Proposal for Two New Genera, *Brevibacillus* gen. nov. and *Aneurinibacillus* gen. nov.

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16S rRNA gene sequences of the type strains of 11 species belonging to the *Bacillus brevis* and *Bacillus aneurinolyticus* groups were determined. On the basis of the results of gene sequence analyses, these species were separated into two clusters. The *B. brevis* cluster included 10 species, namely, *Bacillus brevis*, *Bacillus agri*, *Bacillus centrosporus*, *Bacillus choshinensis*, *Bacillus parabrevis*, *Bacillus reuszeri*, *Bacillus formosus*, *Bacillus borstelensis*, *Bacillus laterosporus*, and *Bacillus thermoruber*. *Bacillus aneurinolyticus* and *Bacillus migulanus* belonged to the *B. aneurinolyticus* cluster. Moreover, the two clusters were phylogenetically distinct from other *Bacillus*, *Ampibacillus*, *Sporalactobacillus*, *Paenibacillus*, and *Alicyclobacillus* species. On the basis of our data, we propose reclassification of the *B. brevis* cluster as *Brevibacillus* gen. nov. and reclassification of the *B. aneurinolyticus* cluster as *Aneurinibacillus* gen. nov. By using 16S rRNA gene sequence alignments, two specific PCR amplification primers were designed for differentiating the two new genera from each other and from other aerobic, endospore-forming organisms.

The aerobic, rod-shaped, endospore-forming genus *Bacillus* is a systematically diverse taxon (5). The members of this genus exhibit a wide range of DNA base compositions, and the major amino acid compositions of the cell walls of these organisms vary (6, 22, 32). Analyses of 16S rRNA gene sequences have identified at least eight phylogenetic groups in the genus *Bacillus* (2, 3, 7, 20, 22–24, 33, 38). Two of these groups have been reclassified as new genera. One genus, the genus *Alicyclobacillus* (38), consists of thermoaciduric species that contain rarely encountered cellular ω-cyclic fatty acids. The other new genus, the genus *Paenibacillus* (3), was distinguished on the basis of the results of slot blot hybridization in which a specific probe was used.

Recent taxonomic studies have shown that strains previously assigned to *Bacillus brevis* should be separated into nine species, namely, *Bacillus brevis*, *Bacillus agri*, *Bacillus centrosporus*, *Bacillus choshinensis*, *Bacillus parabrevis*, *Bacillus reuszeri*, *Bacillus formosus*, *Bacillus borstelensis*, and *Bacillus migulanus* (18, 19, 28, 29, 34). A closely related species, *Bacillus aneurinolyticus*, has also been revived by Shida et al. (30). On the basis of the results of comparisons of their phenotypic characteristics, chemosystematic profiles, and conserved specific S-layer proteins, the 10 species mentioned above were separated into two groups (28–30, 34). One group, designated the *Bacillus brevis* group, included all of the species derived from *Bacillus brevis* except *Bacillus migulanus*. The other group, called the *Bacillus aneurinolyticus* group, included *Bacillus aneurinolyticus* and *Bacillus migulanus*. These groups corresponded closely to the groups generated by a numerical analysis of electrophoretic whole-cell protein patterns (31). Moreover, the results of phylogenetic studies demonstrated that *Bacillus laterosporus* and *Bacillus thermoruber* fell into the “*Bacillus brevis*” cluster or rRNA group 4 (2, 7, 23, 24), and *Bacillus aneurinolyticus* was judged to be closely related to the *Bacillus brevis* clade (2, 7).

The observations described above raised interesting ques-

![FIG. 1. Phylogenetic relationships of Bacillus species and some related organisms based on 16S rRNA gene sequences. The branching pattern was generated by the neighbor-joining method. The numbers are bootstrap values which are greater than 700. The thick lines indicate the *Bacillus brevis* cluster and the *Bacillus aneurinolyticus* cluster. Bar = 0.01 nucleotide substitution per site.](image-url)

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TABLE 1. Bacterial strains used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source*</th>
<th>History*</th>
<th>Nucleotide sequence accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacillus brevis group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus brevis JCM 2503T</td>
<td>1</td>
<td>DSMZ 30T from ATCC 8246T</td>
<td>D78457</td>
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<td>Bacillus agri NRRL NRS-1210T</td>
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<td>C. Lamanna strain 13</td>
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</tr>
<tr>
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<td>2</td>
<td>B. S. Henry strain 120</td>
<td>D78458</td>
</tr>
<tr>
<td>Bacillus choshinensis HPD52</td>
<td>3</td>
<td>H. Takagi et al., from soil, protein producer ( = JCM 8505T = IFO 15518T = CIP 103838T = DSMZ 8552T)</td>
<td>D78459</td>
</tr>
<tr>
<td><strong>Bacillus parabrevis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus parabrevis IFO 12334T</td>
<td>4</td>
<td>ATCC 10027T from N. R. Smith strain 605T from J. R. Porter from G. Bredemann (= JCM 8506T = CIP 103840T)</td>
<td>D78463</td>
</tr>
<tr>
<td>Bacillus reuszeri NRRL NRS-1206T</td>
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<td>H. W. Reuszer Army strain 39 ( = JCM 9170T = IFO 15719T = CIP 104543T)</td>
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<tr>
<td>Bacillus formosus NRRL NRS-863T</td>
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<td>D78461</td>
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<tr>
<td>Bacillus thermotolerans DSMZ 7064T</td>
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<td>P. L. Manachini strain BT2</td>
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<td>Bacillus aneurinolyticus group</td>
<td></td>
<td></td>
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<tr>
<td>Bacillus aneurinolyticus group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacillus aneurinolyticus ATCC 12856T</td>
<td>6</td>
<td>Y. Ito from R. Kimura ( = IAM 1077T = JCM 9024T = IFO 15520T = CIP 104054T)</td>
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</tr>
<tr>
<td>Bacillus migulanus ATCC 9999T</td>
<td>6</td>
<td>NCTC 7096T from R. Syngle from Moscow, gramicidin S producer ( = JCM 8504T = IFO 15520T = CIP 103841T)</td>
<td>D78462</td>
</tr>
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<td>Y. Niimura strain Ep01</td>
<td>D82062</td>
</tr>
</tbody>
</table>

* Data obtained from the DDBJ-GenBank-EMBL database.

**Materials and Methods**

Bacterial strains. The bacterial strains used in this study are listed in Table 1. All working stock preparations except the working stock preparation for a Bacillus thermoruber strain were cultured on T2 agar plates (37) for 24 h at 30°C. The Bacillus thermoruber strain was cultured on TER/l agar plates (17) for 24 h at 4°C. The strains were stored at room temperature.

Chemotaxonomic characterization of Bacillus thermoruber. The isoprenoid quinones of Bacillus thermoruber DSMZ 7064T (T = type strain) were analyzed by the method described by Komagata and Suzuki (16). A Western blot (immunoblot) analysis of whole-cell proteins was performed as described by Towbin et al. (36). Rabbit antisera against the S-layer proteins of Bacillus choshinensis HPD31 and Bacillus migulanus KA S232 were prepared as described by Takagi et al. (34) and Abe and Kimoto (1), respectively.

Cloning and sequencing of 16S rRNA genes. Chromosomal DNA was prepared as described previously (34). PCR amplification of the 16S rRNA gene from chromosomal DNA was carried out by the method of Fox et al. (10). Oligonucleotides 5'-CCTGGAATTCAGATCGAGTGGATGATCCGGCTCATG (primer 27FC: 5' end of the 16S rRNA gene) and 5'-CGTTCCTCCCTAGGTTCTGTACCTTC-3' (primer 1596R: 3' end of the 16S rRNA gene) were used as primers for PCR as described by W isotzek et al. (38), with some modifications. The amplified 16S rRNA genes were digested with Kho1 and HindIII and cloned into PGE-M (Pharmacia), Prokera Co., Madison, Wis.) that was digested with the same enzymes. The cloned plasmids were used for sequencing templates. Sequencing was carried out by the method of Sanger et al. (27) by using a Taq DyeDeoxy terminator cycle sequencing kit (Pharmacia Biotech), 4.0 pl of a 1.25 mM deoxynucleoside triphosphate solution, and 0.25 pl of a solution containing forward and reverse primers at a concentration of 0.1 mM. The procedure used involved 1 cycle of denaturation for 0.5 min at 94°C, 25 cycles consisting of extension for 1 min at 72°C and 25 cycles consisting of denaturation for 1.0 min at 94°C, annealing for 1.5 min at 58°C, and extension for 1.5 min at 72°C, and 1 cycle of extension for 5.0 min at 72°C. The PCR products were analyzed by electrophoresis on a 1.2% agarose gel with TAE buffer (20).

Nucleotide sequence accession numbers. The 16S rRNA gene sequences determined in this study have been deposited in the DDBJ-GenBank database under the accession numbers shown in Table 1.

**Results**

Chemotaxonomic characterization of Bacillus thermoruber. The name Bacillus thermoruber was revived by Manachini et al. (17). This organism was reported to grow at 45 to 48°C and to produce a red pigment. Bacillus thermoruber DSMZ 7064T contained menaquinone-7, which accounted for more than 95% of the total menaquinones. A Western blot analysis showed that Bacillus thermoruber DSMZ 7064T contained protein that cross-reacted with anti-serum against the S-layer protein of Bacillus choshinensis and
not with antisera against the S-layer protein of Bacillus migulanus (data not shown). Other chemosystematic data for Bacillus thermoderber are similar to data for members of the Bacillus brevis group (28, 29, 34). Therefore, Bacillus thermodeterber is a member of the Bacillus brevis group. The separate position of this organism within the Bacillus brevis group was established by its levels of DNA relatedness (data not shown), its levels of 16S rRNA gene sequence similarity (data not shown), and its DNA base composition (17).

Phylogenetic relationship. Nucleotide sequences (1,419 to 1,422 bp) of the 16S rRNA genes of the type strains of Bacillus brevis, Bacillus agri, Bacillus centrosporus, Bacillus choshinensis, Bacillus parabrevis, Bacillus reussieri, Bacillus formosus, Bacillus borstelensis, Bacillus laterosporus, Bacillus aneurinolyticus, and Bacillus migulanus were determined. These sequences were compared with those of 28 other Bacillus species, 3 Paenibacillus species, Amphibacillus xylanus (determined in this study), Sporolactobacillus inulinus, and Alicyclobacillus acidocaldarius. The levels of sequence similarity among 10 species belonging to the Bacillus brevis group (Bacillus brevis, Bacillus agri, Bacillus centrosporus, Bacillus choshinensis, Bacillus parabrevis, Bacillus reussieri, Bacillus formosus, Bacillus borstelensis, Bacillus laterosporus, and Bacillus thermoderber) were more than 93.2%, and the levels of sequence similarity between these 10 species and the other species were less than 91.3% (data not shown). In addition, the level of sequence similarity between the members of the Bacillus aneurinolyticus group (Bacillus aneurinolyticus and Bacillus migulanus) was 98.6%, and the levels of sequence similarity between these two species and other species were less than 91.3%. All 10 species belonging to the Bacillus brevis group were placed in a robust (100% of the bootstrap values) monophyletic cluster (the Bacillus brevis cluster), and the two species belonging to the Bacillus aneurinolyticus group were placed in another equally robust monophyletic cluster (the Bacillus aneurinolyticus cluster) (Fig. 1). These two clusters were clearly separated from the other clusters containing Bacillus, Amphibacillus, Sporolactobacillus, Paenibacillus, and Alicyclobacillus species.

Identification of the Bacillus brevis group and the Bacillus aneurinolyticus group by 16S rRNA gene amplification. The PCR primers used to detect members of the Bacillus brevis and Bacillus aneurinolyticus groups were designed by using the 16S rRNA gene sequence alignments. Primer BREV174F, which was designed to detect the Bacillus brevis cluster, covered positions 148 to 174 (Fig. 2), and primer ANEU506F, which was designed to detect the Bacillus aneurinolyticus cluster, covered positions 482 to 506 (Fig. 3). The results of 16S rRNA gene amplification of 32 Bacillus, Amphibacillus, Sporolactobacillus, Paenibacillus, and Alicyclobacillus species in which the detection primers were used are shown in Fig. 4. Using primers BREV174F and 1377R resulted in a 1.2-kb PCR fragment with the type strains of all of the species belonging to the Bacillus brevis cluster, but not with the type strains of the species belonging to other clusters. With primers ANEU506F and 1377R, the type strains of the species belonging to the Bacillus aneurinolyticus cluster produced an amplified 0.8-kb fragment, but the type strains of the 30 species belonging to other clusters did not. In addition, a 1.3-kb fragment was amplified in all of the strains tested with primers 27FC and 1377R (data not shown).

DISCUSSION

In previous studies, phenotypic characteristics, chemotaxonomic profiles, and the nature of S-layer proteins intimated that there were two taxonomically distinct groups, namely, the Bacillus brevis group and the Bacillus aneurinolyticus group (18, 19, 28–30, 34). The Bacillus brevis group can be characterized
by serologically related S-layer proteins. Similarly, the important characteristics of the *Bacillus aneurinolyticus* group are the unique serologically related S-layer proteins of the members of this group. Thiamine hydrolase-mediated decomposition of thiamine is another distinguishing trait of the *Bacillus aneurinolyticus* group.

In the present study, sequence analysis of the 16S rRNA gene provided data that support the existence of the *Bacillus brevis* and *Bacillus aneurinolyticus* groups. For example, sequence comparisons performed with members of the *Bacillus brevis* group and the *Bacillus aneurinolyticus* group revealed intragroup similarity values of more than 93.2 and 98.6%, respectively. In contrast, the levels of similarity between members of these groups and members of previously described genera are flat, smooth, and yellowish gray, and no soluble pigment is produced on nutrient agar. Most all of the species are strictly aerobic. *Brevibacillus laterosporus* is facultatively anaerobic.

Recent reports (4, 9, 13, 14) have shown that PCR amplification of 16S rRNA gene fragments is useful for identification of some bacterial strains with specific primers. Forman et al. (9) reported that this procedure was suitable and useful for rapid and specific identification of members of the genus *Francisella* at the genus, species, and subspecies levels. In this study, we developed a rapid method for identifying two genera, the genera *Brevibacillus* and *Aneurinibacillus*, by PCR amplification of 16S rRNA gene fragments with specific primers. The detection primers were highly specific for these genera. After strains are assigned to the genus *Brevibacillus* or the genus *Aneurinibacillus* by this method, numerical analyses based on electrophoretic whole-cell protein profiles (31) and DNA-DNA hybridization data (19, 28, 30, 34) are useful for identifying the organisms to the species level. In addition, the PCR amplification method is rapid, simple, and efficient. Thus, this method is recommended as a method that is convenient and useful in taxonomic studies of aerobic, endospore-forming rods.

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The salient characteristics of the seven genera of aerobic, endospore-forming rods are shown in Table 2.

**Description of Brevibacillus gen. nov.** *Brevibacillus* (Bre-vi-bac-il-i-us). L. adj. *brevis*, short; L. dim. *bacillus*, small rod; M. L. masc. *n. Brevibacillus*, short, small rod.) Cells are rod shaped (0.7 to 0.9 by 3.0 to 5.0 μm). Gram positive or gram variable. Motile by means of peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia. Colonies of 10 species are flat, smooth, and yellowish gray, and no soluble pigment is produced on nutrient agar.

Almost all of the species are strictly aerobic. *Brevibacillus laterosporus* is facultatively anaerobic.

Catalase positive (*Brevibacillus thermoruber* is weakly catalase positive). Oxidase variable.
The Voges-Proskauer reaction (production of acetyl methylcarbinol) is negative, and the pH in Voges-Proskauer broth is higher than 7.0.

Hydrogen sulfide and indole are not produced.

Nitrate reduction to nitrite is variable.

Decomposition of tyrosine is variable.

Growth at pH 5.6 or 5.7 and at 50°C is variable. Optimum growth temperature of nine species (all species except Brevibacillus thermonuber) is 30°C. The optimum growth temperature of Brevibacillus thermonuber is 45 to 48°C. Growth is inhibited by 5% NaCl.

Acid but no gas is produced from various sugars.

Specific S-layer protein is present.

The major cellular fatty acids are iso-C_15:0 and anteiso-C_15:0 acids or just iso-C_15:0 acid.

The major quinone is menaquinone 7.

The G+C content ranges from 42.8 to 57.4 mol%.

The levels of 16S rRNA gene sequence similarity are more than 93.2% for the members of this genus. The 16S rRNA gene fragment is amplified by PCR by using primers BREV174F and 1377R.

The type species is Brevibacillus brevis.

Description of Brevibacillus brevis (Migula 1900) comb. nov.
The description of Brevibacillus brevis comb. nov. is identical to the descriptions given by Claus and Berkeley (5), Nakamura (18), and Takagi et al. (34). The type strain is strain JCM 2503 (= ATCC 8246 = CCM 2050 = CIP 52.86 = DSMZ 30 = IFO 15304 = NRRL B-14602 = LMG 7123 = NCIMB 9372).

Description of Brevibacillus agri (Nakamura 1993) comb. nov.
The description of Brevibacillus agri comb. nov. is identical to the descriptions given by Nakamura (19) and Shida et al. (29). The type strain is strain NRRL NRS-1219 (= JCM 9067 = DSMZ 6348 = IFO 15538).

Description of Brevibacillus centrosporus (Nakamura 1993) comb. nov.
The description of Brevibacillus centrosporus comb. nov. is identical to the description given by Nakamura (19). The type strain is strain NRRL NRS-664 (= JCM 9071 = IFO 15540).

Description of Brevibacillus choshinensis (Takagi et al. 1993) comb. nov.
The description of Brevibacillus choshinensis comb. nov. is identical to the descriptions given by Takagi et al. (34) and Shida et al. (28). The type strain is strain HPD52 (= JCM 8505 = IFO 15518 = CIP 103838 = DSMZ 8552 = ATCC 51359 = NCIMB 13345).

Description of Brevibacillus parabrevis (Takagi et al. 1993) comb. nov.
The description of Brevibacillus parabrevis comb. nov. is identical to the descriptions given by Takagi et al. (34) and Shida et al. (28). The type strain is strain IFO 12334 (= JCM 8506 = CIP 103840 = ATCC 10027 = NCIMB 13346).

Description of Brevibacillus reuszeri (Shida et al. 1995) comb. nov.
The description of Brevibacillus reuszeri comb. nov. is identical to the descriptions given by Shida et al. (28) and Nakamura (19). The type strain is strain NRRL NRS-1206 (= JCM 9170 = IFO 15719 = CIP 104543).

Description of Brevibacillus formosus (Shida et al. 1995) comb. nov.
The description of Brevibacillus formosus comb. nov. is identical to the descriptions given by Shida et al. (28)
**TABLE 2. Salient characteristics of the genera of aerobic, endospore-forming rods**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Brevibacillus&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Aneurinibacillus&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Bacillus&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Sporolactobacillus&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Amphibacillus&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Alicyclobacillus&lt;sup&gt;f&lt;/sup&gt;</th>
<th>Paenibacillus&lt;sup&gt;e&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>No. of species</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Spore shape</td>
<td>Oval</td>
<td>Oval</td>
<td>Oval or spherical</td>
<td>Swollen or not swollen</td>
<td>Oval</td>
<td>Oval</td>
<td>Oval</td>
</tr>
<tr>
<td>Sporangia</td>
<td>Swollen</td>
<td>Swollen</td>
<td>Swollen</td>
<td>Swollen</td>
<td>Swollen</td>
<td>Swollen or not swollen</td>
<td>Swollen</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of thiamine</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Production of:</td>
<td>Acetylmethylcarbinol</td>
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<td>−</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
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<td>Lactic acid</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>−</td>
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<td>pH in Voges-Proskauer broth</td>
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<td>&gt;7.0</td>
<td>v (7.0 to 9.5)</td>
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<td>9.0</td>
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<tr>
<td>pH</td>
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<td>7.0</td>
<td>v (15 to 55)</td>
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<td>65</td>
<td>30</td>
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<tr>
<td>Temp (°C)</td>
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<td>37</td>
<td>v (7.0 to 9.5)</td>
<td>30</td>
<td>37</td>
<td>65</td>
<td>30</td>
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<tr>
<td>Major isoprenoid quinone</td>
<td>Anteiso-C&lt;sub&gt;16&lt;/sub&gt;:iso-C&lt;sub&gt;15&lt;/sub&gt;,iso-C&lt;sub&gt;16&lt;/sub&gt;</td>
<td>MK-7</td>
<td>Anteiso-C&lt;sub&gt;15&lt;/sub&gt;:iso-C&lt;sub&gt;16&lt;/sub&gt;</td>
<td>None</td>
<td>MK-7</td>
<td>MK-7</td>
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</tr>
<tr>
<td>Major cellular fatty acids</td>
<td>Anteiso-C&lt;sub&gt;16&lt;/sub&gt;:iso-C&lt;sub&gt;15&lt;/sub&gt;,iso-C&lt;sub&gt;16&lt;/sub&gt;:iso-C&lt;sub&gt;15&lt;/sub&gt;,iso-C&lt;sub&gt;16&lt;/sub&gt;</td>
<td>Anteiso-C&lt;sub&gt;15&lt;/sub&gt;:iso-C&lt;sub&gt;16&lt;/sub&gt;</td>
<td>Anteiso-C&lt;sub&gt;15&lt;/sub&gt;:iso-C&lt;sub&gt;16&lt;/sub&gt;</td>
<td>Anteiso-C&lt;sub&gt;15&lt;/sub&gt;:iso-C&lt;sub&gt;16&lt;/sub&gt;</td>
<td>Anteiso-C&lt;sub&gt;15&lt;/sub&gt;:iso-C&lt;sub&gt;16&lt;/sub&gt;</td>
<td>Anteiso-C&lt;sub&gt;15&lt;/sub&gt;:iso-C&lt;sub&gt;16&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Levels of intragenus 16S rRNA gene amplification</td>
<td>&gt;93.2</td>
<td>98.6</td>
<td>NT</td>
<td>100</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
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</table>
<sup>a</sup> Data from references 28 and 34.<br><sup>b</sup> Data from this study.<br><sup>c</sup> Data from reference 30.<br><sup>d</sup> Data from reference 5.<br><sup>e</sup> Data from reference 21.<br><sup>f</sup> Data from reference 38.<br><sup>g</sup> Data from references 2, 11, and 12.<br><sup>h</sup> v, variable reaction; −, negative reaction; +, positive reaction; NT, not tested.<br><sup>i</sup> MK-7, menaquinone 7.
and Nakamura (19). The type strain is strain NRRL NRS-863 (= JCM 9169 = IFO 15716 = CIP 104544).

**Description of Brevibacillus borstelensis** (Shida et al. 1995) comb. nov. The description of *Brevibacillus borstelensis* comb. nov. is identical to the description given by Shida et al. (28) and Nakamura (19). The type strain is strain NRRL NRS-818 (= JCM 9022 = IFO 15714 = CIP 104545).

**Description of Brevibacillus laterosporus** (Laubach 1905) comb. nov. The description of *Brevibacillus laterosporus* comb. nov. is identical to the description given by Claus and Berkeley (5). The type strain is strain JCM 2496 (= ATCC 64 = CCM 2116 = CIP 52.83 = DSM 25 = IFO 15654 = IAM 12455 = LMG 6921 = NCIMB 9367).

**Description of Brevibacillus thermoruber** (Manachini et al. 1985) comb. nov. The description of *Brevibacillus thermoruber* comb. nov. is identical to the description given by Manachini et al. (17). A specific S-layer protein is present in this organism (this study). The major quinone is menaquinone 7 (this study). The type strain is strain DSMZ 7064.

**Description of Aneurinibacillus gen. nov. Aneurinibacillus** (A.neu.rि.ni.ba.cil’1us. M. L. n. aeurinum, thiamine; L. dim. n. bacillus, small rod; M. L. masc. n. Aneurinibacillus, thiamine-decomposing small rod.) Cells are rod shaped (0.7 to 0.9 by 3.0 to 5.0 μm). Gram positive. Motile by means of peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia. Colonies are flat, smooth, and yellowish gray, and no soluble flagella. Ellipsoidal spores are formed in swollen sporangia. Strictly aerobic. Catalase positive (Aneurinibacillus aneurinolyticus is weakly catalase positive). Oxidase variable. The Voges-Proskauer reaction (production of acetyl methylcarbinol) is negative, and the pH in Voges-Proskauer broth is higher than 7.0.

Dihydroxyacetone, hydrogen sulfide, and indole are not produced. Nitrates are reduced to nitrite. Casein, gelatin, starch, Tween 20, Tween 40, Tween 60, Tween 80, urea, and hippurate are not hydrolyzed. Hydrolysis of DNA is variable.

Tyrosine is decomposed. Thiamin is decomposed by thiamin hydrolyase. Phenylalanine is deaminated. Citrate, propionate, alginone, gluconate, malonate, and tartrate are not utilized. Utilization of acetate, fumarate, lactate, succinate, L-glutamate, L-aspartate, L-malate, and α-ketoglutarate is variable. Nitrate is not utilized, and utilization of ammonium is variable. The egg yolk reaction is positive. Litmus milk is reduced and alkalinized. Growth occurs at 20°C to 50°C and at pHs 5.0 to 9.0. The optimum growth temperature and pH are 37°C and 7.0, respectively. Growth occurs in the presence of 2% NaCl and 0.001% lysozyme. Growth is variable in the presence of 0.02% sodium azide. Growth is inhibited in the presence of 5% NaCl. Production of acid from D-fructose, sucrose, trehalose, D-xylose, mannitol, Tween 80, urea, and hippurate are not hydrolyzed. Hydrolysis of DNA is variable.

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**REFERENCES**


The level of 16S rRNA gene sequence similarity for the members of this genus is 98.6%. The 16S rRNA gene fragment is amplified by PCR by using primers ANEU506 and 1377R. The type species is *Aneurinibacillus aneurinolyticus*. The description of *Aneurinibacillus aneurinolyticus* (Shida et al. 1994) comb. nov. The description of *Aneurinibacillus aneurinolyticus* comb. nov. is identical to the description given by Shida et al. (30). The type strain is strain ATCC 12856 (= IAM 1079 = JCM 9024 = IFO 15521 = CIP 104007).

**Description of Aneurinibacillus migulanus** (Takagi et al. 1993) comb. nov. The description of *Aneurinibacillus migulanus* comb. nov. is identical to the description given by Takagi et al. (34) and Shida et al. (30). The type strain is strain ATCC 9999 (= JCM 8504 = IFO 15520 = CIP 103841).


