisms. An efficient method of discovering novel bioactive metabolites is through the isolation of new micro-organisms, and numerous approaches have been attempted to date (Huck et al., 1991; Iwai & Takahashi, 1992; Jiang & Xu, 1996; Nioh et al., 1995; Nonomura & Hayakawa, 1988; Suzuki et al., 1998; Takeuchi & Hatano, 1999). In a previous report, a new isolation method using agar medium containing oxidant scavengers, such as superoxide dismutase (SOD) or SOD plus catalase, was described (Takahashi et al., 2003). Using this method, we succeeded in increasing the number of bacterial strains isolated from soil samples.

Among 45 strains isolated from paddy soil in Saitama Prefecture, Japan, using this new isolation method, 20 strains belonged to the class Actinobacteria. Three strains were irregular rods, found to have meso-diaminopimelic acid in the peptidoglycan; in the phylogenetic tree based on 16S rRNA gene sequences, they were loosely associated with Ornithinicoccus hortensis. Ornithinicoccus hortensis cells are cocci containing ornithine in their peptidoglycan.

In this paper, we report on the morphological, physiological and biochemical characteristics, cell composition, DNA–DNA hybridization and 16S rRNA gene sequences of the three isolates in comparison with those of Ornithinicoccus hortensis. On the basis of the characteristics studied, these isolates represent a novel genus and species, Oryzihumus leptocrescens gen. nov., sp. nov.

Strains KV-628T, KV-641 and KV-656 were isolated from soil samples collected from a paddy field in Saitama Prefecture, Japan. Soil samples (2 g) were suspended in 18 ml sterile water and then mixed. Soil particles were allowed to sediment, the liquid phase was diluted $10^5$ times and $100 \mu l$ was spread onto the surface of four kinds of GPM agar media (Pridham & Gottlieb, 1948) with yeast nitrogen base sources at 1% (w/v) was determined in carbon-utilization tests grown on GPM medium at 27°C. The strains were catalase-positive, irregular rod-shaped bacteria with meso-diaminopimelic acid in the peptidoglycan; in the phylogenetic tree based on 16S rRNA gene sequences, they were loosely associated with Ornithinicoccus hortensis. Ornithinicoccus hortensis cells are cocci containing ornithine in their peptidoglycan.

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supplemented with the following antibiotics: erythromycin, aztreonam, rifampicin, novobiocin, streptomycin and chloramphenicol.

Whole-cell hydrolysates were analysed for diaminopimelic and chloramphenicol.

Strains KV-628T, KV-641 and KV-656 were isolated from isolation GPM agar medium supplemented with SOD, catalase or SOD plus catalase. PCR amplification was performed to determine if these strains were members of the Actinobacteria. This test was established to distinguish strains of the Actinobacteria from both Gram-negative bacteria and Gram-positive bacteria with low-G+C DNA contents by taking advantage of the specific insertion of a 100 bp sequence in domain III of the 23S rRNA gene of the Actinobacteria. The PCR was designed to amplify a ~380 bp fragment from the Actinobacteria and a ~270 bp fragment from other bacteria (Roller et al., 1992; Yu et al., 2002). Amplicons of these strains were all ~350 bp in length, and the results showed that all three of the isolated strains were members of the Actinobacteria.

Nearly complete 16S rRNA gene sequences were determined for the three isolated strains. A database search demonstrated that these strains belonged to the suborder Micrococcineae of the family Intrasporangiaceae. It was clear from the phylogenetic tree (Fig. 1) that the three strains formed a monophyletic clade loosely associated with Ornithinicoccus hortensis. The sequence similarity values among KV-628T, KV-641 and KV-656 were above 99-9%, whereas the sequence similarity values between these three strains and Ornithinicoccus hortensis were below 95-6%. Sequence similarity values for KV-628T and other Intrasporangiaceae family members were as follows: Terrabacter tumescens, 95-2%; Knoellia sinensis, 95-2%; Intrasporangium calvum, 94-8%; Janibacter limosus, 94-8%; Tetrasphaera japonica, 94-8%; Arsenicicoccus bolidensis, 94-8%; Terracoccus luteus, 94-4%; and Ornithinimicrobium humphilum, 93-7%.

The three strains were also examined for a set of phenotypic and chemotaxonomic characteristics. Strains KV-628T, KV-641 and KV-656 were very similar in terms of morphological and chemotaxonomic characteristics. The three strains were irregular rods and the cell diameters varied from 0.4 to 0.9 μm by 0.9 to 1.9 μm (Fig. 2). The cells of the three strains were Gram-positive, catalase-positive and showed no motility. The strains were able to grow at 15–37 °C and at pH 4–9, but at pH 9 only trace growth was observed. NaCl in 1/5 nutrient agar medium was tolerated up to 4%, while only trace growth was observed at 5% NaCl.

The physiological characteristics are given in the species description. The three isolated strains were very similar in terms of carbon-source assimilation and enzyme content.

The DNA G+C content of KV-628T, KV-641 and KV-656 was 72–73 mol% (with the HPLC nucleoside method). The cell-wall peptidoglycan of KV-628T, KV-641 and KV-656 contained meso-diminopimelic acid, alanine and glutamic acid at molar ratios of 1.0:1.6:1.0, 1.0:1.7:1.0 and 1.0:1.7:1.0, respectively. The three isolated strains contained a peptidoglycan of type Ala (Schleifer & Kandler, 1972). The predominant menaquinone was MK-8(H4). The acyl type was acetyl. Mycolic acids were not detected. The
predominant cellular fatty acids were iso-C14:0, iso-C15:0, anteiso-C15:0 and iso-C16:0 (Table 1).

To confirm that these strains belong to a novel species, DNA–DNA hybridization relatedness was determined. The levels of DNA–DNA relatedness among the three isolated strains and Ornithinicoccus hortensis were determined. The values among the three isolated strains were 90–101%, whereas those between the three strains and Ornithinicoccus hortensis were below 25%. The values were well below the 70% cut-off point recommended by Wayne et al. (1987) for species classification.

At present, the family Intrasporangiaceae contains nine genera: Arsenicicoccus (Collins et al., 2004), Intrasporangium (Kalakoutskii et al., 1967), Janibacter (Martin et al., 1997), Knoellia (Groth et al., 2002), Terrabacter (Collins et al., 1989), Terracoccus (Prauser et al., 1997), Tetrasphaera (Maszenan et al., 2000), Ornithinicoccus (Groth et al., 1999) and Ornithinimicrobium (Groth et al., 2001). Table 2 shows the differential characteristics of the members of the family Intrasporangiaceae. The genera Janibacter, Knoellia and Tetrasphaera also have meso-diaminopimelic acid in their cell walls, but Janibacter, Knoellia (iso-C15:0, iso-C17:0, iso-C16:0 and anteiso-C17:0) and Tetrasphaera differ in terms of cellular fatty acids.

On the basis of the distinct phylogenetic position of the three novel isolates within the family Intrasporangiaceae, as well as their differential characteristics in terms of cell morphology and cell-wall murein type, a novel genus and species, Oryzihumus leptocrescens gen. nov., sp. nov., is proposed.

**Description of Oryzihumus gen. nov.**

*Oryzihumus* (Ory.zi.hu’mus. L. fem. n. oryza rice; L. masc. n. humus soil; N.L. masc. n. Oryzihumus rice soil).

Table 1. Cellular fatty acid composition (%) of strains KV-628T, KV-641 and KV-656

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>KV-628T</th>
<th>KV-641</th>
<th>KV-656</th>
</tr>
</thead>
<tbody>
<tr>
<td>i-C13:0</td>
<td>-</td>
<td>0:5</td>
<td>-</td>
</tr>
<tr>
<td>i-C14:0</td>
<td>12:9</td>
<td>7:7</td>
<td>4:8</td>
</tr>
<tr>
<td>C14:0</td>
<td>-</td>
<td>1:0</td>
<td>2:8</td>
</tr>
<tr>
<td>i-C15:0</td>
<td>23:1</td>
<td>40:1</td>
<td>27:3</td>
</tr>
<tr>
<td>ai-C15:0</td>
<td>3:1</td>
<td>10:7</td>
<td>14:5</td>
</tr>
<tr>
<td>i-C16:0</td>
<td>35:8</td>
<td>13:7</td>
<td>9:4</td>
</tr>
<tr>
<td>C16:0</td>
<td>0:6</td>
<td>0:7</td>
<td>5:2</td>
</tr>
<tr>
<td>i-C17:0</td>
<td>0:5</td>
<td>0:9</td>
<td>0:6</td>
</tr>
<tr>
<td>ai-C17:0</td>
<td>1:4</td>
<td>5:0</td>
<td>5:0</td>
</tr>
</tbody>
</table>

Fig. 1. Phylogenetic tree, derived from 16S rRNA gene sequences, created using the neighbour-joining method and $K_{\text{auc}}$ values. Only values above 50% significance are indicated. The solid circles indicate that the corresponding nodes are also recovered in the maximum-likelihood tree. The tree was unrooted and *Rarobacter faecitabidus* was used as an outgroup.
Table 2. Differential characteristics of strain KV-628T and related taxa

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Cell morphology</th>
<th>Wall diaminoo acid</th>
<th>DNA G+C content (mol%)</th>
<th>Murein type</th>
<th>Major menaquinone</th>
<th>Fatty acid type</th>
</tr>
</thead>
<tbody>
<tr>
<td>KV-628T</td>
<td>Irregular rods</td>
<td>meso-A2pm</td>
<td>72–73</td>
<td>A1γ</td>
<td>MK-8(H4)</td>
<td>SAI</td>
</tr>
<tr>
<td>Arsenicicoccus*</td>
<td>Cocci</td>
<td>L-Orn</td>
<td>72</td>
<td>A3γ</td>
<td>MK-8(H4)</td>
<td>SIU</td>
</tr>
<tr>
<td>Intrasporangium†</td>
<td>Hyphae</td>
<td>L-Orn</td>
<td>66</td>
<td>A3γ</td>
<td>MK-8</td>
<td>SAI</td>
</tr>
<tr>
<td>Janibacter§</td>
<td>Coccolid to rod-shaped</td>
<td>meso-A2pm</td>
<td>70</td>
<td>A1γ</td>
<td>MK-8(H4)</td>
<td>SIU</td>
</tr>
<tr>
<td>Knoblia§</td>
<td>Coccolid to rod-shaped</td>
<td>meso-A2pm</td>
<td>68–69</td>
<td>A1γ</td>
<td>MK-8(H4)</td>
<td>SAI</td>
</tr>
<tr>
<td>Terrabacter‡</td>
<td>Coccolid</td>
<td>L-Orn</td>
<td>70–73</td>
<td>A3γ</td>
<td>MK-8(H4)</td>
<td>SAI</td>
</tr>
<tr>
<td>Terracoccus§</td>
<td>Coccolid</td>
<td>L-Orn</td>
<td>73</td>
<td>A3γ</td>
<td>MK-8(H4)</td>
<td>SAI</td>
</tr>
<tr>
<td>Tetrasphaera¶</td>
<td>Coccolid</td>
<td>meso-A2pm</td>
<td>68–71</td>
<td>A1γ</td>
<td>MK-8(H4)</td>
<td>SIU</td>
</tr>
<tr>
<td>Ornithinicumisco♦</td>
<td>Coccolid</td>
<td>L-Orn</td>
<td>72</td>
<td>A4β</td>
<td>MK-8(H4)</td>
<td>SAI</td>
</tr>
<tr>
<td>Ornithinimicrobium★★</td>
<td>Coccolid</td>
<td>L-Orn</td>
<td>70</td>
<td>A4β</td>
<td>MK-8(H4)</td>
<td>SAI</td>
</tr>
</tbody>
</table>

*Data from Collins et al. (2004).
†Data from Schumann et al. (1997).
‡Data from Martin et al. (1997).
§Data from Groth et al. (2002).
∥Data from Prauser et al. (1999).
¶Data from Maszenan et al. (2000).
#Data from Collins et al. (2004).
**Data from Groth et al. (2001).

Gram-positive, catalase-positive, aerobic, non-motile irregular rods. The peptidoglycan is of the A type of direct cross-linkage and contains meso-diaminopimelic acid, alanine and glutamic acid. The acyl type of the glycan chain of peptidoglycan is acetyl. Mycolic acids are absent. The major menaquinone is MK-8(H4). The DNA G+C content is 72–73 mol%. Phylogenetically, this genus is a member of the family Intrasporangiaceae, suborder Micrococccineae. The type species is Oryzihumus leptocrescens.

Description of Oryzihumus leptocrescens sp. nov.

Oryzihumus leptocrescens (lep.to.cre’sens. Gr. adj. leptos thin, fine, delicate, slender; L. part. adj. crescens growing; N.L. part. adj. leptocrescens slender growing).

Cells are irregular rods 0.4–0.9 μm by 0.9–1.9 μm. Colonies are pale yellow. Aerobic to microaerophilic. Growth occurs between pH 4 and 9 and at 15 and 37 °C. In 1/5 nutrient agar medium, NaCl is tolerated up to 5%. D-Glucose, maltose, sucrose and trehalose are assimilated, but L-ribo- and D-xylose are not. Susceptible to erythromycin (15 μg ml⁻¹), rifampicin (30 μg ml⁻¹), novobiocin (30 μg ml⁻¹), streptomycin (10 μg ml⁻¹) and chloramphenicol (30 μg ml⁻¹). Not susceptible to aztreonam (100 μg ml⁻¹). Esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-Bl-phosphohydrolase, β-galactosidase, β-glucosidase and β-glucosidase are detected by the API ZYM enzyme assay; alkaline phosphatase, cysteine arylamidase, trypsin, chymotrypsin, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are not detected. A weak reaction for lipase (C14) is detected. Variable reactions for α-galactosidase are detected. The DNA G+C content is 72–73 mol%.

Habitat: paddy soil in Japan. The type strain is KV-628T (= NRRL B-24347T = JCM 12835T = NBRC 100762T).

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References


