Paenibacillus rhizoplanae sp. nov., isolated from the rhizosphere of Zea mays

Peter Kämpfer,¹,* Hans-Jürgen Busse,² John A. McInroy,³ Chia-Hui Hu,³ Joseph W. Kloepper³ and Stefanie P. Glaeser¹

Abstract

A Gram-stain-positive, aerobic, endospore-forming bacterial strain isolated from the rhizosphere of Zea mays was studied to determine its detailed taxonomic position. Based on 16S rRNA gene sequence similarity comparisons, strain JJ-64T was shown to be a member of the genus Paenibacillus, most closely related to the type strains of Paenibacillus silagei (99 %) and Paenibacillus borealis (97.5 %). 16S rRNA gene sequence similarity to all other Paenibacillus species was ≤ 97.5 %. DNA–DNA hybridization values to the type strains of P. silagei and P. borealis were 51 % (reciprocal 25 %) and 31 % (reciprocal 37 %), respectively. The presence of meso-diaminopimelic acid as the diagnostic diamino acid of the peptidoglycan, the major quinone MK-7 and the polyamine pattern with spermidine as the major component were well in line with the characteristics of the genus Paenibacillus. Furthermore, the polar lipid profile of strain JJ-64T with the predominant lipids diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine and two unidentified aminophospholipids reflected the close phylogenetic relatedness to P. silagei. Major fatty acids were iso- and anteiso-branched components. Physiological and biochemical characteristics allowed the further phenotypic differentiation of strain JJ-64T from the most closely related species. Thus, strain JJ-64T represents a novel species of the genus Paenibacillus, for which the name Paenibacillus rhizoplanae sp. nov. is proposed. The type strain is JJ-64T (=LMG 29875T=CCM 8725T).

The genus Paenibacillus, initially proposed by Ash et al. [1], now accommodates more than 100 species isolated from various sources. Interestingly, species of Paenibacillus have been isolated often as endophytes [2–5] and from other plant-associated environments such as the rhizosphere [6–15], seeds [16] or the phyllosphere [17, 18] of plants. In general, endospore-forming bacilli, including members of the genus Paenibacillus, are of particular interest for their capacity to promote plant growth and form endospores [19].

Field-grown maize plants (Zea mays) grown in Dunbar, Nebraska, USA, were manually uprooted a few weeks after planting. Roots, approximately 15 cm in length, were separated from the surrounding soil by vigorous shaking such that only the most tightly adhering soil remained. Bacteria were collected from the root surface by immersing the root in sterile water followed by plating dilutions on nutrient agar (Sigma-Aldrich). Strain JJ-64T was initially isolated at Auburn University in this manner.

Subsequent cultivation of strain JJ-64T was performed on tryptone soy agar (TSA; Oxoid) at 25 °C for 24 h. Cell morphology and motility was observed under a Zeiss light microscope at a magnification of 1000×, using cells that had been grown for 3 days at 25 °C on TSA. Gram-staining was performed by the modified Hucker method according to Gerhardt et al. [20]. The KOH test was carried out according to Moaledj [21]. Physiological characterization was done according to the methods described by Kämpfer et al. [22] and Kämpfer [23]. In addition, the presence of urease was tested on urea agar (Merck) supplemented with 2 % urea according to the manufacturer’s instructions [24]. Indole and sulphide production was tested on SIM agar according to the instructions of the manufacturer (Merck).

For phylogenetic analysis, the 16S rRNA gene of strain JJ-64T was PCR amplified and sequenced with universal 16S rRNA gene sequence targeting primers 27F and 1492R [25]. The final corrected 16S rRNA gene sequence of strain JJ-64T had a size of 1485 nt and spanned gene termini 8–1475 (numbering according to Brosius et al. [26]). The phylogenetic relationship of strain JJ-64T was analysed in ARB release 5.2 [27] using the ‘All-Species Living Tree’ Project LTP; [28] database release LTPs123 (September 2015). The 16S rRNA gene sequence of strain JJ-64T and all sequences of type strains of novel Paenibacillus species proposed since the LTP database release 2015 obtained from GenBank (NCBI) were aligned with SINA (v1.2.9) according to the

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SILVA seed alignment ([www.arb-silva.de; [29]]) or downloaded with the ARB-Silva search tool from the database SSU 128 ([www.arb-silva.de/search]), and implemented in the LTP database. Pairwise sequence similarities were calculated with the ARB neighbor-joining tool without the use of an evolutionary model. A maximum-likelihood tree was calculated with RAxML v7.04 [30], GTR-GAMMA and rapid bootstrap analysis and a maximum-parsimony tree with DNAPARS v3.6 [31]. Both trees were calculated with 100 re-samplings (bootstrap analysis; [32]) and based on 16S rRNA gene sequences between gene termini 105 and 1366 (Escherichia coli numbering, [26]). First, trees were calculated including all Paenibacillus species and subsequently only neighbouring species and the cluster of Paenibacillus species containing the type species were selected for further tree calculations. Strain J)J-64$^T$ showed highest 16S rRNA gene sequence similarity to the type strains of Paenibacillus silagei (99.0 %) followed by Paenibacillus borealis (97.6 %). Sequence similarities to all other Paenibacillus species were below 97.5 %. Strain JJ-64$^T$ formed, independent of the applied treeing method, a distinct cluster with the type strain of P. silagei (Fig. 1). The cluster was supported by high bootstrap values. The two strains formed a broader distinct cluster with the type strains of ‘Paenibacillus salmicaen’, Paenibacillus julianii, Paenibacillus graminis and Paenibacillus riograndensis. The respective type strains shared between 96.0 and 97.2 % pairwise 16S rRNA gene sequence similarity with strain JJ-64$^T$. The type strain of P. borealis was only loosely attached to that cluster although it shared a higher 16S rRNA gene sequence similarity (97.6 %) with strain JJ-64$^T$.

Detailed analysis of the 16S rRNA gene sequence alignment showed that the main reason for the distinct relationship between strain JJ-64$^T$ and the type strains of the six above listed species was a specific sequence stretch between 16S rRNA gene sequence positions 1003 and 1024 (according to the E. coli numbering) which differed from the respective sequence of all other Paenibacillus type strains including the type strain of P. borealis.

The genomic DNA G+C content of strain JJ-64$^T$ was determined by the DNA melting temperature method established by Gonzales & Saiz-Jimenez [33] as described previously by Glaeser et al. [34]. The genomic DNA G+C content determined for strain JJ-64$^T$ was 52.9 mol%, which was within the range reported for the two next closest related species, 52.2±0.7 mol% for P. silagei LOOC204$^T$ [35] and 53.6 % for P. borealis KK19$^T$ [36]. All three values were higher than the G+C content determined for the type species Paenibacillus polymyxa (43–46 mol%; confirmed by genome sequence-based data).

DNA–DNA hybridization was applied according to Ziemke et al. [37] using genomic DNA extracted by the method of Pitcher et al. [38]. Hybridization of the genomic DNA of strain JJ-64$^T$ and P. silagei DSM 101993$^T$ and P. borealis LMG 21603$^T$ resulted in values of 51 % (reciprocal 25 %) and 31 % (reciprocal 37 %), respectively.

Biomass used for analyses of the diagnostic diamino acid of the peptidoglycan, polyamines, the quinone system and polar lipids was grown in PYE broth (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) at 28 °C. Quinones and polar lipids were extracted and analysed by applying the integrated procedure reported by Tindall [39, 40] and Altenburger et al. [41]. The diagnostic diamino acid of the peptidoglycan was analysed according to Schumann [42] and in accordance with other species of the genus Paenibacillus meso-diaminopimelic acid was detected. The quinone system was composed of menaquinones MK-7 (94.4 %) and MK-6 (5.6 %), which is also in line with other species of the genus. The polar lipid profile (Fig. 2) was composed of the major lipids diphosphatidylglycerol, phosphatidylglycerol, phosphatidyethanolamine, phosphatidylserine and two unidentified aminophospholipids (APL1, APL2). In addition, four minor polar lipids (L1–L4) without a functional group were detected. This polar lipid profile showed a high degree of similarity to that of P. silagei LOOC204$^T$ mainly in the presence of major lipids. Only the presence of an unidentified phospholipid and minor amounts of an unidentified aminolipid and two lipids without a functional group in P. silagei LOOC204$^T$ differentiated the two strains. On the other hand the absence of any glycolipid distinguished strain JJ-64$^T$ from the type species of the genus, P. polymyxa [43].

Polyamines were extracted and analysed from cells harvested at the late exponential growth phase as reported by Busse and Auling [44] and Altenburger et al. [45]. HPLC analysis was carried out using the equipment described by Stolz et al. [46]. The polyamine pattern showed the major compound spermidine [35.1 µmol (g dry weight)$^{-1}$], minor amounts of spermine [1.8 µmol (g dry weight)$^{-1}$] and traces of cadaverine and putrescine [each less than 0.1 µmol (g dry weight)$^{-1}$]. Similar polyamine patterns were also reported for other Paenibacillus species, including the type species of the genus, P. polymyxa, and Paenibacillus chartarius, Paenibacillus vulgaris and Paenibacillus cucumis [47–50].

The fatty acids were extracted and analysed as described by Kämpfer and Kroppenstedt [51]. Strains were grown under identical conditions (TSA, 72 h of incubation at 28 °C) and cells used for extraction were taken from colonies of the same size. Fatty acids were identified with Sherlock version 2.11, TSBA40 Rev. 4.1.

The fatty acids comprised mainly iso- and anteiso-branched components and the fatty acid profile was very similar to those of the most closely related Paenibacillus species. The detailed fatty acid profile is shown in Table 1.

The results of the physiological characterization, performed using methods described previously [22, 23], are given in Table 2 and in the species description.

Based on the summary of genotypic, phenotypic and chemotaxonomic results we describe a novel species of the genus Paenibacillus, for which the name Paenibacillus rhizoplanae is proposed. The Minimal Standards for describing
The mum temperature for growth is 28°C. Weak growth is observed on MacConkey agar. Optimal growth is on TSA (after 48 h) are circular, convex and beige. No other cell inclusions are detected. Colonies show no motility. Oval endospores are formed in a central plane, the region of the root epidermis of a plant where soil particles and bacteria adhere.

Cells (with rounded ends) stain Gram-positive. No chains or filaments are observed after growth on TSA at 28°C for 48 h. Cells are 2.0–3.0 μm in length and 0.8–1.0 μm in width and show no motility. Oval endospores are formed in a central position. No other cell inclusions are detected. Colonies grown on TSA (after 48 h) are circular, convex and beige with a shiny appearance and have an average diameter of 2–3 mm. Weak growth is observed on MacConkey agar. Optimum temperature for growth is 28–30°C; growth occurs between 10 and 36°C but not at 4 or 45°C. Optimal pH for growth is 7–8; growth occurs between pH 6.0 and 9.5. Grows in the presence of 1–3 % (w/v) NaCl (but not 4 % or above) in TS broth. Tests for catalase and oxidase activities are positive. Tests for production of indole and sulphide, urease, gelatinase, β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and citrate utilization, and gelatin, starch and casein hydrolysis are negative. No acid formation from the sugars or sugar-related compounds D-ribose, D-arabinose, D-xylose, D-mannitol, D-mannose, melibiose, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose or D-xylose is observed. Several sugars or sugar-related compounds are utilized as sole carbon source according to the method of Kämpfer et al. [22]: L-arabinose, arbutin, p-arbutin, cellobiose, D-fructose, myo-inositol, D-galactose, D-glucuronate, glucose, maltose, D-maltitol, D-mannose, melibiose, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose or D-xylose is observed. Several sugars or sugar-related compounds are utilized as sole carbon source according to the method of Kämpfer et al. [22]: L-arabinose, arbutin, p-arbutin, cellobiose, D-fructose, myo-inositol, D-galactose, D-glucuronate, glucose, maltose, D-maltitol, D-mannose, D-mannitol, melibiose, ribose, salicin, sucrose, D-sorbitol, trehalose and D-xylose. Acetate, N-acetyl-D-glucosamine, cis-aconitate, trans-aconitate, adipate, D-adenitol, 4-aminobutyrate,
azelate, citrate, itaconate, malate, mesaconate, 2-oxogluta-rate, propionate, putrescine, pyruvate and L-rhamnose are not utilized as sole carbon source. The quinone system contains mainly menaquinone MK-7 and small amounts of MK-6. The polar lipid profile is composed of the major lipids diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, PS, phosphatidylserine; APL1, APL2, unidentified aminophospholipids; L1 L4, unidentified polar lipids lacking any functional group.

Table 1. Cellular fatty acid compositions of strain JJ-64T and related species of the genus Paenibacillus

<table>
<thead>
<tr>
<th>Saturated:</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>11.2</td>
<td>14.6</td>
<td>15.0</td>
</tr>
<tr>
<td>C16:0</td>
<td>21.5</td>
<td>27.4</td>
<td>31.2</td>
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</table>

<table>
<thead>
<tr>
<th>Branched:</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>iso-C14:0</td>
<td>4.3</td>
<td>5.1</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>6.9</td>
<td>5.3</td>
<td>TR</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>46.9</td>
<td>40.1</td>
<td>42.1</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>6.7</td>
<td>7.4</td>
<td>11.8</td>
</tr>
<tr>
<td>anteiso-C17:0</td>
<td>2.5</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2. Differential phenotypic characteristics between strain JJ-64T and related species of the genus Paenibacillus

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>d-Fructose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Ribose</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>N-Acetyl-galactosamine</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Maltitol</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

amounts of four unidentified lipids (L1, L2, L3, L4) are detected. meso-Diaminopimelic acid is the characteristic diamino diacid of the peptidoglycan and spermidine is the predominant compound in the polypeptide pattern. Major fatty acids are anteiso-C15:0, C16:0, C14:0, iso-C16:0 and iso-C15:0. In addition, iso-C14:0 and anteiso-C17:0 are detected.

The type strain, JJ-64T (=LMG 29875T=CCM 8725T), was isolated from the root surface of a field-grown corn plant.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Ethical statement

No experiments with humans or animals were carried out.

References


24. Christensen WB. Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and shigella types. *J Bacteriol* 1946;52:461–466.


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