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# A Polyphasic Reassessment of the Genus *Aneurinibacillus*, Reclassification of *Bacillus thermoaerophilus* (Meier-Stauffer et al. 1996) as *Aneurinibacillus thermoaerophilus* comb. nov., and Emended Descriptions of *A. aneurinilyticus* corrig., *A. migulanus*, and *A. thermoaerophilus*

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**Fifty-three strains representing 25 species of aerobic endospore-forming bacteria, of which 11 (37 strains) belong to the genera *Aneurinibacillus* and *Brevibacillus* (*Bacillus* rRNA group 4 of Ash et al. [Lett. Appl. Microbiol. 13:202–206, 1991]), were characterized genotypically by amplified ribosomal DNA restriction analysis (ARDRA) and/or phenotypically by fatty acid methyl ester analysis, sodium dodecylsulfate-polyacrylamide gel electrophoresis of whole-cell proteins, pyrolysis mass spectrometry, 99 API Biotype 100 assimilation tests, and 16 other routine phenotypic tests. ARDRA revealed that *Aneurinibacillus aneurinilyticus*, *Aneurinibacillus migulanus*, and *Bacillus thermoaerophilus* formed a cluster quite separate from *Brevibacillus* species, supporting the distinction of both genera and the transfer of *B. thermoaerophilus* to the genus *Aneurinibacillus*. Two of the species, *A. aneurinilyticus* (the type species) and *A. migulanus*, are phenotypically and genotypically quite similar but can be distinguished from each other by several phenotypic characters. Phenotypic differentiation at the generic level is also described.**

*Bacillus aneurinolyticus* [sic] was described by Aoyama (1) as a thiamine-decomposing organism from human feces but was omitted from the Approved Lists of Bacterial Names (25) because of the paucity of representative strains. (We will use the corrected species epithet *aneurinilyticus* according to Appendix 9B of the International Code of Nomenclature of Bacteria [1990].) Phenotypically, this organism is known to resemble *Bacillus brevis* and related taxa (3), and studies on the 16S rRNA sequences of the type strains of these two species suggest that *B. aneurinilyticus* represents a distinct evolutionary line close to that of *B. brevis* (2) or that it diverged early from the *B. brevis* line (5, 6).

The taxonomy of *B. brevis* has been modified by assigning *B. brevis* strains to a number of new species, such as *Bacillus agri* and *Bacillus centrosporus* (17); *Bacillus migulanus*, *Bacillus choshinensis*, *Bacillus parabrevis*, and *Bacillus galactophilus* (27); and *Bacillus reuszeri*, *Bacillus formosus*, and *Bacillus borstelensis* (22). Also, the name *B. aneurinilyticus* has been revived (20), and that of *B. galactophilus* has been recognized to be a synonym of *B. agri* (21). The thermophilic species *Bacillus thermoaerophilus* has been described to include sugar beet isolates and *B. brevis* ATCC 12990 (16).

Recently, two new genera were created on the basis of a 16S rDNA gene sequence analysis (of the type strains only) to accommodate the above-mentioned and allied species; the genus *Aneurinibacillus* contains *A. aneurinilyticus* and *A. migulanus*, while the genus *Brevibacillus* contains *Brevibacillus brevis* and the species derived from it (see above), *Brevibacillus laterosporus* and *Brevibacillus thermoruber* (23). *B. thermoaerophilus* was not included in the latter study.

As a continuation of the collaborative taxonomic study by some members of the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of the Genus *Bacillus* and Related Organisms (13), a polyphasic study of the new genus *Aneurinibacillus* that also included representative strains of the new genus *Brevibacillus* as well as *B. thermoaerophilus* and some other *Bacillus* species was undertaken in order to clarify the positions of the strains at different taxonomic levels and to identify phenotypic characters allowing distinction among them.

## MATERIALS AND METHODS

**Strains and media.** The designations of the strains, their origins, and the different methods applied to each are shown in Table 1. Unless otherwise stated, strains were grown on nutrient agar with 1% (wt/vol) glucose (pH 7.4) (NAG) at 30°C for 24 to 48 h, except for *B. thermoaerophilus*, which was grown at 55°C. The strains were checked for purity by plating and phase-contrast microscopic examination and were maintained both as lyophilized cultures and as sporulated cultures on NAG slopes containing 5 mg of $MnSO_4 \cdot 4H_2O$ per liter (to enhance sporulation). Slopes were incubated for 48 h or longer until spores could be observed by microscopy, and they were then stored at 4°C.

**DNA preparation.** For amplified ribosomal DNA restriction analysis (ARDRA), total genomic DNA was purified by a slight modification of the method of Pitcher et al. (18), as described previously (11). The DNA of *A. aneurinilyticus* LMG 15531$^T$ and *A. migulanus* LMG 15427$^T$ for DNA-DNA binding experiments (see below) was prepared from 6 to 14 g (wet weight) of cells grown in Roux flasks containing 130 ml of Trypticase soy agar (TSA). For DNA preparation, the method of Pitcher et al. (18) was modified as follows. Ten milliliters of cell suspension in TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) was treated with 250 mg of lysozyme at 37°C for 0.5 h. Then TE buffer was added to bring the volume up to 50 ml, and the cells were lysed by adding 20 ml of guanidium-EDTA-Sarkosyl reagent (see reference 18). Lysed cells were vigorously shaken in 1 M NaCl and then treated with ammonium acetate at a final concentration of 2.2 M. After removal of proteins and cell debris by a double chloroform extraction (involving gentle shaking for 20 min) and centrifugation for 40 min at 23,500 × *g*, the DNA was precipitated from the aqueous phase with 0.54 volumes of isopropanol, spooled on a glass rod, and dissolved in 0.1× SSC

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TABLE 1. Designations and sources of *Aneurinibacillus*, *Brevibacillus*, and *Bacillus* strains used, and an overview of the different characterization methods applied to them

| Strain[a] | LMG number | Other designation(s)[b] | Source[b] | Method(s) applied | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | ARDRA | API | PAGE | FAME | Py-MS |
| *Aneurinibacillus aneurinilyticus* | LMG 15531$^{T}$ | B0205$^{T}$, ATCC 12856$^{T}$, JCM 9024$^{T}$ | Gordon | + | + | + | + | + |
| *A. aneurinilyticus* | LMG 12387 | B0202, ATCC 11376, NRS 1443 | Gordon | + | + | + | + | |
| *A. aneurinilyticus* | LMG 15532 | B0206, NRRL NRS-1448 | Gordon | + | + | + | + | + |
| *A. aneurinilyticus* | LMG 15533 | B0207, NRRL NRS-1449 | Gordon | + | + | + | + | + |
| *A. aneurinilyticus* | LMG 15534 | B0208, NRRL NRS-1450 | Gordon | + | + | + | + | + |
| *A. aneurinilyticus* | LMG 15535 | B0209, NRRL NRS-1451 | Gordon | + | + | + | + | + |
| *A. aneurinilyticus* | LMG 15967 | B4045, ATCC 11376, JCM 9023 | ATCC | + | + | + | + | + |
| *A. aneurinilyticus* | LMG 16001 | DSM 5562 | DSM | + | + | + | + | + |
| *A. aneurinilyticus* | LMG 16008 | B4027, IFO 3115, JCM 7539 | IFO | + | + | + | + | + |
| *A. aneurinilyticus* | LMG 17160 | NCIMB 10059 | NCIMB | + | + | + | + | |
| *A. aneurinilyticus* | LMG 17161 | NCIMB 10060 | NCIMB | + | + | + | + | |
| *A. aneurinilyticus* | LMG 17162 | NCIMB 10061 | NCIMB | + | + | + | + | |
| *A.* [*aneurinilyticus*] | LMG 17164 | NCIMB 10057 | NCIMB | + | + | + | + | |
| *A. migulanus* | LMG 15427$^{T}$ | B0270$^{T}$, NCTC 7096$^{T}$, ATCC 9999$^{T}$ | NCTC | + | + | + | + | + |
| *A. migulanus* | LMG 16098 | B4036, NRRL NRS-606 | Nakamura | + | + | + | + | + |
| *Bacillus amyloliquefaciens* | LMG 9814$^{T}$ | DSM 3034$^{T}$ | DSM | + | | | | |
| *B. azotoformans* | LMG 9581$^{T}$ | CCM 2849$^{T}$, ATCC 29788$^{T}$ | CCM | + | | | | |
| *B. badius* | LMG 7122$^{T}$ | DSM 23$^{T}$ | DSM | + | + | + | + | + |
| *B. badius* | LMG 12332 | B0201, NRS 1407 | Gordon | + | + | + | + | |
| *B. cereus* | LMG 6923$^{T}$ | DSM 31$^{T}$ | DSM | + | | | | |
| *B. circulans* | LMG 13261$^{T}$ | B0004$^{T}$, DSM 11$^{T}$ | DSM | + | | | | |
| *B. firmus* | LMG 7125$^{T}$ | DSM 12$^{T}$ | DSM | + | | | | |
| *B. licheniformis* | LMG 6933$^{T}$ | DSM 13$^{T}$ | DSM | + | | | | |
| *B. megaterium* | LMG 7127$^{T}$ | DSM 32$^{T}$ | DSM | + | | | | |
| *B. simplex* | LMG 11160$^{T}$ | DSM 1321$^{T}$ | DSM | + | | | | |
| *B. sphaericus* | LMG 7134$^{T}$ | B0012$^{T}$, DSM 28$^{T}$ | DSM | + | | | | |
| *B. subtilis* | LMG 7135$^{T}$ | DSM 10$^{T}$ | DSM | + | | | | |
| *B. thermoaerophilus* | LMG 17165$^{T}$ | DSM 10154$^{T}$ | DSM | + | + | + | + | |
| *B. thermoaerophilus* | LMG 17166 | DSM 10155 | DSM | + | + | + | + | |
| *B. thuringiensis* | LMG 7138$^{T}$ | DSM 2046$^{T}$ | DSM | + | | | | |
| *Brevibacillus agri* | LMG 15103$^{T}$ | CCUG 31345$^{T}$, NRRL NRS-1219$^{T}$ | CCUG | + | + | + | + | + |
| *B. agri* | LMG 15102 | CCUG 31344, NRRL B-1157 | CCUG | + | | + | + | |
| *B. agri* | LMG 15592 | B4001, NRRL B-1157 | Nakamura | | + | | | + |
| *B. borstelensis* | LMG 16009$^{T}$ | B4029$^{T}$, IFO 15714$^{T}$ | IFO | + | + | + | + | + |
| *B. borstelensis* | LMG 15599 | B4009, NRRL NRS-1373 | Nakamura | + | + | + | + | + |
| *B. brevis* | LMG 16703$^{T}$ | ATCC 8246$^{T}$ | ATCC | + | + | + | + | + |
| [*B. brevis*] | LMG 12423 | B0117, Gibson strain 442 | Gibson | + | + | + | + | + |
| *B.* [*brevis*] | LMG 15431 | B0629, Wellcome 2934 | Goodfellow | + | + | | + | + |
| *B. brevis* | LMG 17054 | ATCC 11031 | ATCC | + | + | | + | |
| *B. brevis* | LMG 17055 | ATCC 35690 | ATCC | | + | | | |
| *B. centrosporus* | LMG 15106$^{T}$ | CCUG 31347$^{T}$, NRRL NRS-664$^{T}$ | CCUG | + | + | + | + | + |
| *B. centrosporus* | LMG 15602 | B4015, NRRL NRS-632 | Nakamura | + | + | + | + | + |
| *B. choshinensis* | LMG 15968$^{T}$ | B4046$^{T}$, ATCC 51359$^{T}$, JCM 8505$^{T}$ | ATCC | + | + | + | + | + |
| *B. choshinensis* | LMG 16096 | B4033, NRRL NRS-378 | Nakamura | + | + | + | + | + |
| *B. formosus* | LMG 16010$^{T}$ | B4039$^{T}$, NRRL NRS-863$^{T}$ | Nakamura | + | + | + | + | + |
| *B. formosus* | LMG 16101 | B4040, NRRL NRS-910 | Nakamura | + | + | + | + | |
| *B. laterosporus* | LMG 16000$^{T}$ | B4035$^{T}$, DSM 25$^{T}$, ATCC 64$^{T}$ | DSM | + | + | + | + | + |
| *B. laterosporus* | LMG 15436 | B0116, Gibson 1066 "*B. orpheus*" | Gibson | + | + | + | + | |
| *B. parabrevis* | LMG 15971$^{T}$ | B4047$^{T}$, ATCC 10027$^{T}$ | ATCC | + | + | + | + | + |
| *B. parabrevis* | LMG 15428 | B0271, Gibson 108, NCTC 7577 | NCTC | + | + | + | + | + |
| *B. reuszeri* | LMG 16012$^{T}$ | B4042$^{T}$, NRRL NRS-1206$^{T}$ | Nakamura | + | + | + | + | + |
| *B. reuszeri* | LMG 16105 | B4043, NRRL NRS-1207 | Nakamura | + | + | + | + | + |
| *Sporosarcina ureae* | LMG 17366$^{T}$ | B0819$^{T}$, CCM 684$^{T}$ | CCM | + | | | | |

[a] Names shown in brackets have uncertain taxonomic species or genus statuses; e.g., *A. aneurinilyticus* LMG 17164 belongs to *A. migulanus* according to Shida et al. (20).

[b] Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; B, N. A. Logan *Bacillus* collection, Glasgow Caledonian University, Glasgow, United Kingdom; CCM, Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic; CCUG, Culture Collection of the University of Göteborg, Göteborg, Sweden; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; Gibson, T. Gibson collection, held at Glasgow Caledonian University, Glasgow, United Kingdom; Goodfellow, M. Goodfellow, University of Newcastle, Newcastle upon Tyne, United Kingdom; Gordon, R. E. Gordon, Rutgers University, New Brunswick, N.J.; IFO, Institute for Fermentation Osaka collection, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research, Saitama, Japan; LMG, Laboratorium voor Microbiologie collection, Universiteit Gent, Ghent, Belgium; Nakamura, L. K. Nakamura, U.S. Department of Agriculture, Peoria, Ill.; NCIMB, National Collection of Industrial and Marine Bacteria, Aberdeen, United Kingdom; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, United Kingdom; NRRL, Northern Regional Research Laboratory collection, Peoria, Ill.; Wellcome, Wellcome Research Laboratories, Beckenham, Kent, United Kingdom.

(10× SSC is 1.5 M NaCl plus 0.15 M trisodium citrate, pH 7.0). The dissolved DNA was treated with RNase A (final concentration, 100 μg/ml in 1× SSC) for 1 h at 37°C. The RNase was removed by a single chloroform extraction and centrifugation (as described above), and the DNA was precipitated with 0.54 volumes of isopropanol and spooled on a glass rod. After consecutive washing steps in 70, 80, 90, and 95% ethanol and short periods of air drying, the DNA was dissolved in a small volume of 0.1× SSC containing a few drops of chloroform and stored at −80°C.

All DNA preparations were visually inspected for DNA integrity by 1% (wt/vol) agarose gel electrophoresis and were measured spectrophotometrically at 234, 260, and 280 nm to check the DNA purity as indicated by an optical density ratio, $OD_{260}/OD_{260}$, of between 0.45 and 0.70 and an $OD_{260}/OD_{280}$ ratio of between 1.7 and 2.0.

**DNA-DNA binding.** DNA-DNA binding reactions were determined spectrophotometrically by the initial renaturation method of De Ley et al. (4), and the results were expressed as percentages as described by Willems et al. (31).

**ARDRA.** Enzymatically amplified 16S ribosomal DNA (rDNA) was obtained from each strain by PCR and analyzed by digestion with five restriction enzymes (*Hae*III, *Dpn*II, *Rsa*I, *Bfa*I, and *Tru*9I) as described previously (11). The restriction patterns for each strain were combined, and the combined normalized patterns were subjected to numerical analysis with the Gelcompar 4.0 software (Applied Maths, Kortrijk, Belgium), using the Dice coefficient and the unweighted pair group method using arithmetic averages (UPGMA) clustering algorithm.

**Gas chromatographic analysis of fatty acid methyl esters (FAME).** Cells were grown on TSA at 28°C for 24 h as recommended by the manufacturers of the Microbial Identification System (MIS) (Microbial ID, Inc., Newark, Del.), except for *B. thermoaerophilus*, which was grown at 55°C. Methods for fatty acid extraction, methyl ester preparation, and methyl ester separation by gas chromatography were described previously (29). The fatty acid methyl ester profiles were identified and clustered by using the MIS software (version 4.0).

**Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) of whole-cell proteins.** Cells for SDS-PAGE were obtained after 24 h of growth on NAG at 30°C, except for those of *B. thermoaerophilus*, which was grown at 30 and 55°C. Methods for preparation and electrophoresis of SDS protein extracts and for UPGMA cluster analysis of the protein profiles were described previously (19, 30).

**Phenotypic characterization and numerical analysis.** As the methods of Gordon et al. (8) and Logan and Berkeley (15) are unable to separate *A. aneurinilyticus*, *A. migulanus*, members of the genus *Brevibacillus*, and *Bacillus badius* satisfactorily because most of these species are unreactive in most of the tests, the organisms were characterized by using carbon source assimilation tests in the API Biotype 100 system (bioMérieux, Marcy l'Etoile, France). Strains were cultivated for 24 to 48 h on TSA, cells were harvested in sterile distilled water, and suspensions were prepared in Biotype Medium 2 (containing 31 growth factors) to turbidities equivalent to the no. 3 McFarland standard and then inoculated into test strips for the API Biotype 100 system, which comprises 99 carbon source assimilation tests and one negative control. Assimilation reactions at 30°C (55°C for strains of *B. thermoaerophilus*) were followed for 4 days (2 days for strains of *B. thermoaerophilus*) and scored 4, 3, 2, 1 (for positive results after 1, 2, 3, and 4 days, respectively), or 0 (for negative reactions). Strains were grown overnight at 30°C (55°C for strains of *B. thermoaerophilus*) in nutrient broth for inoculation of media for four other phenotypic observations: hydrolysis of casein and gelatin and growth at 20 and 55°C were tested by the methods of Gordon et al. (8) and scored as 1 (positive) or 0 (negative). Twelve characteristics of vegetative-cell and sporangial morphologies were observed as described by Logan and Berkeley (15) and scored as 1 (positive) or 0 (negative). Redundant tests (i.e., positive or negative for all strains) were excluded, and the data were subjected to numerical taxonomic analysis with the GENSTAT 5 program. Similarities were calculated by using the general similarity coefficient of Gower ($S_G$) (9). Negative matches in the API Biotype 100 tests were excluded, and test ranges were set higher than those observed to compensate for bias attributable to negative results from weakly reacting strains (14). Results were examined by UPGMA cluster analysis and expressed at 2.5% intervals.

**Pyrolysis mass spectrometry (Py-MS).** Each strain was grown on duplicate plates of nutrient agar for 24 to 48 h, and duplicate samples were taken from each plate for analysis. Preparation and Py-MS of the bacterial samples and normalization, cluster analysis based on the $S_G$ coefficient, and canonical variates analysis of the data with the GENSTAT statistical software were performed as described previously (7, 10).

## RESULTS AND DISCUSSION

**A polyphasic study of the genus *Aneurinibacillus*.** In a numerical analysis of combined ARDRA patterns (Fig. 1), the two species of *Aneurinibacillus*, *A. aneurinilyticus* (13 strains) and *A. migulanus* (2 authentic strains and an erroneously identified *Brevibacillus brevis* strain, LMG 12423), were recovered in one cluster at a similarity level of 91%. 16S rDNA sequence similarities of 98.6% (23) and 99.7% (16), respectively, were reported for the type strains of these two species. In the ARDRA dendrogram, the two strains of the thermophilic species *B. thermoaerophilus* were the nearest neighbors of this cluster, with a similarity level of 68%, confirming again the results of 16S rDNA sequence comparison of Meier-Stauffer et al. (16) based on the type strains only. The representatives of the genera *Brevibacillus*, *Paenibacillus*, and *Bacillus* rRNA groups 1 and 2 were further removed; *Brevibacillus* strains showed a somewhat higher relationship of 50% to *Aneurinibacillus* strains and *B. thermoaerophilus* (Fig. 1). With ARDRA, a reliable allocation of strains to the different genera split off from the genus *Bacillus* or to the remaining *Bacillus* rRNA groups can thus be accomplished. Furthermore, our data indicate that *B. thermoaerophilus* shows a closer relationship to the genus *Aneurinibacillus* than to all other genera and *Bacillus* rRNA groups investigated.

In the FAME analysis, *A. aneurinilyticus*, *A. migulanus*, and, surprisingly, *B. badius* were shown to have quite similar fatty acid profiles, while that of *B. thermoaerophilus* was different. The latter result can be at least partly explained by the difference in cultivation temperature; *B. thermoaerophilus* strains could not be grown at the normal mesophilic temperature of 28°C as recommended by the manufacturers of the MIS identification system and were cultivated at 55°C. Indeed, a shift in fatty acid composition toward longer-chain fatty acids and lower ratios of unsaturated fatty acids to saturated fatty acids for the thermophylic strains compared to the mesophilic strains (as can be deduced from Table 2) have been mentioned as being linked with a higher growth temperature (26).

The remarkable high degree of similarity between the fatty acid profile of *B. badius* and those of the mesophilic strains of *Aneurinibacillus*, however, remains unexplained. Nevertheless, 16S rDNA sequence comparisons (2, 5) and ARDRA (this paper) clearly locate *B. badius* in, or at least at the periphery of, the main *Bacillus* rRNA group, group 1 (2). Finally, all taxa can be distinguished (Table 2) by qualitative and quantitative differences in fatty acid composition. It should be noted here that *A.* [*aneurinilyticus*] LMG 17164 (see footnote *a* in Table 1) showed a typical *A. aneurinilyticus* fatty acid profile yet behaved as an outlier of the *Aneurinibacillus*-*B. badius* group in other phenotypic analyses (see below). Hence, it was assumed that this strain was misidentified at the species level (20), and its fatty acid profile was not included when the mean fatty acid profile of *A. migulanus* was calculated (Table 2).

In the analysis of API Biotype 100 and other phenotypic characters, *A. migulanus*, *B. thermoaerophilus*, and *B. badius* strains, as well as most strains of *A. aneurinilyticus*, were recovered in separate but closely related clusters which merged at 57.5% $S_G$ and then joined with the *Brevibacillus* cluster at 42.5% $S_G$ (Fig. 2). Most strains of the genera *Aneurinibacillus*, *Bacillus*, and *Brevibacillus* have been recovered in single-species clusters, but the separations of several species are not distinct; this is due to the existence of considerable within-species variation in the API Biotype 100 tests. Although the separation of the main *A. aneurinilyticus* cluster from the clusters of *A. migulanus*, *B. thermoaerophilus*, and *B. badius* is modest (largely due to the two atypical strains LMG 17160 and LMG 17162), several phenotypic characters can be found to separate these species from each other and from *Brevibacillus* species on the basis of the strains examined in this study (Table 3). Strain LMG 12423, which was received as *Brevibacillus brevis*, showed a phenotypic profile characteristic of *A. migulanus* and was recovered in the *A. migulanus* cluster (Fig. 2); we therefore reclassify it here as a strain of *A. migulanus.*

Two other strains received as *Aneurinibacillus* species were not recovered in their respective clusters, and the characters of

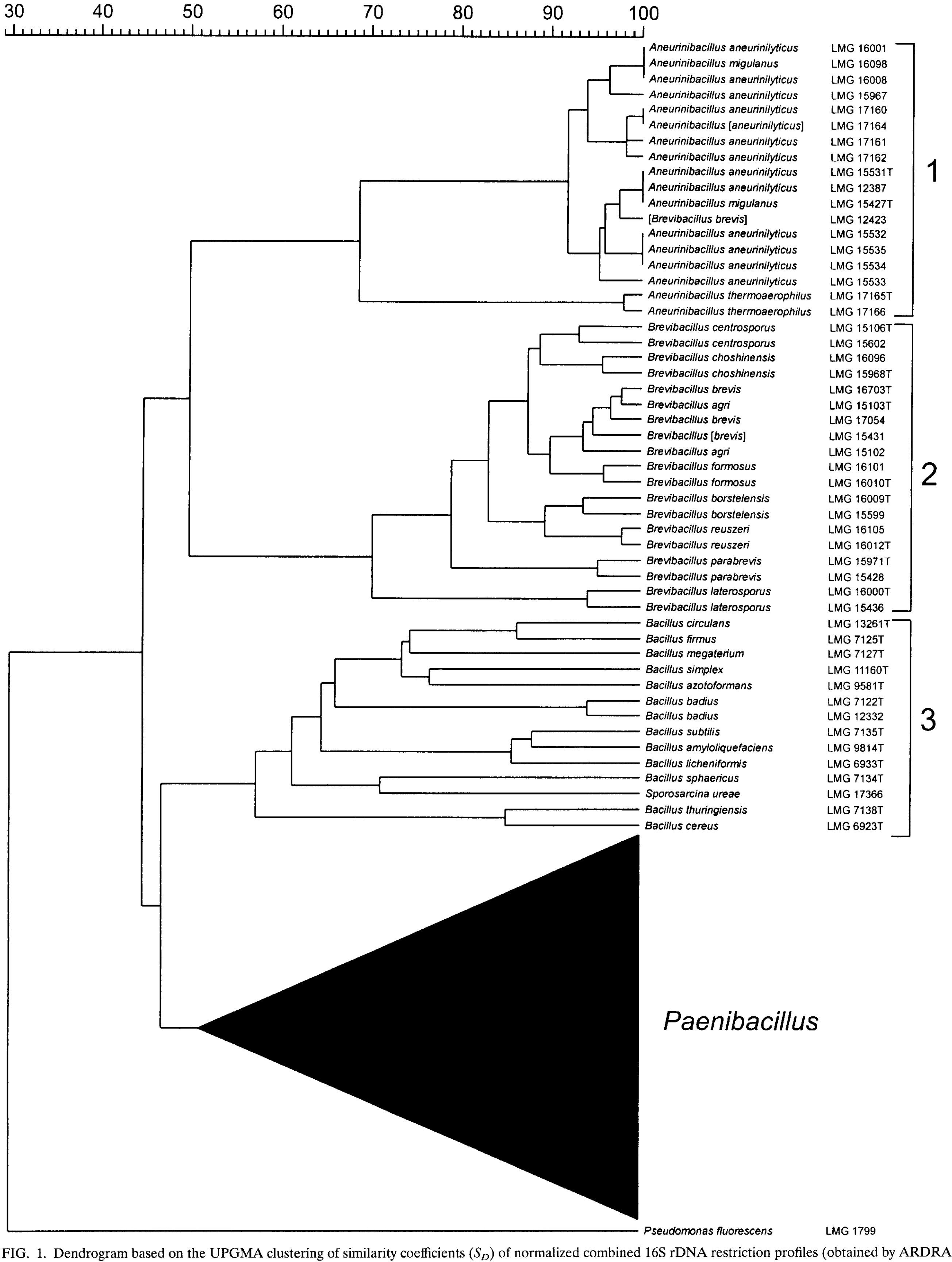

FIG. 1. Dendrogram based on the UPGMA clustering of similarity coefficients ($S_D$) of normalized combined 16S rDNA restriction profiles (obtained by ARDRA) of 13 *A. aneurinilyticus* strains, 2 *A. migulanus* strains, 2 *A. thermoaerophilus* strains, 20 representatives of the genus *Brevibacillus*, 14 representatives of *Bacillus* rRNA groups 1 and 2, and 77 representatives of the genus *Paenibacillus*. Data for *Paenibacillus* strains were taken from reference 12. The restriction enzymes used were *Hae*III, *Dpn*II, *Rsa*I, *Bfa*I, and *Tru*9I. *Pseudomonas fluorescens* LMG 1799 was used as an outgroup reference. T's indicate the positions of type strains. Clusters 1, 2, and 3 represent *Aneurinibacillus* species, *Brevibacillus* species, and *Bacillus* rRNA groups 1 and 2 (2), respectively. Misidentified strains are shown in brackets.

TABLE 2. Comparison of the fatty acid profiles of 13 *A. aneurinilyticus*, 2 *A. migulanus*, 2 *A. thermoaerophilus*, and 2 *B. badius* strains as measured by gas chromatographic analysis of their methyl esters

| Species | Mean % (range) for fatty acid[a]: | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 14:0 iso | 14:0 | 15:0 iso | 15:0 anteiso | 15:1 ω6c | 15:0 | 16:1 ω7C alcohol | 16:1 iso H | 16:0 iso | 16:1 ω11c | 16:1 ω5c | 16:0 | iso 17:1 ω10c | 17:0 iso | 17:0 anteiso | 17:1 ω6c | Summed feature 2[b] | Summed feature 4[c] | Summed feature 5[d] |
| *A. aneurinilyticus* | 3.7 (1.4–8.8) | 2.6 (1.6–4.0) | 57.2 (41.9–66.3) | 1.3 (0.0–3.7) | | 0.8 (0.0–1.6) | | | 2.3 (0.5–4.2) | 10.7 (8.0–13.5) | 0.8 (0.0–1.8) | 5.0 (2.2–8.5) | 2.6 (1.5–3.4) | 2.0 (1.0–3.4) | | | 1.4 (0.9–2.8) | 6.0 (2.7–10.3) | 2.1 (1.0–3.5) |
| *A. migulanus* | 9.0 (8.3–9.6) | 1.9 (1.8–2.0) | 48.6 (48.3–48.9) | 1.3 (1.3–1.3) | 0.9 (0.8–1.0) | 2.8 (2.3–3.3) | 1.4 (1.4–1.4) | 1.5 (1.3–1.7) | 6.5 (6.5–6.6) | 6.5 (6.4–6.6) | 1.1 (1.1–1.2) | 3.5 (3.4–3.6) | 1.9 (1.7–2.2) | 2.1 (2.1–2.1) | | 1.6 (1.4–1.7) | 1.4 (1.3–1.6) | 4.9 (4.8–5.0) | 2.4 (2.4–2.5) |
| *A. thermoaerophilus* | | | 58.5 (50.3–66.8) | 5.1 (0.0–10.3) | | 2.2 (0.9–3.4) | | | 3.8 (3.5–4.1) | | | 2.2 (1.8–2.5) | | 23.6 (23.4–23.8) | 4.2 (0.0–8.3) | | | | |
| *B. badius* | 1.6 (1.4–1.8) | 1.8 (1.5–2.1) | 55.8 (55.7–55.9) | 6.2 (5.7–6.7) | | 1.7 (1.6–1.8) | 3.5 (3.3–3.7) | | 4.9 (4.6–5.1) | 4.3 (4.1–4.6) | | 2.5 (2.5–2.5) | 5.9 (4.7–7.0) | 3.1 (2.9–3.4) | 2.2 (2.0–2.3) | | | 3.4 (3.0–3.8) | 3.1 (3.0–3.3) |

[a] Only the fatty acids accounting for more than 0.5% of the total fatty acid content are listed. The values in parentheses are the ranges within each group of strains.

[b] Summed feature 2: 15:1 iso H, 15:1 iso I, and/or 13:0 3-OH.

[c] Summed feature 4: 16:1 ω7c and/or 15:0 iso 2-OH.

[d] Summed feature 5: 17:1 iso I and/or 17:1 anteiso B.

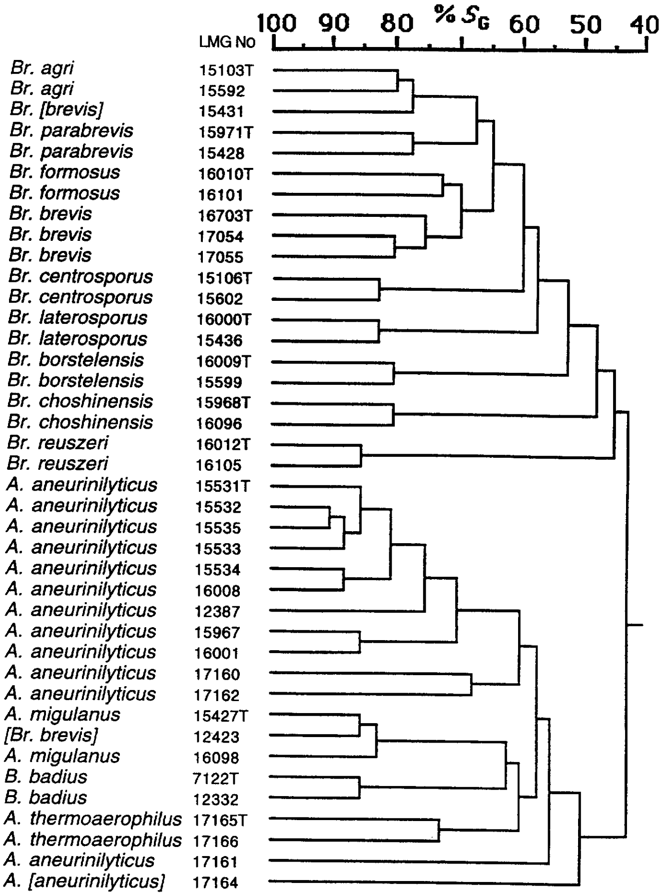

FIG. 2. Phenogram based on the UPGMA clustering of similarity coefficients ($S_G$) of 13 strains received as *A. aneurinilyticus*, 2 received as *A. migulanus*, 2 *A. thermoaerophilus* strains, 2 *B. badius* strains, and 21 representatives of the genus *Brevibacillus* (*Br.*) based on 115 phenotypic characters. T's indicate the positions of type strains. Misidentified strains are shown in brackets.

these organisms were disregarded in the construction of Table 3. *A. aneurinilyticus* LMG 17161 was an outlier of the main *Aneurinibacillus-B. badius* group, although it behaved as a typical *A. aneurinilyticus* strain in other analyses. *A. [aneurinilyticus]* LMG 17164 was also recovered as an outlier, joining the *Aneurinibacillus-B. badius* group at 50% $S_G$. This strain was received as *A. aneurinilyticus* but reclassified as a strain of *A. migulanus* by Shida et al. (20). It was found here to have a typical *A. aneurinilyticus* FAME profile. It might be an intermediate strain. One organism received as *Brevibacillus brevis*, LMG 15431, was recovered in the *Brevibacillus agri* cluster (Fig. 2) and may be identified as a member of the latter species.

In the dendrogram based on SDS-PAGE of cell proteins (Fig. 5), the *A. aneurinilyticus* and *A. migulanus* strains formed a heterogeneous cluster at 77% similarity. The division of this cluster into two subclusters was mainly caused by the number (one or two) of dense, high-molecular-weight bands in the $7.7 \times 10^4$ to $8.2 \times 10^4$ zone. Twelve of the 13 *B. aneurinolyticus* strains investigated contained two such bands, which is consistent with the S-layer protein data reported by Shida et al. (20). Although *B. badius* LMG 12332 joined at the periphery of one of the subclusters, on visual inspection its protein profile showed greater resemblance to that of *B. badius* LMG 7122$^T$, except for a dense protein band with a molecular weight of $8.0 \times 10^4$ (Fig. 5). Indeed, by exclusion of the $7.7 \times 10^4$ to $8.2 \times 10^4$ molecular weight zone from numerical analysis (data not shown), the *A. aneurinilyticus-A. migulanus* cluster formed

TABLE 3. Phenotypic characters that differentiate the studied strains of *Aneurinibacillus* species, *Brevibacillus*[a] species, and *B. badius*

| Character[b] | *A. aneurinilyticus* | *A. migulanus* | *A. thermoaerophilus* | *B. badius* | *Brevibacillus agri* | *Brevibacillus brevis* | *Brevibacillus formosus* | *Brevibacillus parabrevis* | *Brevibacillus laterosporus* | *Brevibacillus borstelensis* | *Brevibacillus centrosporus* | *Brevibacillus choshinensis* | *Brevibacillus reuszeri* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parasporal bodies | − | − | − | − | [c]− | − | − | − | + | − | − | − | − |
| Hydrolysis of: | | | | | | | | | | | | | |
| Casein | − | − | + | + | + | + | + | + | + | + | − | − | − |
| Gelatin | − | − | + | + | + | + | + | + | + | + | − | − | − |
| Growth at: | | | | | | | | | | | | | |
| 55°C | − | − | + | − | − | − | − | − | − | − | − | − | − |
| 20°C | − | + | − | + | v[c] | − | + | + | v | − | + | + | + |
| Assimilation of: | | | | | | | | | | | | | |
| 4-Aminobutyrate | − | + | v | + | − | v | v | − | − | − | − | − | + |
| L-Aspartate | + | + | + | + | + | + | + | + | + | − | + | + | + |
| Ethanolamine | + | + | + | − | + | v | + | + | v | + | − | v | − |
| D-Fructose | − | + | v | − | + | + | + | − | + | + | − | − | v |
| Fumarate | + | + | + | + | + | v | + | − | + | + | + | v | − |
| D-Gluconate | v | + | − | − | + | + | + | + | − | + | + | − | + |
| D-Glucosamine | − | − | − | − | − | + | − | v | − | − | + | − | − |
| Glutarate | + | v | − | + | − | − | − | − | − | − | − | − | − |
| DL-Glycerate | − | − | v | − | − | − | − | − | + | + | − | − | + |
| Glycerol | + | + | + | + | + | + | + | + | + | + | − | − | − |
| 2-Ketoglutarate | v | v | + | + | + | v | + | + | v | − | + | − | + |
| DL-Lactate | + | + | + | + | + | − | + | − | − | − | + | − | + |
| Lactulose | − | − | − | − | − | − | − | − | − | − | − | − | + |
| D-Malate | v | − | + | − | − | − | − | − | − | − | − | − | − |
| Maltose | − | − | − | − | + | + | + | + | + | − | − | − | − |
| *N*-Acetyl-D-glucosamine | − | − | + | − | + | + | + | + | + | − | + | + | + |
| Putrescine | + | + | + | + | + | − | − | + | − | − | v | v | + |
| Quinate | − | − | + | − | − | − | − | − | − | − | − | − | − |
| L-Sorbose | − | − | + | − | − | − | − | − | − | − | − | − | − |
| Sucrose | v | − | − | + | + | + | + | + | − | − | − | − | − |
| L-Tartrate | − | − | + | − | − | − | − | − | − | − | − | − | − |
| D-Trehalose | v | − | − | − | + | + | + | + | + | − | − | − | − |
| Tricarballylate | − | − | + | − | − | − | − | − | − | − | − | − | − |
| D-Turanose | − | − | v | − | + | + | + | + | − | − | − | − | − |
| L-Tyrosine | − | + | + | − | v | − | − | v | − | − | − | − | − |

[a] One strain of *Brevibacillus thermoruber* could be distinguished from the other species by its production of a red-orange pigment, positive results for hydrolysis of casein and gelatin, growth at 55°C, and negative results for all the other characters listed.

[b] With the exceptions of spore morphology, hydrolysis of casein and gelatin, and growth at 55 and 20°C, all characters were determined by tests in the API Biotype 100 system.

[c] v, Between-strain variation.

at 88% similarity, with the *A. migulanus* type strain linking at 81% similarity, and was quite distinct (76% similarity) from the two tightly (93% similarity) clustered *B. badius* strains. The SDS-PAGE profiles of the *A. aneurinilyticus* and *A. migulanus* strains clearly differed from those of the *Brevibacillus* species (Fig. 5). The SDS-PAGE profiles of the two *B. thermoaerophilus* strains, grown either at 30 or 55°C, showed little resemblance to each other or to those of the other species examined and were characterized by a dense band of variable high molecular weight (Fig. 5), probably corresponding to the glycosylated S-layer proteins described previously (16).

An initial Py-MS analysis showed that the *Aneurinibacillus* strains were very closely related to, but nevertheless distinct from, strains of the *Brevibacillus* species examined. Removal of a number of outlying strains of *B. badius* and *B. laterosporus*, which alleviates the compression of other groups that is known to be caused by outliers (24), led to the results presented in Fig. 3. The genus *Aneurinibacillus* is distinct from the genus *Brevibacillus* and from *B. badius*, but Py-MS analysis does not allow separation of *A. aneurinilyticus* and *A. migulanus*. Similarly, the two-dimensional canonical variates analysis plot of the data (Fig. 4) indicates that these two species overlap each other but are separate from the other organisms examined.

Since our polyphasic data showed a very close relationship between *A. aneurinilyticus* and *A. migulanus*, and SDS-PAGE of whole-cell proteins and Py-MS (two techniques known to delineate taxa at the species and subspecies levels [24 and 28]) did not enable a reliable separation of the two species, we reexamined the DNA-DNA homology between the respective type strains. This was also prompted by the contradictory DNA homology values (ranging from 0 to 38%) for the type strains reported in the literature (20, 27). In our study, a DNA homology value of 40.5% ± 1.5% (mean ± standard deviation of two determinations) was measured, which confirms one of the previously reported data (27) and also confirms the validity of *A. aneurinilyticus* and *A. migulanus* as separate but highly related species. Nevertheless, this indicates the importance of the DNA-DNA hybridization method used for achieving taxonomic conclusions.

**Conclusion.** Members of the genus *Aneurinibacillus* (*B. thermoaerophilus* included) can be distinguished phylogenetically (by ARDRA and complete 16S rDNA sequence comparison

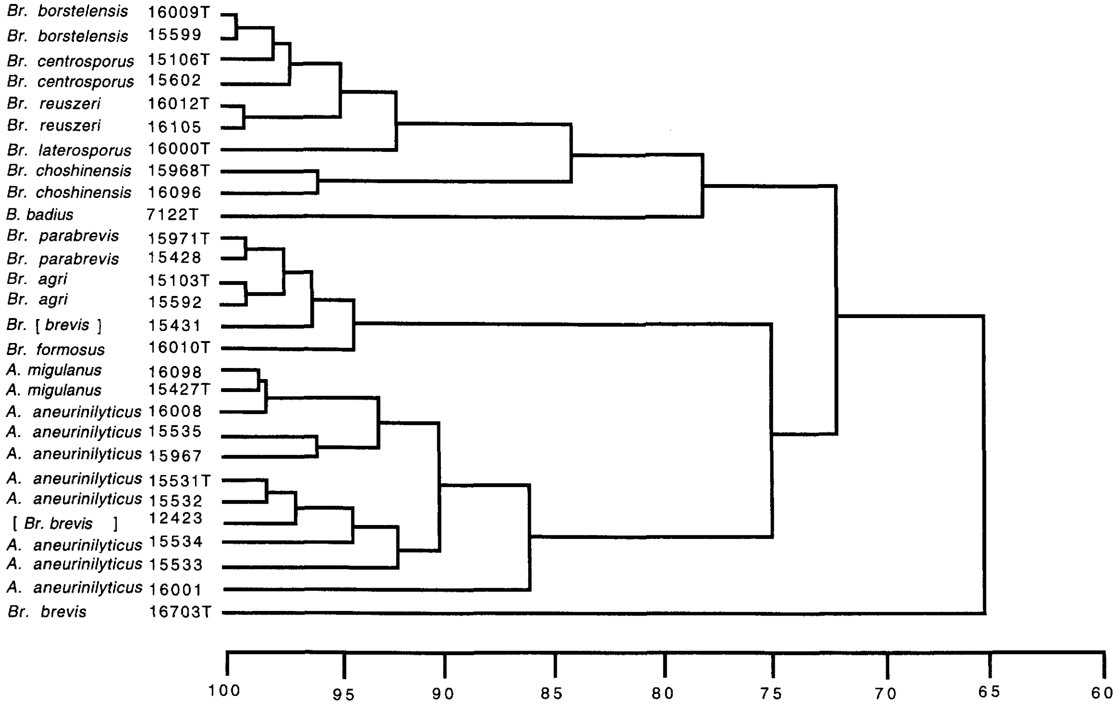

FIG. 3. Dendrogram based on the UPGMA clustering of similarity coefficients ($S_G$) of Py-MS data from 8 *A. aneurinilyticus* and 2 *A. migulanus* strains, 1 *B. badius* strain, and 17 representatives of the genus *Brevibacillus* (*Br.*) T's indicate the positions of type strains. Misidentified strains are shown in brackets.

[this paper and, e.g., references 16 and 23) from *Brevibacillus* species. Although only a relatively small number of phenotypic characters separate the genus *Aneurinibacillus* from the genus *Brevibacillus* and from *B. badius* (a member of *Bacillus* rRNA group 1), a phenotypic differentiation is possible. These arguments favor the reclassification of *B. thermoaerophilus* as *Aneurinibacillus thermoaerophilus* comb. nov. Emended descriptions of the genus *Aneurinibacillus* and its three species, *A. aneurinilyticus*, *A. migulanus*, and *A. thermoaerophilus*, follow.

**Emended description of the genus *Aneurinibacillus*.** *Aneurinibacillus* (A.neu.ri.ni.ba.cil'lus. M. L. n. *aneurinum*, thiamine; L. dim. n. *bacillus*, small rod, *Bacillus*, a genus of endospore-forming rods; M. L. masc. *Aneurinibacillus*, a genus of thiamine-decomposing, endospore-forming rods). The descriptions below are based on our own observations and previous descriptions of the species belonging to this genus (16, 20, 27). Although the two species *Aneurinibacillus aneurinilyticus* and *Aneurinibacillus migulanus* are retained, it should be noted that these two species are difficult to distinguish by routine tests.

Gram-positive, rod-shaped cells, 0.5 to 1.0 μm by 2.0 to 6.0 μm, and motile by peritrichous flagella. Ellipsoidal spores, one per cell, are borne centrally, paracentrally, and subterminally and swell the sporangia slightly or not at all. Strictly aerobic. Growth on routine media such as nutrient agar and Trypticase soy agar. Decompose thiamine. Catalase positive, weakly positive, or negative. Nitrate reduction variable. Casein, gelatin, and Tween 80 hydrolysis variable. Starch and urea are not hydrolyzed; indole is not produced. Growth temperatures range from 20 to 60°C. Growth at pH 5.5 to 9.0. Growth occurs in the presence of 2% NaCl but not 5% NaCl. Few carbohydrates are assimilated, and acid is produced weakly if at all from them; amino acids and some organic acids are used as carbon sources. The following carbon sources are assimilated in the API Biotype 100 system: D-alanine, 5-aminovalerate, L-aspartate, ethanolamine, fumarate, L-glutamate, glycerol, 3-hydroxybutyrate, DL-lactate, L-proline, and putrescine. The major cellular fatty acid components (ranges [percent of total] are given in parentheses) are 15:0 iso (41.9 to 66.8%), 16:0 iso (0.5 to 6.6%), 16:0 (1.8 to 8.5%), and 17:0 iso (1.0 to 23.8%).

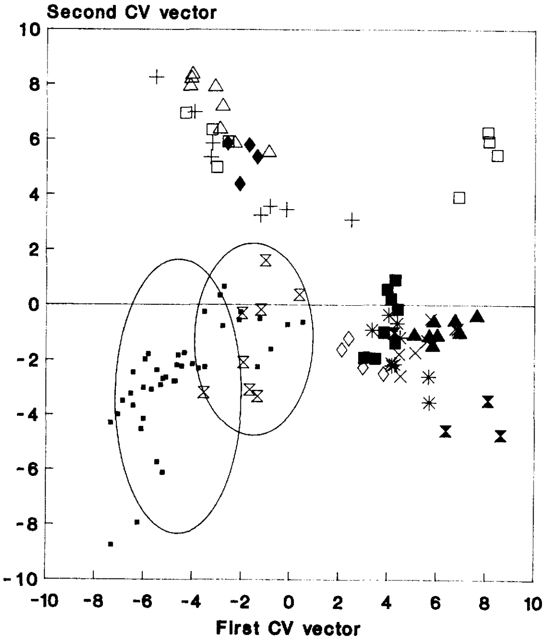

FIG. 4. A canonical variates (CV) analysis plot of Py-MS data for strains of *A. aneurinilyticus* (▪), *A. migulanus* (⧖), *Brevibacillus agri* (+), *B. badius* (⧗), *Brevibacillus brevis* (□), *Brevibacillus borstelensis* (×), *Brevibacillus centrosporus* (✱), *Brevibacillus choshinensis* (■), *Brevibacillus laterosporus* (◇), *Brevibacillus parabrevis* (△), *Brevibacillus formosus* (◆), and *Brevibacillus reuszeri* (▲).

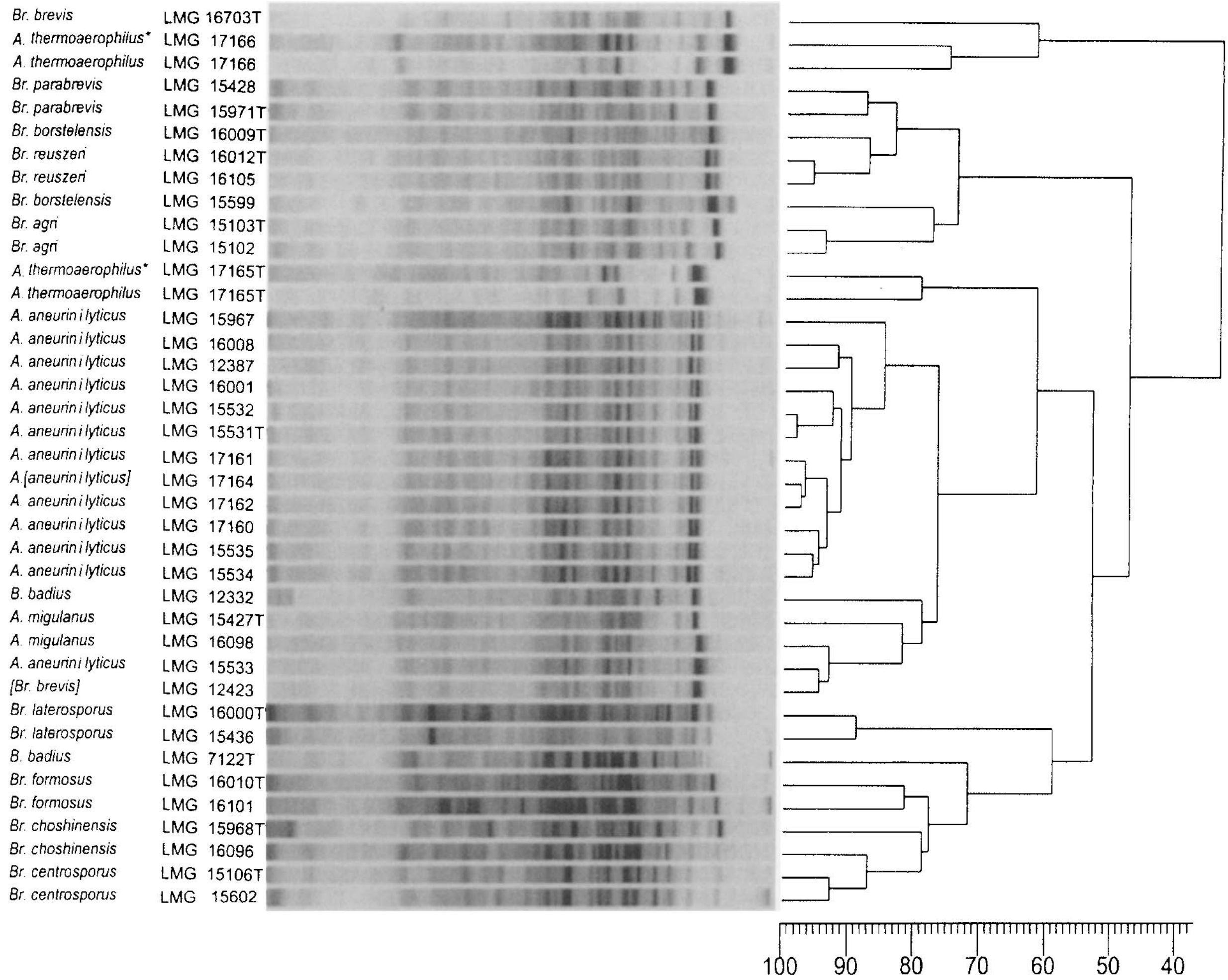

FIG. 5. Normalized computer profiles of SDS-PAGE analysis of whole-cell proteins of 13 *A. aneurinilyticus* strains, 3 *A. migulanus* strains, 2 *A. thermoaerophilus* strains, 2 *B. badius* strains, and 18 representatives of the genus *Brevibacillus* (*Br.*). The dendrogram was based on the UPGMA clustering of the correlations (*r*) of the total-protein profiles. T's indicate the positions of type strains. Misidentified strains are shown in brackets. Asterisks indicate that the cells were grown at 30°C.

The major quinone is menaquinone 7. A specific S-layer protein is present. The G+C content ranges from 42 to 47 mol%.

The type species is *Aneurinibacillus aneurinilyticus*, represented by type strain LMG 15531$^T$ (= ATCC 12856$^T$ = B0205$^T$ = NRRL NRS-1589$^T$).

**Emended description of *Aneurinibacillus aneurinilyticus* corrig.** *Aneurinibacillus aneurinilyticus* (an.eur.in.*i*.ly'tic.us. M. L. n. *aneurinum*, thiamine; M. L. adj. *lyticus*, dissolving; M. L. adj. *aneurinilyticus*, decomposing thiamine). The following characters are in addition to those given above for the genus. Colonies on nutrient agar after 48 h at 37°C are flat, 0.5 to 2 mm in diameter, round or irregular in shape, with slightly crenate edges, and they are glossy, translucent, and creamy greyish. Colonies become whitish and opaque as their component cells sporulate. Vegetative cells are 0.7 to 0.9 μm by 3.0 to 5.0 μm. Weakly catalase positive. Growth temperatures range from 25 to 50°C. Growth at pH 5.0 to 9.0. Optimum temperature for growth, 37°C; optimum pH for growth, 7.0. Nitrate is reduced to nitrite. Casein, gelatin, and Tweens 20, 40, 60, and 80 are not hydrolyzed.

The following carbon source, in addition to those listed in the genus description above, is assimilated in the API Biotype 100 system: glutarate. Assimilation of *N*-acetyl-D-glucosamine, adonitol, L-alanine, D-cellobiose, D-gluconate, 2-ketoglutarate, α-lactose, D- and L-malate, α-D-melibiose, 1-O-methyl-β-D-glucopyranoside, phenylacetate, propionate, D-ribose, L-serine, succinate, sucrose, D-tartrate, and D-trehalose varies among strains. The following carbon sources are not assimilated: *cis*- and *trans*-aconitate, esculin, 4-aminobutyrate, L-arabinose, D- and L-arabitol, benzoate, betain, caprate, caprylate, citrate, *m*-coumarate, dulcitol, *i*-erythritol, β-D-fructose, α-L-fucose, D-galactose, D-galacturonate, β-gentiobiose, gentisate, D-glucose, D-glucosamine, D-glucuronate, DL-glycerate, histamine, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, hydroxyquinoline-β-glucuronide, *myo*-inositol, itaconate, 2-keto-D-gluconate, 5-keto-D-gluconate, lactulose, D-lyxose, malonate, maltitol, maltose, maltotriose, D-mannitol, D-mannose, D-melezitose, 1-O-methyl-α-galactopyranoside, 1-O-methyl-β-galactopyranoside, 1-O-methyl-α-D-glucopyranoside, 3-O-methyl-D-glucopyranose, mucate, palatinose, 3-phenylpropionate, protocatechuate, quinate, D-raffinose, α-L-rhamnose, D-saccharate, D-sorbitol, L-sorbose, D-tagatose, L-tartrate, *meso*-tartrate, tricarballylate, trigonelline, tryptamine, L-tryptophan, D-turanose, L-tyrosine, xylitol, and D-xylose. The major cellular fatty acid components (ranges [percent of total] are given in parentheses) are 14:0 iso (1.4 to 8.8%); 14:0 (1.6 to 4.0%); 15:0 iso (41.9 to 66.3%); 16:0 iso (0.5 to 4.2%); 16:1 ω11c (8.0 to 13.5); 16:0 (2.2 to 8.5%); iso 17:1 ω10c (1.5 to 3.4%); 17:0 iso (1.0 to 3.4%); 15:1 iso H and/or 15:1 iso I, and/or 13:0 3-OH (0.9 to 2.8%); 16:1 ω7c and/or 15:0 iso 2-OH (2.7 to 10.3%); and 17:1 iso I and/or 17:1 anteiso B (1.0 to 3.5%). The G+C content ranges from 41.1 to 43.4 mol% (as determined by high-performance liquid chromatography [HPLC] [20]).

The type strain is LMG 15531$^{T}$ (= ATCC 12856$^{T}$ = B0205$^{T}$ = NRRL NRS-1589$^{T}$), and this strain has the characteristics of the species described above. It has a G+C content of 42.9 mol%. This strain assimilates 2-ketoglutarate, L-malate, phenylacetate, propionate, D-ribose, L-serine, and succinate.

**Emended description of *Aneurinibacillus migulanus*.** *Aneurinibacillus migulanus* (mi.gu.la'nus. M. L. adj. *migulanus*, referring to the German bacteriologist W. Migula, who contributed to bacterial taxonomy). The following characters are in addition to those given above for the genus. Colonies on nutrient agar after 48 h at 37°C are flat, 2 to 3 mm in diameter, with crenate edges, and they are translucent, yellowish grey, and glossy. Colonies become creamy white and opaque as their component cells sporulate. Vegetative cells are 0.5 to 1.0 μm by 2.0 to 6.0 μm. Catalase positive. Growth temperatures range from 20 to 50°C. Growth at pH 5.5 to 9.0. Nitrate is reduced to nitrite. Casein, gelatin, and Tweens 20, 40, 60, and 80 are not hydrolyzed. The following carbon sources, in addition to those listed in the genus description above, are assimilated in the API Biotype 100 system: L-alanine, 4-aminobutyrate, β-D-fructose, D-gluconate, phenylacetate, propionate, D-ribose, L-serine, succinate, and L-tyrosine. Assimilation of *cis*- and *trans*-aconitate, glutarate, *myo*-inositol, 2-ketoglutarate, and L-malate varies among strains. The following carbon sources are not assimilated: *N*-acetyl-D-glucosamine, adonitol, esculin, L-arabinose, D- and L-arabitol, benzoate, betain, caprate, caprylate, D-cellobiose, citrate, *m*-coumarate, dulcitol, *i*-erythritol, α-L-fucose, D-galactose, D-galacturonate, β-gentiobiose, gentisate, D-glucose, D-glucosamine, D-glucuronate, DL-glycerate, histamine, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, hydroxyquinoline-β-glucuronide, itaconate, 2-keto-D-gluconate, 5-keto-D-gluconate, α-lactose, lactulose, D-lyxose, D-malate, malonate, maltitol, maltose, maltotriose, D-mannitol, D-mannose, α-D-melibiose, D-melezitose, 1-O-methyl-α-galactopyranoside, 1-O-methyl-β-galactopyranoside, 1-O-methyl-β-D-glucopyranoside, 1-O-methyl-α-D-glucopyranoside, 3-O-methyl-D-glucopyranose, mucate, palatinose, 3-phenylpropionate, protocatechuate, quinate, D-raffinose, α-L-rhamnose, D-saccharate, D-sorbitol, L-sorbose, sucrose, D-tagatose, D- and L-tartrate, *meso*-tartrate, D-trehalose, tricarballylate, trigonelline, tryptamine, L-tryptophan, D-turanose, xylitol, and D-xylose. The major cellular fatty acid components (ranges [percent of total] are given in parentheses) are 14:0 iso (8.3 to 9.6%); 14:0 (1.8 to 2.0%); 15:0 iso (48.3 to 48.9%); 15:0 anteiso (1.3%); 15:1 ω6c (0.8 to 1.0%); 15:0 (2.3 to 3.3%); 16:1 ω7c alcohol (1.4%); 16:1 iso H (1.3 to 1.7%); 16:0 iso (6.5 to 6.6%); 16:1 ω11c (6.4 to 6.6%); 16:1 ω5c (1.1 to 1.2%); 16:0 (3.4 to 3.6%); iso 17:1 ω10c (1.7 to 2.2%); 17:0 iso (2.1%); 17:1 ω6c (1.4 to 1.7%); 15:1 iso H and/or 15:1 iso I, and/or 13:0 3-OH (1.3 to 1.6%); 16:1 ω7c and/or 15:0 iso 2-OH (4.8 to 5.0%); and 17:1 iso I and/or 17:1 anteiso B (2.4 to 2.5%). The G+C content ranges from 42.5 to 43.2 mol% (as determined by HPLC [27]).

The type strain is LMG 15427$^{T}$ (= ATCC 9999$^{T}$ = B0270$^{T}$ = NCTC 7096$^{T}$), and this strain has the characteristics of the species described above. It has a G+C content of 42.5 mol%. This strain assimilates glutarate, *myo*-inositol, 2-ketoglutarate, and L-malate.

**Emended description of *Aneurinibacillus thermoaerophilus* comb. nov.** The following characters are in addition to those given above for the genus or are those in which members of this species differ from the characters given for the genus.

Colonies on nutrient agar after 24 h at 55°C are flat, 1 to 10 mm in diameter, irregular in shape, with a tendency to swarm across the surface of the agar, and they are matte, translucent, and creamy greyish in appearance, becoming whitish and opaque in their centers. Vegetative cells are 1.0 to 1.2 μm by 3.5 to 5.5 μm. Catalase production is variable. Growth temperatures range from 40 to 60°C, and growth occurs from pH 7.0 to 8.0. Hydrolyzes casein, gelatin, and Tween 80, and produces acid from glucose and glycerol. Nitrate is not reduced. The following carbon sources, in addition to those listed in the genus description above, are assimilated in the API Biotype 100 system: *N*-acetyl-D-glucosamine, esculin, L-alanine, 2-ketoglutarate, D- and L-malate, phenylacetate, propionate, quinate, D-ribose, L-sorbose, succinate, L-tartrate, *meso*-tartrate, tricarballylate, and L-tyrosine. Assimilation of *trans*-aconitate, 4-aminobutyrate, D-arabitol, citrate, β-D-fructose, D-galacturonate, α-D-glucose, DL-glycerate, histamine, D-lyxose, D-mannitol, α-D-melibiose, 3-O-methyl-D-glucopyranose, 3-phenylpropionate, L-serine, D-tartrate, trigonelline, D-turanose, and D-xylose varies among strains. The following carbon sources are not assimilated: *cis*-aconitate, adonitol, L-arabinose, L-arabitol, benzoate, betain, caproate, caprylate, D-cellobiose, *m*-coumarate, dulcitol, *i*-erythritol, α-L-fucose, D-galactose, β-gentiobiose, gentisate, D-gluconate, D-glucosamine, D-glucuronate, glutarate, L-histidine, 3-hydroxybenzoate, 4-hydroxybenzoate, hydroxyquinoline-β-glucuronide, *myo*-inositol, itaconate, 2-keto-D-gluconate, 5-keto-D-gluconate, α-lactose, lactulose, malonate, maltitol, maltose, maltotriose, D-mannose, D-melezitose, 1-O-methyl-α-galactopyranoside, 1-O-methyl-β-galactopyranoside, 1-O-methyl-β-D-glucopyranoside, 1-O-methyl-α-D-glucopyranoside, mucate, palatinose, protocatechuate, D-raffinose, α-L-rhamnose, D-saccharate, D-sorbitol, sucrose, D-tagatose, D-trehalose, tryptamine, L-tryptophan, and xylitol. The major cellular fatty acid components (ranges [percent of total] are given in parentheses) are 15:0 iso (50.3 to 66.8%), 15:0 anteiso (10.3%; may be absent), 15:0 (0.9 to 3.4%), 16:0 iso (3.5 to 4.1%), 16:0 (1.8 to 2.5%), 17:0 iso (23.4 to 23.8%), and 17:0 anteiso (8.3%; may be absent). The G+C content ranges from 46.3 to 46.7 mol% (as determined by HPLC [16]).

The type strain is LMG 17165$^{T}$ (= DSM 10154$^{T}$), and this strain has the characteristics of the species described above. It has a G+C content of 46.7 mol% and assimilates D-arabitol, D-galacturonate, α-D-glucose, DL-glycerate, histamine, D-lyxose, D-mannitol, 3-0-methyl-D-glucopyranose, D-tartrate, D-turanose, and D-xylose.

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