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

OSAMU SHIDA, HIROAKI TAKAGI, KIYOSHI KADOWAKI, AND KAZUO KOMAGATA

Research Laboratory, Higeta Shoyu Co., Ltd., Choshi, Chiba 288, and Department of Agricultural Chemistry, Faculty of Agriculture, Tokyo University of Agriculture, Setagaya-ku, Tokyo 156, Japan

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*, *Amphibacillus*, *Sporolactobacillus*, *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 clusters. The *B. brevis* cluster included 10 species, namely, *Bacillus brevis*, *Bacillus agri*, *B. centrosporus*, *B. choshinensis*, *B. parabrevis*, *B. reuszeri*, *B. formosus*, *B. borstelensis*, *B. laterosporus*, and *B. thermoruber*. *Bacillus aneurinolyticus* and *B. migulanus* belonged to the *B. aneurinolyticus* cluster. Moreover, the two clusters were phylogenetically distinct from other *Bacillus*, *Amphibacillus*, *Sporolactobacillus*, *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 observations described above raised interesting ques-

---

* Corresponding author. Mailing address: Research Laboratory, Higeta Shoyu Co., Ltd., 2-8 Chu-cho, Choshi, Chiba 288, Japan. Phone: 81-479-22-1180. Fax: 81-479-24-3422. Electronic mail address: LDX05744@niftyserve.or.jp.
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><em>Bacillus brevis</em> JCM 2503T</td>
<td></td>
<td></td>
<td>D78457</td>
</tr>
<tr>
<td><em>Bacillus agri</em> NRRL NRS-1210T</td>
<td>1</td>
<td>from NCTC 2611T from W. W. Ford strain 27B</td>
<td>D78454</td>
</tr>
<tr>
<td><em>Bacillus centrosorus</em> NRRL NRS-664T</td>
<td>2</td>
<td></td>
<td>D78458</td>
</tr>
<tr>
<td><em>Bacillus choshinensis</em> 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><em>Bacillus parabrevis</em> 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><em>Bacillus reuszeri</em> NRRL NRS-1206T</td>
<td>5</td>
<td>H. W. Reuszer Army strain 39 (= JCM 9170T = IFO 15719T = CIP 104543T)</td>
<td>D78464</td>
</tr>
<tr>
<td><em>Bacillus formosus</em> NRRL NRS-863T</td>
<td>2</td>
<td>J. R. Porter from G. Bredemann (= JCM 9169T = IFO 15716T = CIP 104545T)</td>
<td>D78460</td>
</tr>
<tr>
<td><em>Bacillus borstelensis</em> NRRL NRS-818T</td>
<td>2</td>
<td></td>
<td>D78456</td>
</tr>
<tr>
<td><em>Bacillus laterosporus</em> JCM 2496T</td>
<td>1</td>
<td></td>
<td>D78461</td>
</tr>
<tr>
<td><em>Bacillus thermoruber</em> DSMZ 7064T</td>
<td>5</td>
<td>P. L. Manachini strain BT2</td>
<td>Z26921T</td>
</tr>
<tr>
<td><em>Bacillus aneurinolyticus</em> group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus aneurinolyticus</em> ATCC 12856T</td>
<td>6</td>
<td>Y. Ito from R. Kimura (= IAM 1077T = JCM 9024T = IFO 15502T = CIP 104545T)</td>
<td>D78455</td>
</tr>
<tr>
<td><em>Bacillus migulanus</em> ATCC 9999T</td>
<td>6</td>
<td>NCTC 7096T from R. Syng from Moscow, gramicidin S producer (= JCM 8504T = IFO 15520T = CIP 103841T)</td>
<td>D78462</td>
</tr>
<tr>
<td><em>Amphibacillus xylinus</em> JCM 7361T</td>
<td>1</td>
<td>Y. Niimura strain Ep01</td>
<td>D82062</td>
</tr>
</tbody>
</table>

**TABLE 1.** Bacterial strains used in this study


**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 TSA agar plates (17) for 24 h at 4°C. The strains were stored at room temperature.

**Chemosystematic characterization of Bacillus thermoruber.** The isoprenoid quinones of *Bacillus thermoruber* DSMZ 7064T (T = type strain) was 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 brevis* strain were cultured on T2 agar plates (37) for 24 h at 30°C.

**Comparison of 16S rRNA gene sequences.**

Previously published 16S rRNA gene sequences were obtained from the EMBL-GenBank-DDBJ database. Multiple sequences were aligned, nucleotide substitution rates (K_{eq} values) (15) were calculated, a neighbor-joining phylogenetic tree (25) was constructed, and a bootstrap analysis with 1000 replicates to evaluate the phylogenetic tree topology (8) was performed by using the CLUSTAL W program (35). Alignment gaps and unidentified base positions were not taken into account in the calculations.

**Identification of strains belonging to the *Bacillus brevis* group and the *Bacillus aneurinolyticus* group by 16S rRNA gene amplification.** Strains belonging to the *Bacillus brevis* group and the *Bacillus aneurinolyticus* group were identified by 16S rRNA gene amplification by using specific detection primers and PCR. The sequences of forward detection primers BREV174F and ANEU506F were 5′-A GACCGGGTAAACATAGGAAACATTAT-3′ and 5′-GAAACCGCCGGGAT GACCTCCCGGTTC-3′, respectively. The sequence of reverse primer 1377R was 5′-GGCATGCTGATCCGCGAT ACTAGC-3′; this sequence covered the conserved region of the 16S rRNA gene at positions 1401 to 1377. The primers were designed by considering aligned sequences of the 16S rRNA gene.

Approximately 0.2 ng of chromosomal DNA was subjected to a PCR in a 25-μL (total volume) reaction mixture containing 0.1 μL of Taq DNA polymerase (Pharmacia Biotech, Uppsala, Sweden), 2.5 μL of 10× Taq DNA polymerase buffer (Pharmacia Biotech), 4.0 μL of a 1.25 mM deoxynucleoside triphosphate solution, and 0.25 μL 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 denaturation for 1 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 (26).

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

**RESULTS**

**Chemosystematic 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 antiserum against the S-layer protein of *Bacillus choshinensis* and
not with antiserum against the S-layer protein of *Bacillus migulanus* (data not shown). Other chemosystematic data for *Bacillus thermoruber* were similar to data for members of the *Bacillus brevis* group (28, 29, 34). Therefore, *Bacillus thermoruber* 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 reuzieri*, *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 reuzieri*, *Bacillus formosus*, *Bacillus borstelensis*, *Bacillus laterosporus*, and *Bacillus thermoruber*) 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*, *Alicyclobacillus acidocaldarius*, and *Alicyclobacillus acidocaldarius*.

**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* clusters were designed by using the 16S rRNA gene sequence alignments. Primer BREVBACILLUS GEN. NOV. AND ANEUHACILLUS GEN. NOV. 941
b) 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 were consistently less than 91.3%. These intragroup and intergroup similarity values indicate that the *Bacillus brevis* and *Bacillus aneurinolyticus* groups are cohesive and distinct from each other and from previously described genera. Furthermore, examinations of the 16S rRNA gene sequences also revealed sequence segments that are group specific.

The results of analyses based on 16S rRNA gene sequences demonstrated that the *Bacillus brevis* and *Bacillus aneurinolyticus* groups represent two taxa that are phylogenetically distinct from each other and from the other genera studied (namely, the genera *Bacillus*, *Sporolactobacillus*, *Paenibacillus*, *Amphibacillus*, and *Alicyclobacillus*). (Fig. 1). Interestingly, the *Bacillus brevis* and *Bacillus aneurinolyticus* groups are monophyletically related to each other than to the other genera. On the basis of the accumulated phenotypic characteristics, chemosystematic profiles, 16S rRNA gene sequences, and phylogenetic data, we propose two new genera, *Brevibacillus* gen. nov. for the 10 species in the *Bacillus brevis* cluster and *Aneurinibacillus* gen. nov. for the 2 species in the *Bacillus aneurinolyticus* cluster.

Previous 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. Formsma 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.

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 ба-циллус*) L. adj. *brevis*, short; L. dim. *n. 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.

![FIG. 3. Sequence of the detection primer for members of the *Bacillus aneurinolyticus* group (the *Bacillus aneurinolyticus* cluster) (primer ANEU506F) and alignment of the 16S rRNA gene sequences of *Bacillus* species and related organisms. Dashes indicate nucleotides identical to *Bacillus aneurinolyticus* nucleotides. Boldface letters indicate nucleotides identical to the nucleotides of primer ANEU506F and the primer regions of other *Bacillus* species and related organisms. Abbreviations are explained in the legend to Fig. 2.](image-url)
The Voges-Proskauer reaction (production of acetylmethylcarbinol) 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. Hydrolysis of tyrosine is variable. Growth at pH 5.6 or 5.7 and at 50°C is variable. Optimum growth occurs at pH 7.0. The 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.

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 IF0 12334 (= JCM 8506 = CIP 103840 = ATCC 10027 = NCIMB 13346).

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 IF0 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></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of species</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Spore shape</td>
<td>Oval</td>
<td>Swollen</td>
<td>Swollen</td>
<td>Swollen or not swollen</td>
<td>Oval</td>
<td>Oval</td>
<td>Oval</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>V</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Hydrolysis of thiamine</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Production of Acetylmethylcarbinol</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>pH in Voges-Proskauer broth</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Optimum growth conditions</td>
<td>30 to 48</td>
<td>37</td>
<td>70</td>
<td>70</td>
<td>70</td>
<td>37</td>
<td>65</td>
</tr>
<tr>
<td>Major menaquinone</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
<td>MK-7</td>
</tr>
<tr>
<td>Major cellular fatty acids</td>
<td>Antiser-C15:0 and iso-C15:0</td>
<td>Antiser-C15:0 and iso-C15:0</td>
<td>Antiser-C15:0 and iso-C15:0</td>
<td>Antiser-C15:0 and iso-C15:0</td>
<td>Antiser-C15:0 and iso-C15:0</td>
<td>Antiser-C15:0 and iso-C15:0</td>
<td>Antiser-C15:0 and iso-C15:0</td>
</tr>
<tr>
<td>Levels of intragenus 16S rRNA gene sequence alignment</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>16S rRNA gene amplification by PCR with Primer BREVI176F</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Cross-reaction with antiser against Brevibacillus clausii, Antiser-C15:0, and iso-C15:0</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>46-57</td>
<td>42-43</td>
<td>39</td>
<td>36-38</td>
<td>32-46</td>
<td>39</td>
<td>36-40</td>
</tr>
</tbody>
</table>

- Data from references 28 and 34.
- Data from reference 30.
- Data from reference 35.
- Data from reference 31.
- Data from reference 32.
- Data from reference 33.
- Data from reference 2.
- Data from references 2, 11, and 12.
- Data from reference 36.
- Data from reference 38.

+ Positive reaction; NT, not tested.
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.r.i.ni.ba.cil.lus: M. L. n. aerineum, 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 pigment is produced on nutrient agar. Strictly aerobic. Catalase positive (Aneurinibacillus aerineolyticus is weakly catalase positive). Oxidase variable. The Voges-Proskauer reaction (production of acetyl-methyl-carbinol) 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. Hydrolsis of DNA is variable. Tyrosine is decomposed. Thiamin is decomposed by thiamin hydrolase. Phenylalanine is deaminated. Citrate, propionate, alginate, 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 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 fructose, sucrose, trehalose, d-ribose, glyceral, d-sorbitol, and l-sorbose is variable, and no gas is produced. No acid or gas is produced from d-glucose, d-arabinose, d-galactose, maltose, lactose, d-sylose, mannitol, d-cellobiose, salicin, d-mannose, melibiose, l-thamnose, raffinose, inositol, erythritol, adonitol, and starch. A specific S-layer protein is present. The major cellular fatty acids are iso-C₈:0, iso-C₈:0, and C₁₆:0 acids. The major quinone is menaquinone 7. The G+C content ranges from 41.1 to 43.4 mol%.

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 ANEU506F and 1377R. The type species is Aneurinibacillus aerineolyticus.

Description of Aneurinibacillus aerineolyticus (Shida et al. 1994) comb. nov. The description of Aneurinibacillus aerineolyticus comb. nov. is identical to the description given by Shida et al. (30). The type strain is strain ATCC 12856 (= IAM 1071 = 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 = IAM 15520 = CIP 103841).

ACKNOWLEDGMENTS

We thank K. Sano, R. Wakamatsu, and H. Shimada for technical assistance.

REFERENCES


