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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*, *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 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-

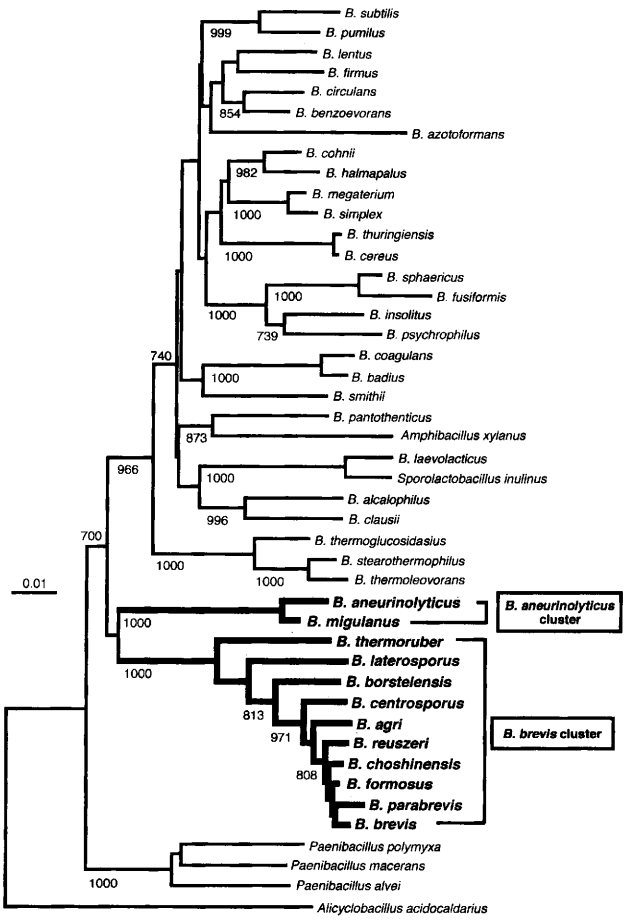

FIG. 1. Phylogenetic relationships of *Bacillus* species and some related organisms based on 16S rRNA gene sequences. The branching pattern was generated by the neighbor-joining method. The numbers are bootstrap values which are greater than 700. The thick lines indicate the *Bacillus brevis* cluster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

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TABLE 1. Bacterial strains used in this study

Strain	Source[a]	History[b]	Nucleotide sequence accession no.
Bacillus brevis group			
Bacillus brevis JCM 2503^T	1	DSMZ 30^T from ATCC 8246^T from N. R. Smith strain 604^T from J. R. Porter from NCTC 2611^T from W. W. Ford strain 27B	D78457
Bacillus agri NRRL NRS-1219^T	2	C. Lamanna strain 13	D78454
Bacillus centrosporus NRRL NRS-664^T	2	B. S. Henry strain 120	D78458
Bacillus choshinensis HPD52^T	3	H. Takagi et al., from soil, protein producer (= JCM 8505^T = IFO 15518^T = CIP 103838^T = DSMZ 8552^T)	D78459
Bacillus parabrevis IFO 12334^T	4	ATCC 10027^T from N. R. Smith strain 605^T from J. R. Porter from G. Bredemann (= JCM 8506^T = CIP 103840^T)	D78463
Bacillus reuszeri NRRL NRS-1206^T	2	H. W. Reuszer Army strain 39 (= JCM 9170^T = IFO 15719^T = CIP 104543^T)	D78464
Bacillus formosus NRRL NRS-863^T	2	J. R. Porter from G. Bredemann (= JCM 9169^T = IFO 15716^T = CIP 104544^T)	D78460
Bacillus borstelensis NRRL NRS-818^T	2	J. R. Porter (= JCM 9022^T = IFO 15716^T = CIP 104545^T)	D78456
Bacillus laterosporus JCM 2496^T	1	CCM 2116^T from R. E. Gordon	D78461
Bacillus thermoruber DSMZ 7064^T	5	P. L. Manachini strain BT2	Z26921[c]
Bacillus aneurinolyticus group			
Bacillus aneurinolyticus ATCC 12856^T	6	Y. Ito from R. Kimura (= IAM 1077^T = JCM 9024^T = IFO 15521^T = CIP 104007^T)	D78455
Bacillus migulanus ATCC 9999^T	6	NCTC 7096^T from R. Synge from Moscow, gramicidin S producer (= JCM 8504^T = IFO 15520^T = CIP 103841^T)	D78462
Amphibacillus xylanus JCM 7361^T	1	Y. Niimura strain Ep01	D82062

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[b] NCTC, National Collection of Type Cultures, Central Public Health Laboratory Service, London, United Kingdom; CIP, Collection des Bactéries de l'Institut Pasteur, Paris, France; CCM, Czech Collection of Microorganisms, Masaryk University, Bruno, Czech Republic; IAM, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan.

[c] Data obtained from the DDBJ-GenBank-EMBL database.

tions concerning the phylogenetic relationship and status of the *Bacillus brevis* group and the *Bacillus aneurinolyticus* group. To address these questions, we determined the 16S rRNA gene sequences of the species in these two groups and compared them with available homologous sequences of other *Bacillus* species.

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/1 agar plates (17) for 24 h at 45°C. The strains were stored at room temperature.

Chemosystematic characterization of *Bacillus thermoruber*. The isoprenoid quinones of *Bacillus thermoruber* DSMZ 7064^T (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′-CTGGGATCCATTTACTCGAGAGTTTGATCCTGGCT CAG-3′ (primer 27FC; 5′ end of the 16S rRNA gene) and 5′-GGTTCCCCTA AGCTTACCTTGTTACGACTTC-3′ (primer 1490RC; 3′ end of the 16S rRNA gene) were used as primers for PCR as described by Wisotzkey et al. (38), with some modifications. The amplified 16S rRNA genes were digested with *Xho*I and *Hin*dIII and cloned into pGEM-7zf(+) (Promega Co., Madison, Wis.) that was digested with the same enzymes. The cloned plasmids were used for sequencing templates. Sequencing was carried out as described by Sanger et al. (27) by using a *Taq* DyeDeoxy terminator cycle sequencing kit (Perkin-Elmer Co., Foster City, Calif.) and a model ABI 373 automatic DNA sequencer (Perkin-Elmer Co.). Seven sequencing primers were used as described by Fox et al. (10).

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_{nuc} values) (15) were calculated, a neighbor-joining phylogenetic tree (25) was constructed, and a bootstrap analysis with 1,000 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 GACCGGGATAACATAGGGAAACTTAT-3′ and 5′-GAACCGCCGGGAT GACCTCCCGGTC-3′, respectively. The sequence of reverse primer 1377R was 5′-GGCATGCTGATCCGCGATTACTAGC-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 U 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 1.0 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 (26).

Nucleotide sequence accession numbers. The 16S rRNA gene sequences determined in this study have been deposited in the DDBJ-EMBL-GenBank 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 7064^T contained menaquinone 7, which accounted for more than 95% of the total menaquinones.

A Western blot analysis showed that *Bacillus thermoruber* DSMZ 7064^T contained protein that cross-reacted with antiserum against the S-layer protein of *Bacillus choshinensis* and

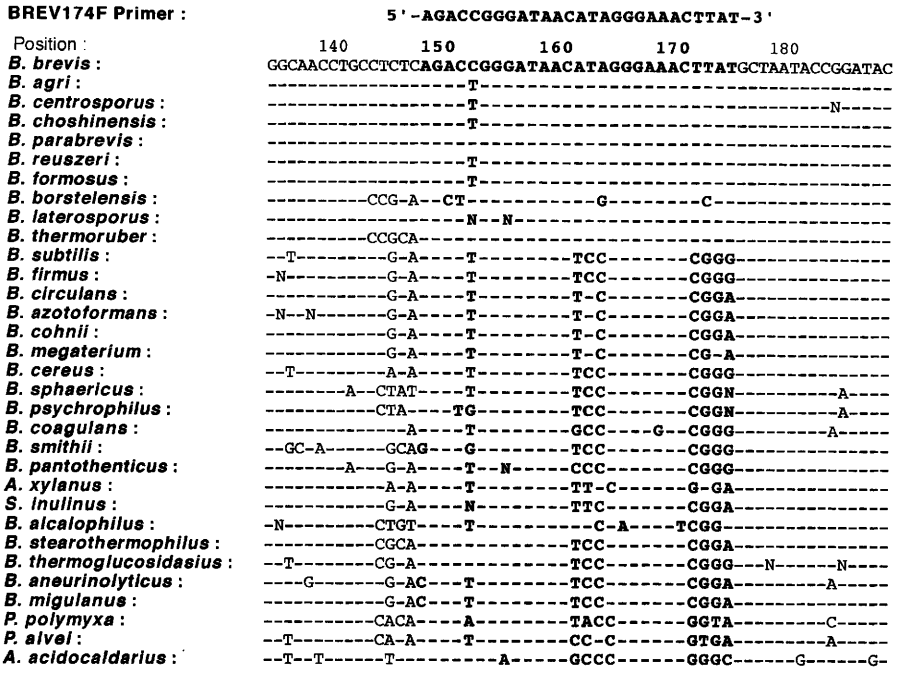

FIG. 2. Sequence of the detection primer for members of the *Bacillus brevis* group (the *Bacillus brevis* cluster) (primer BREV174F) and alignment of the 16S rRNA gene sequences of *Bacillus* species and related organisms. Dashes indicate nucleotides identical to nucleotides of *Bacillus brevis*. Boldface letters indicate nucleotides identical to the nucleotides of primer BREV174F and the primer regions of other *Bacillus* species and related organisms. Abbreviations: *B.*, *Bacillus*; *A. xylanus*, *Amphibacillus xylanus*; *S.*, *Sporolactobacillus*; *P.*, *Paenibacillus*; *A. acidocaldarius*, *Alicyclobacillus acidocaldarius*.

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 reuszeri*, *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 reuszeri*, *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*, 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* clusters 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

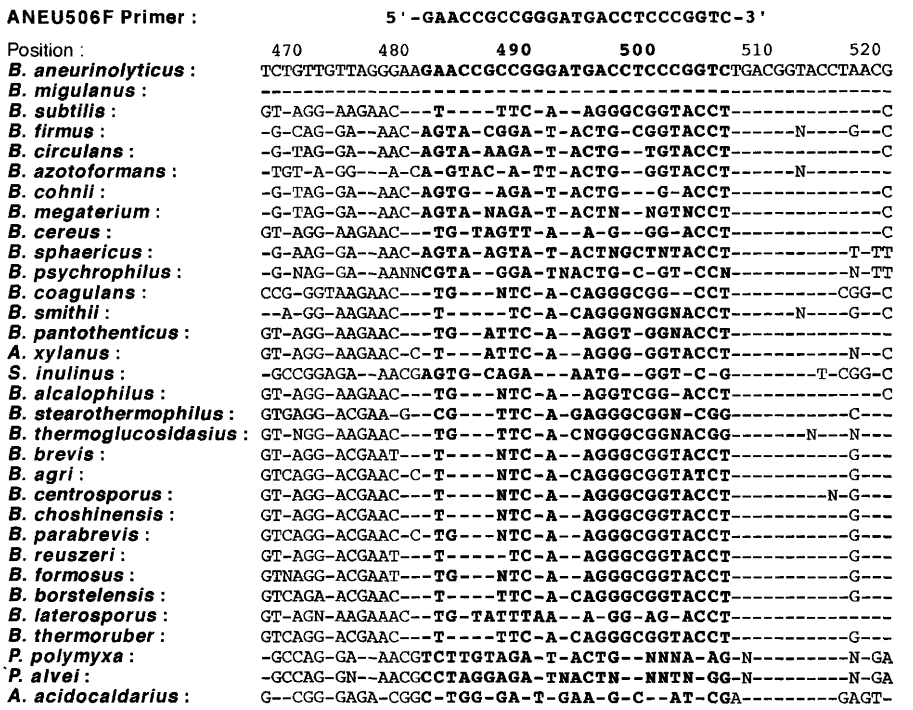

FIG. 3. Sequence of the detection primer for members of the *Bacillus aneurinolyticus* group (the *Bacillus aneurinolyticus* cluster) (primer ANEU506F) and alignment of the 16S rRNA gene sequences of *Bacillus* species and related organisms. Dashes indicate nucleotides identical to *Bacillus aneurinolyticus* 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.

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 more closely 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. Forsman 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.ba.cil'lus. 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.

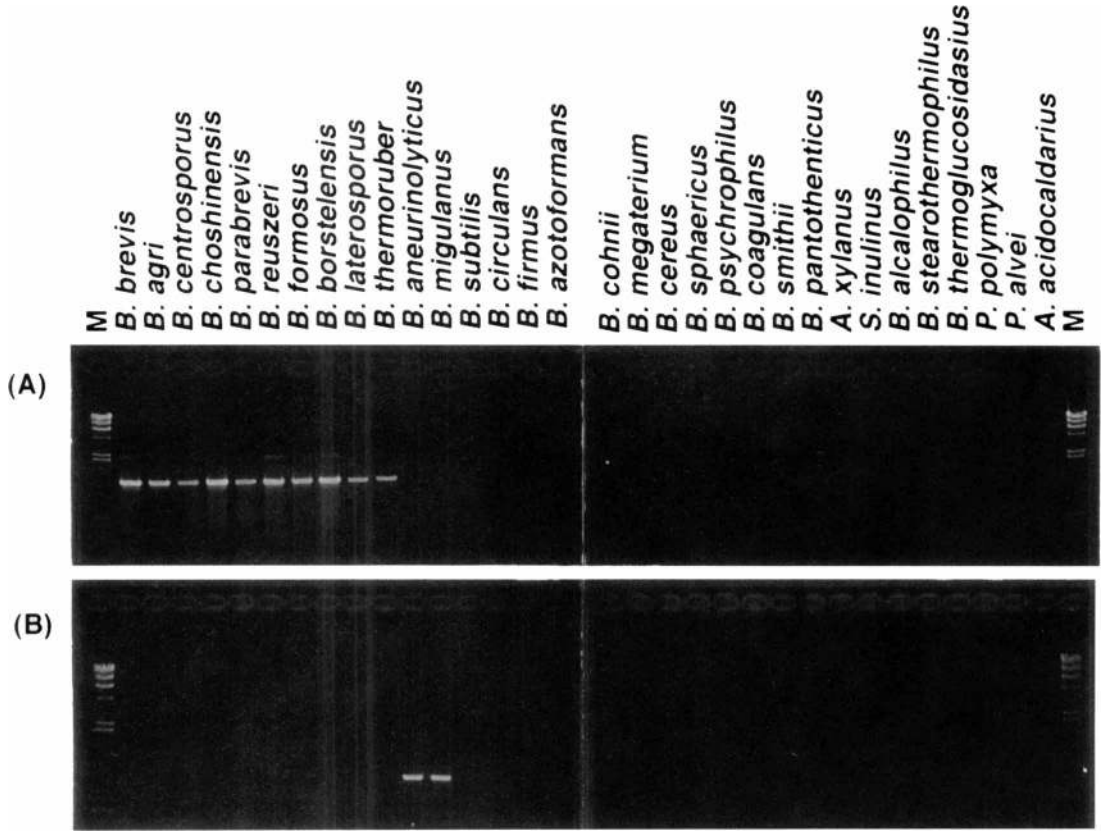

FIG. 4. Amplification of the 16S rRNA gene by PCR with detection primers. (A) Primers BREV174F and 1377R. (B) Primers ANEU506F and 1377R. All of the strains tested were type strains. Lanes M contained *Hin*dIII-digested λ DNA as a molecular weight marker. Abbreviations are explained in the legend to Fig. 2.

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 casein, gelatin, and starch is variable.

Decomposition 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 thermoruber*) is 30°C. The optimum growth temperature of *Brevibacillus thermoruber* 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

Characteristic	*Brevibacillus*[a,b]	*Aneurinibacillus*[b,c]	*Bacillus*[d]	*Sporolactobacillus*[d]	*Amphibacillus*[e]	*Alicyclobacillus*[f]	*Paenibacillus*[g]
No. of species	10	2		1	1	3	11
Spore shape	Oval	Oval	Oval or spherical	Oval	Oval	Oval	Oval
Sporangia	Swollen	Swollen	Swollen or not swollen	Swollen	Swollen	Swollen or not swollen	Swollen
Anaerobic growth	v[h]	−	v	+	+	−	+
Catalase activity	+	+	+	−	−	+	+
Hydrolysis of thiamine	−	+	NT	NT	NT	NT	NT
Production of:							
Acetylmethylcarbinol	−	−	v	NT	NT	v	v
Lactic acid	NT	NT	v	+	+	NT	NT
pH in Voges-Proskauer broth	>7.0	>7.0	v	NT	NT	NT	<6.0
Optimum growth conditions							
pH	7.0	7.0	v (7.0 to 9.5)	7.0	9.0	3.0	7.0
Temp (°C)	30 to 48	37	v (15 to 55)	30	37	65	30
Major isoprenoid quinone	MK-7[i]	MK-7	MK-7	MK-7	None	MK-7	MK-7
Major cellular fatty acids	Anteiso-$C_{15:0}$ and iso-$C_{15:0}$ or iso-$C_{15:0}$	Iso-$C_{15:0}$, $C_{16:0}$, iso-$C_{16:0}$	v	Anteiso-$C_{15:0}$, iso-$C_{15:0}$, $C_{14:0}$	Anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{16:0}$, iso-$C_{15:0}$	ω-Alicyclic acids	Anteiso-$C_{15:0}$
Levels of intragenus 16S rRNA gene sequence similarity (%)	>93.2	98.6	NT	100[j]	100[b]	>92.7	>92.0
16S rRNA gene amplification by PCR with[b]:							
Primer BREV174F	+	−	−	−	−	−	−
Primer ANEU506F	−	+	−	−	−	−	−
Cross-reaction with antisera against S-layer protein from:							
Brevibacillus choshinensis[a,b]	+	−	−	−	NT	NT	−
Aneurinibacillus migulanus[c]	−	+	−	−	NT	NT	−
G+C content (mol%)	46–57	42–43	32–69	39	36–38	52–60	40–54

[a] Data from references 28 and 34.
[b] Data from this study.
[c] Data from reference 30.
[d] Data from reference 5.
[e] Data from reference 21.
[f] Data from reference 38.
[g] Data from references 2, 11, and 12.
[h] v, variable reaction; −, negative reaction; +, positive reaction; NT, not tested.
[i] MK-7, menaquinone 7.
[j] Datum from reference 2.

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 descriptions 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 = DSMZ 25 = IFO 15654 = IAM 12455 = LMG 6931 = 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.ri.ni.ba.cil'lus. M. L. n. *aneurinum*, 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 aneurinolyticus* is weakly catalase positive). Oxidase variable.

The Voges-Proskauer reaction (production of acetylmethylcarbinol) is negative, and the pH in Voges-Proskauer broth is higher than 7.0.

Dihydroxyacetone, hydrogen sulfide, and indole are not produced.

Nitrate is 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 hydrolase.

Phenylalanine is deaminated.

Citrate, propionate, alginate, gluconate, malonate, and tartrate are not utilized. Utilization of acetate, fumarate, lactate, succinate, L-glutamate, L-asparatate, 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 D-fructose, sucrose, trehalose, D-ribose, glycerol, D-sorbitol, and L-sorbose is variable, and no gas is produced. No acid or gas is produced from D-glucose, L-arabinose, D-galactose, maltose, lactose, D-xylose, mannitol, D-cellobiose, salicin, D-mannose, melibiose, L-rhamnose, raffinose, inositol, erythritol, adonitol, and starch.

A specific S-layer protein is present.

The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$, and $C_{16:0}$ 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 aneurinolyticus*.

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 1077 = 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 descriptions given by Takagi et al. (34) and Shida et al. (30). The type strain is strain ATCC 9999 (= JCM 8504 = IFO 15520 = CIP 103841).

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