Phylogenetic Interrelationships of Round-Spore-Forming Bacilli Containing Cell Walls Based on Lysine and the Non-Spore-Forming Genera *Caryophanon*, *Exiguobacterium*, *Kurthia*, and *Planococcus*

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The 16S rRNA gene sequences of “*Bacillus aminovorans*” and several species considered to be phylogenetically related to the group 2 bacilli of Ash et al. (C. Ash, J. A. E. Farrow, S. Wallbanks, and M. D. Collins, *Lett. Appl. Microbiol.* 13:202–206, 1991) were determined. A comparative analysis of the sequence data revealed that the round-spore-forming group 2 bacilli, together with some asporogenous taxa (the genera *Caryophanon*, *Exiguobacterium*, *Kurthia*, *Planococcus*), form a phylogenetically distinct cluster that is only remotely related to *Bacillus subtilis*, the type species of the genus *Bacillus*. Within this cluster, planococci, kurthiae, *Caryophanon* spp., and two lines defined by *Bacillus sphaericus* and *Bacillus fusiformis* and by *Sporosarcina ureae*, *Bacillus pasteurii*, *Bacillus globisporus*, and *Bacillus psychrophilus* were found to be distinct genera. *Exiguobacterium aurantiacum* and *Brevibacterium acetylicum* were found to form a distinct clade, which was peripherally related to this cluster. “*B. aminovorans*” exhibited no specific relationship with the group 2 bacilli or with any of the other reference species examined.

It has been recognized for a long time that the genus *Bacillus* is phenotypically extremely diverse (5). Small-subunit 16S rRNA cataloging (6, 17, 33, 34) and, more recently, sequencing studies (2, 12, 29) have also revealed very considerable phylogenetic heterogeneity within the genus. On the basis of the results of comparative analyses of almost complete 16S rRNA sequences, the genus *Bacillus* has been shown to comprise a minimum of six phylogenetic groups (2). The thermophilic species *Bacillus cycloheptanicus*, *Bacillus acidocaldarius*, and *Bacillus acidoterrestris* have recently been transferred to a new genus, *Alicyclobacillus* (36), and the remainder of the genus *Bacillus* is clearly in need of taxonomic revision.

All of the *Bacillus* species included in the second phylogenetic group of Ash et al. (2), designated the group 2 bacilli, have round spores and a murein based on L-lysine or D-ornithine (33). Members of this group have been shown to be phylogenetically distinct from *Bacillus subtilis*, the type species of the genus, and from all other meso-diaminopimelic acid-containing bacilli examined to date. 16S rRNA oligonucleotide cataloging studies (17, 33, 34) have revealed that the genus *Sporosarcina* (17, 33) and several asporogenous taxa (including the genera *Planococcus* [33] and *Caryophanon* [33]) are phylogenetically related to these round-spore-forming bacilli. Within the overall group, cataloging also revealed additional specific relationships between members of these genera and particular *Bacillus* species (for example, between *Sporosarcina ureae* and *Bacillus pasteurii* and between *Planococcus citreus* and “*Bacillus aminovorans*”) (33). The relationship between *S. ureae* and *Bacillus pasteurii* (17, 33) has been confirmed by the results of sequencing studies (2, 12).

Numerous other taxa exhibit some of the phenotypic traits of the group 2 bacilli. Species of the genus *Kurthia* contain cell walls based on L-lysine (3, 32) and possess similar respiratory quinones (8). Cataloging studies (33), however,

have failed to reveal any close phylogenetic affinity with the group 2 bacilli and their relatives. The alkalophilic bacterium *Exiguobacterium aurantiacum* (10) also has several chemotaxonomic features in common with the group 2 bacilli, but this organism was considered to be sufficiently distinct to warrant a separate genus. It has been suggested (9, 20) that *Brevibacterium acetylicum* may be related to the genus *Exiguobacterium* rather than to *Brevibacterium linens*. The phylogenetic position of the genus *Exiguobacterium* and *Brevibacterium acetylicum* within the gram-positive bacteria has not been investigated.

It is now clear that the species that are included in the group 2 bacilli are only distantly related to *Bacillus subtilis* (2, 12). Although the group 2 bacilli and their relatives have been quite extensively studied by using oligonucleotide cataloging (6, 17, 33, 34), it is now recognized that this technique is not as accurate as full sequence analysis for elucidating precise phylogenetic groups and branching orders (37). It is therefore essential that the detailed interrelationships of the group 2 bacilli and possibly related taxa be resolved with confidence before proposals regarding the taxonomic status of members of this group are made.

In this paper we describe the 16S rRNA gene sequences of “*Bacillus aminovorans*” and six asporogenous species related to the group 2 bacilli. In addition, the results of a comprehensive analysis of the genealogical structure of the group 2 *Bacillus* cluster are described.

MATERIALS AND METHODS

Strains, media, and growth conditions. “*Bacillus aminovorans*” NCIMB 8292^T (T = type strain), *Exiguobacterium aurantiacum* NCIMB 11978^T, *Kurthia zopfii* NCIMB 9878^T and *Kurthia gibsonii* NCIMB 9758^T were grown on nutrient agar no. 2 (25 g/liter; catalog no. CM67; Oxoid). *Brevibacterium acetylicum* NCIMB 9889^T was grown on coryneform agar, which contained (per liter of distilled water) 10 g of a tryptic digest of casein, 5 g of yeast extract, 5 g of D-glucose, 5 g of NaCl, and 15 g of agar (pH 7.2). *Caryophanon latum*

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FIG. 1. NJ tree for group 2 bacilli and reference strains. Abbreviations: *B. anthracis* 1*, *Bacillus anthracis*, *Bacillus cereus*, “*Bacillus medusa*,” *Bacillus mycoides*, and *Bacillus thuringiensis*; *B. subtilis* 2*, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, *Bacillus atrophaeus*, *Bacillus lautus*, *Bacillus lentimorbus*, *Bacillus licheniformis*, *Bacillus popilliae*, and *Bacillus pumilus*; *B. megaterium* 3*, *Bacillus fastidiosus*, “*Bacillus maroccanus*,” *Bacillus megaterium*, *Bacillus psychrosaccharolyticus*, and *Bacillus simplex*; *B. circulans* 4*, *Bacillus benzo-evorans*, *Bacillus circulans*, *Bacillus firmus*, and *Bacillus lentus*; *B. coagulans* 5*, *Bacillus coagulans* and *Bacillus methanolicus*; *B.*, *Bacillus*; *S.*, *Sporosarcina*; *K.*, *Kurthia*; *Ca.*, *Caryophanon*; *D.*, *Desulfotomaculum*; *M.*, *Marinococcus*; *E.*, *Exiguobacterium*; *Br.*, *Brevibacterium*; *P.*, *Planococcus*.

NCIMB 9533^T and *Caryophanon tenue* NCDO 2324^T were grown on caryophanon agar, which contained (per liter of 10 mM Tris-HCl buffer [pH 7.8]) 2 g of a tryptic digest of casein, 2 g of yeast extract, 2 g of soya peptone, 1 g of sodium

acetate · 3H₂O, 1 g of K₂HPO₄, 0.27 g of MgSO₄ · 7H₂O, 0.1 g of sodium glutamate, 0.2 mg of thiamine-HCl, and 0.05 mg of biotin. All strains were cultivated at 25°C.

Extraction of DNA and determination of rRNA gene se-

TABLE 1. Mean levels of sequence similarity of *Caryophanon* spp., *Kurthia* spp., *Exiguobacterium* spp., and "*Bacillus aminovorans*" to representative species of *Bacillus* rRNA groups

Species	% Similarity to:			
	<i>Caryophanon</i> spp.	" <i>Bacillus</i> <i>aminovorans</i> "	<i>Kurthia</i> spp.	<i>Exiguobacterium</i> spp.
<i>Bacillus subtilis</i>	91.1	91.9	89.5	90.0
<i>Bacillus megaterium</i>	91.9	94.6	90.4	90.6
<i>Bacillus badius</i>	93.0	95.7	90.7	90.9
<i>Bacillus pasteurii</i>	93.2	94.1	92.5	90.9
<i>Bacillus sphaericus</i>	96.3	93.3	92.6	89.7
<i>Planococcus citreus</i>	93.4	94.0	91.3	91.6
<i>Bacillus alvei</i>	88.6	88.8	86.1	87.8
<i>Bacillus brevis</i>	89.7	89.5	88.8	88.6
<i>Bacillus stearothermophilus</i>	89.6	91.0	87.9	88.7
<i>Alicyclobacillus cycloheptanicus</i>	83.1	84.4	81.1	82.5

quence. DNA was extracted from cells in the mid-logarithmic growth phase and was purified by the method of Lawson et al. (26). 16S rDNA fragments were generated by PCR (30) amplification by using *Taq* polymerase as previously described (19). The amplified product was purified by using a GENE-CLEAN II kit (Bio 101, Inc., La Jolla, Calif.) and was directly sequenced by using a Sequenase kit (USB Corp., Cleveland, Ohio) (19).

Analysis of sequence data. A total of 86 sequences (2, 12, 29, 36) were aligned by using programs in the Wisconsin molecular biology package (11) and were analyzed by using the Fitch-Margoliash (FM) distance-matrix (DM) method (16) and the neighbor-joining (NJ) method of Saitou and Nei (31). Newly determined sequences and *Bacillus* group 2 sequences (2) were analyzed by using the FM and NJ methods, the bootstrapped (14) DM method with Kimura's (22) parameters, and parsimony (plain, bootstrapped, and branch and bound [14, 27]) methods, using the FITCH, NEIGHBOR, SEQBOOT, DNADIST, CONSENSE, DNAPARS, DNABOOT, and DNAPENNY programs, respectively, of Felsenstein's PHYLIP program package (15). Where appropriate, global rearrangement and random input order options were used. Uncertainties in relationships were assessed by using a reduced data set along with the maximum likelihood method (ML) (13) and the invariance tests of Lake (25) and Cavender (4) contained in the DNAML and DNAINVAR programs of Felsenstein (15). A statistical analysis of the DNAINVAR data was performed by using the formulae of Lake (25) adapted for the Minitab statistical package (Minitab, Inc.).

Nucleotide sequence accession numbers. The sequences which we determined have been deposited in the EMBL data library under the following accession numbers: "*Bacillus aminovorans*" NCIMB 8292^T, X62178; *Brevibacterium acetylicum* NCIMB 9889^T, X70313; *C. latum* NCIMB 9533^T, X70314; *C. tenue* NCDO 2324^T, X70315; *Exiguobacterium aurantiacum* NCIMB 11798^T, X70316; *K. gibsonii* NCIMB 9758^T, X70320; and *K. zopfii* NCIMB 9878^T, X70321.

RESULTS

The almost complete 16S rRNA gene sequences of "*Bacillus aminovorans*," *Brevibacterium acetylicum*, *C. latum*,

C. tenue, *Exiguobacterium aurantiacum*, *K. gibsonii*, and *K. zopfii* were determined by PCR direct sequencing (19). These new sequences were aligned and compared with a data set consisting of 79 sequences, including the sequences of 53 *Bacillus* species and 26 members of other low-G+C-content gram-positive genera. A matrix of similarity values was calculated and subjected to FM and NJ analyses. All of the new strains examined were related to the group 2 bacilli (sensu Ash et al. (2) (Fig. 1). Both *Caryophanon* species were recovered deep within the group 2 bacilli and branched from a common line of descent leading to *Bacillus sphaericus* and *Bacillus fusiformis*. *Exiguobacterium aurantiacum* and *Brevibacterium acetylicum* were recovered together at the periphery of the cluster consisting of group 2 species. The *Kurthia* species were recovered well within the confines of the group 2 *Bacillus* cluster. Other analyses (data not shown) loosely grouped the *Kurthia* species with *Exiguobacterium aurantiacum* and *Brevibacterium acetylicum*. Using the NJ method, we recovered *Bacillus badius* and "*Bacillus aminovorans*" as a clade adjacent to group 2, but the FM analysis placed "*Bacillus aminovorans*" adjacent to the planococci, with *Bacillus badius* removed to the edge of the *Bacillus* group 1 cluster. FM and NJ analyses performed with a reduced data set that included all of the strains closely related to the group 2 bacilli, *Sporosarcina halophila*, and representative strains belonging to the other main *Bacillus* groups described by Ash et al. (2) generally clustered species in the same order in which they appeared when the large data set was used. Augmented FM and NJ analyses (13, 21, 22) produced essentially the same trees, with minor differences in branch lengths (Fig. 2). The mean homology values for the new species compared with representative species belonging to the major phylogenetic groups of Ash et al. (2) are shown in Table 1. The results of bootstrapping the augmented distances from the Kimura (22) model, followed by consensus analysis, supported all of the major groups outlined above (Fig. 2). However, the clades *Exiguobacterium-Kurthia*, *Bacillus badius*-"*Bacillus aminovorans*," and *Bacillus insolitus*-*S. ureae* clades were shown to be unstable and were supported in <70% of the bootstrapped trees.

The results of the parsimony analysis are shown in Fig. 3. All of the major groups obtained with the DM analysis were

FIG. 2. Distance matrix trees for group 2 bacilli. (a) Nonaugmented FM analysis. (b) augmented (Kimura) NJ analysis showing the percentages of occurrence of branches in bootstrapped trees. A., *Alicyclobacillus*. For an explanation of the other abbreviations see the legend to Fig. 1.

(a)

(b)

FIG. 3. Parsimony tree showing the variable branching positions of "*Bacillus aminovorans*" and *Bacillus badius*, along with the percentages of occurrence of branches in bootstrapped trees. Branch lengths were calculated by the DM method from the tree file. For an explanation of the abbreviations see the legends to Fig. 1 and 2.

supported by parsimony data, although some of the branching orders were altered. *Bacillus badius* and "*Bacillus aminovorans*" were recovered as separate lines of descent that were distant from the group 2 bacilli and appeared as a clade in only 39% of the bootstrapped trees. The *Exiguobacterium* clade was adjacent to the group 2 bacilli, together with the genus *Kurthia*, in agreement with the results obtained from branch and bound parsimony analysis (27), which also separated *Bacillus badius* and "*Bacillus aminovorans*" into adjacent lines of descent (data not shown). The results of a bootstrap analysis (14) of the parsimony data supported the following monophyletic groups at a confidence level of 95%: *Planococcus* spp.; *Kurthia* spp.; *Exiguobacterium aurantiacum* and *Brevibacterium acetylicum*; *Bacillus psychrophilus* and *Bacillus globisporus*; *Bacillus sphaericus*

and *Bacillus fusiformis*; and *Caryophanon* spp. The *Bacillus sphaericus*-*Bacillus fusiformis* clade and the *Caryophanon* group occurred in 89% of the bootstrapped parsimony trees. The group that included *S. ureae*, *Bacillus pasteurii*, *Bacillus psychrophilus*, and *Bacillus globisporus* was supported at a confidence level of 87%. *Bacillus insolitus* was attached to this group ca. 50% of the time, indicating an unstable relationship. The association between *Kurthia* spp. and the *Exiguobacterium aurantiacum*-*Brevibacterium acetylicum* group was also supported in ca. 60% of the trees.

A maximum likelihood (13) analysis of the data set used for invariance tests also moved *Bacillus insolitus* to a branching point intermediate between the *S. ureae* group of strains and the *Caryophanon*-*Bacillus sphaericus* line. *Bacillus badius* and "*Bacillus aminovorans*" were split by *P.*

TABLE 2. Invariance analysis for group 2 bacilli and species that may be related to the group 2 bacilli

Species pair	Results of:				
	Parsimony analysis	Background analysis	Chi-square analysis ^a	Significance analysis (%)	L-invariant analysis
<i>Caryophanon latum</i> - <i>Caryophanon tenue</i>	14	2	9.01	99.7	-3,310
<i>Bacillus sphaericus</i> - <i>Bacillus fusiformis</i>	10	1	8.02	99.6	-6,792
<i>Planococcus citreus</i> - <i>Planococcus kocurii</i>	12	2	6.22	98.4	-7,957
<i>Exiguobacterium aurantiacum</i> - <i>Brevibacterium acetylicum</i>	14	5	4.38	96.1	-28,440
<i>Caryophanon tenue</i> - <i>Bacillus sphaericus</i>	10	3	3.21	92.2	-24,519
<i>Bacillus globisporus</i> - <i>Bacillus insolitus</i>	3	0	2.21	85.4	-25,435
<i>Bacillus globisporus</i> - <i>Bacillus pasteurii</i>	5	1	2.06	83.9	-16,759
<i>Bacillus globisporus</i> - <i>Sporosarcina ureae</i>	4	1	1.79	81.4	-21,070
<i>Bacillus insolitus</i> - <i>Bacillus pasteurii</i>	3	0	1.64	79.9	-23,919
<i>Bacillus insolitus</i> - <i>Sporosarcina ureae</i>	3	1	1.13	71.1	-26,674
<i>Bacillus pasteurii</i> - <i>Sporosarcina ureae</i>	4	1	1.53	78.1	-21,056
<i>Bacillus badius</i> -" <i>Bacillus aminovorans</i> "	4	2	1.34	74.6	-29,384
<i>Sporosarcina ureae</i> - <i>Sporosarcina halophila</i>	2	1	0.05	17.1	-41,876
" <i>Bacillus aminovorans</i> "- <i>Bacillus subtilis</i>	5	2	0.82	73.1	-44,204
<i>Bacillus badius</i> - <i>Bacillus subtilis</i>	4	2	0.91	75.4	-32,272
<i>Bacillus megaterium</i> - <i>Bacillus subtilis</i>	8	3	1.83	81.8	-28,792

^a Chi-square analysis was performed with 1 degree of freedom.

citreus, with *Bacillus badius* closer to the group 1 bacilli (data not shown). The results of invariance tests (4, 25) supported the following relationships at a confidence level of 95%: *Exiguobacterium aurantiacum* and *Brevibacterium acetylicum*; *Planococcus* spp.; *Caryophanon* spp.; and *Bacillus sphaericus* and *Bacillus fusiformis*. The relationship between *Caryophanon* spp. and the *Bacillus sphaericus*-*Bacillus fusiformis* group was assessed at a level of significance of ca. 92%. Cavender's L invariants also supported the hypothesis that there is a relationship between *Bacillus psychrophilus* and *Bacillus globisporus*. No other relationships were found to be statistically significant. Details of the results of the invariance analyses performed with group 2 strains are shown in Table 2.

DISCUSSION

16S rRNA cataloging (6, 17, 33, 34) and sequencing (2, 12, 29, 35) studies have done much to clarify the phylogenetic divisions within the genus *Bacillus* and the relationships of these divisions to evolutionarily closely related genera. It is now clear that the concept of the genus *Bacillus*, as currently defined (5), is a phylogenetically meaningless concept and that taxonomic revision and redefinition are urgently needed (2, 33). The changes in the taxonomy of this group began with the removal of the extremely thermophilic, acidophilic bacilli to the new genus *Alicyclobacillus* (36). This was, however, a relatively easy step to take, given the enormous phylogenetic distances between these organisms and *Bacillus subtilis* (Fig. 1). The round-spore-forming bacilli and their asporogenous relatives (the group 2 bacilli) (2, 12) also form a separate taxon when phylogenetic analyses are performed (2, 12; this paper). This group encompasses an evolutionary distance similar to that defined by the genus *Alicyclobacillus* (Fig. 1), and all of the members of the group 2 *Bacillus* cluster have been consistently shown to be phylogenetically distant from *Bacillus subtilis* (Table 1), the type species of the genus *Bacillus*. Indeed, in terms of evolutionary distance values, many asporogenous genera, such as the genera *Brochothrix*, *Carnobacterium*, *Enterococcus*, and *Staphylococcus*, are as closely related to *Bacil-*

lus subtilis as the group 2 bacilli and their relatives are. The concept of retaining the definition of the genus *Bacillus* and its species for the sake of ease of classification and identification while the related asporogenous genera are excluded is flawed by the current vague definition of the genus that it seeks to protect, by the impossibility of redefining the genus *Bacillus* in clear, meaningful terms, and by the phylogenetic evidence. Is it possible, for example, to consider *Bacillus pasteurii* a genuine member of the genus *Bacillus*, while *S. ureae*, its closest phylogenetic relative, is not a member of this genus or to consider *Bacillus alvei*, which is very remote phylogenetically, a member of the genus *Bacillus*, while *Planococcus* or *Staphylococcus* species are not (Fig. 1)? The simplest and most phylogenetically consistent policy would be to exclude the group 2 bacilli from the genus *Bacillus*. This would remove some of the phenotypic inconsistencies, such as murein types and spore shapes, from the definition of the genus *Bacillus*, together with some problematic species. Sufficient evidence has now accumulated to justify this removal. The phylogenetic status of the taxon that includes the group 2 bacilli needs to be resolved to clarify whether it represents a single genus or a group of closely related genera.

All of the phylogenetic analyses revealed considerable internal structure within the overall group comprising the group 2 bacilli. Several of the subdivisions were stable in all of the analyses, including the bootstrapping analysis. *Exiguobacterium aurantiacum* and *Brevibacterium acetylicum* always clustered together, sometimes as a separate clade (Fig. 1 [ML]) and sometimes loosely associated with the genus *Kurthia* (Fig. 3) but always at the boundaries of or outside the cluster that included the group 2 bacilli. Our data support the validity of the genus *Exiguobacterium* as the separate genus proposed by Collins et al. (10) within the overall radiation of genera associated with *Bacillus sensu lato* (Fig. 1) and are consistent with the suggested close affinity between *Brevibacterium acetylicum* and *Exiguobacterium aurantiacum* (9, 20). The phylogenetic distance between *Brevibacterium acetylicum* and *Exiguobacterium aurantiacum* is comparable to that between *Enterococcus faecalis* and *Enterococcus cecorum* (level of similarity,

94.5%) or between *Lactococcus lactis* and *Lactococcus garvieae* (7), which supports the single genus theory. The invariance analysis indicated that there is a significant relationship between *Exiguobacterium aurantiacum* and *Brevibacterium acetylicum*, but that this relationship is not as close as the relationships between the members of established genera, such as the genera *Caryophanon* and *Planococcus*. On the basis of the results of the phylogenetic analysis and the high levels of chemotaxonomic similarity (9) of these organisms, we propose that *Brevibacterium acetylicum* should be reclassified in the genus *Exiguobacterium* as *Exiguobacterium acetylicum* comb. nov. The two *Kurthia* species clustered at the periphery of the group 2 bacilli in all of our analyses, frequently adjacent to the *Caryophanon-Bacillus sphaericus* branch. An apparent relationship to the genus *Exiguobacterium*, another peripheral group, was not supported by the bootstrap analysis results. The results of the invariance analysis (Table 2) (4, 25) did not support the hypothesis that there is a relationship between the genus *Exiguobacterium* and the genus *Kurthia* or the hypothesis that these genera are related to *Bacillus subtilis* or representatives of the group 2 bacilli. Thus, the genus *Kurthia* represents a distinct, somewhat distant line of descent within the overall phylogenetic structure of the group 2 bacilli (Fig. 1).

Stackebrandt et al. (33) found close relationships between *P. citreus* and "*Bacillus aminovorans*" (similarity coefficient, 0.75) and between *Bacillus insolitus* and "*Bacillus aminovorans*" (similarity coefficient, 0.73) by using 16S rRNA oligonucleotide cataloging. We were unable to confirm the second relationship in any of our analyses. However, we were able to confirm the first relationship in the absence of *Bacillus badius* (a peripheral member of the group 1 bacilli) (2) by using FM analysis (data not shown) and more complete sequence data. All of the analyses placed "*Bacillus aminovorans*" and the planococci on separate lines of descent (Fig. 3). The association between *Bacillus badius* and "*Bacillus aminovorans*" was supported approximately 60% of the time by the results of the bootstrapped NJ analysis, was not supported by the results of the FM analysis, and was supported 40% of the time by the results of the bootstrapped parsimony analysis, indicating that the relationship is unstable. Although these species apparently exhibit high levels of sequence homology to the group 2 bacilli (Table 1), treeing analyses always recovered them at the boundaries of, or outside, this latter group. The results of invariance tests did not support relationships of these species to the representative group 2 *Bacillus* species, to *Bacillus subtilis*, or to each other. The presence of meso-diaminopimelic acid in the cell wall mureins of *Bacillus badius* and "*Bacillus aminovorans*" (33), rather than the L-lysine or D-ornithine of the true group 2 bacilli, suggests these species are not members of this group. *Bacillus badius* and "*Bacillus aminovorans*" are clearly not related closely enough to *Bacillus subtilis* (Tables 1 and 2) to be considered true bacilli (2; this paper), nor are they related closely enough to each other to form a single, stable line of descent in phylogenetic analyses. Further studies will be necessary to determine whether these species are indeed truly phylogenetically peripheral to the genus *Bacillus* and warrant separate genera.

Within group 2 proper, four groups centered on the genus *Planococcus*, *S. ureae*, the genus *Caryophanon*, and *Bacillus sphaericus* were recovered in all analyses. These groups were considered to be members of a single cluster in the cataloging study of Stackebrandt et al. (33). With the excep-

tion of *Bacillus insolitus* attached to the *S. ureae* group, the clusters are supported by the results of the bootstrap analysis at a minimum level of 83%. While these four groups could be considered members of a single genus, there are several arguments against this concept. First, the mean homology data shown in Table 1 indicate that these four groups are not particularly closely related to each other. Fox et al. (18) and Kita-Tsukamoto et al. (23) have suggested boundary criteria for families and genera based on sequence homology data. Our four groups could be considered beyond the boundary for species belonging to the same genus according to these criteria, with the exception of the *Caryophanon-Bacillus sphaericus-Bacillus fusiformis* line of descent (Table 1). Second, although the branching positions of the four groups may change with respect to the main evolutionary line, according to the analysis used, internally they are stable and supported by the results of bootstrapping analysis. The branching order of the *S. ureae* group is itself stable to analysis (Fig. 1 through 3 [ML]). Third, the results of invariance analysis do not support very close relationships between the groups (Table 2). A single genus based on these four groups would not, in our opinion, represent much taxonomic progress. There would still be the problem of reconciling gross morphological, physiological, and biochemical differences with the known phylogeny, albeit on a smaller, more manageable scale. We believe that each of the four groups is sufficiently distinct to warrant a separate genus.

S. ureae, *Bacillus pasteurii*, *Bacillus psychrophilus*, and *Bacillus globisporus* clustered together in all of the analyses and formed a stable line of descent, supported by bootstrapping data, on the main tree. The branching order within this line of descent was identical in all analyses. This stability could be used as evidence for assigning these species to a single redefined genus, the genus *Sporosarcina*. *Bacillus insolitus* was usually associated with this group, but often it was in an adjacent, rather than internal, position (Fig. 2 and 3 [ML]). However, the results of invariance analyses indicate that *S. ureae*, *Bacillus pasteurii*, *Bacillus psychrophilus*, and *Bacillus insolitus* are not particularly closely related. This is perhaps surprising, given the stability of the line of descent as determined by DM and other analyses and the relatively short genetic distances between the species. The valid species *S. halophila* (Claus et al.) is phylogenetically distant from *S. ureae* (12) (Fig. 1 through 3 and Table 2). *S. halophila* is not sufficiently close to *Bacillus subtilis* to warrant transfer to the genus *Bacillus* sensu stricto (Table 2), nor is it related to any other known genus in the overall radiation of gram-positive bacteria examined to date (12) (Fig. 1). Krych et al. (24) showed that *Bacillus sphaericus* contains six DNA-DNA homology groups and that *Bacillus sphaericus*, as exemplified by the type strain, exhibited the lowest levels of homology to the other five groups. DNA homology group 2b (24) corresponded to *Bacillus sphaericus* subsp. *fusiformis*, and this taxon was elevated to species status by Priest et al. (28). No proposals for separate specific status have been made so far for the other homology groups, although a strain described as "*Bacillus rotans*" was recovered in DNA homology group III (24) and strains pathogenic for mosquito larvae also appear to form a distinct group (1). Judging from the data of Krych et al. (24), it is probable that all six of the homology groups are members of the genus centered on *Bacillus sphaericus*. The genus *Caryophanon* (Peshkoff) and the *Bacillus sphaericus* group share a common departure point from the main tree, and this relationship was apparent in all of our analyses except the invariance

analysis. However, these taxa are sufficiently distinct from each other to warrant separate generic status for the two groups. The revival of the species *C. tenue* (Trentini) was justified. In this study, *C. tenue* was found to be closely related to, but distinct from, *C. latum*. Both *Planococcus* species were significantly related as determined by all analyses and were distinct from the other groups. "*Bacillus aminovorans*" was not found to be related to the planococci. The separate status of the genus *Planococcus* should therefore be retained.

Bacillus rRNA group 2 has been shown to be stable to phylogenetic analysis (2, 12; this paper) and to consist of a cluster of related genera. Therefore, it seems likely that this group should be given the phylogenetic status of a family.

Taxonomic proposal. We propose that *Brevibacterium acetylicum* should be transferred to the genus *Exiguobacterium* as *Exiguobacterium acetylicum* (Levine and Soppel-and) comb. nov. A full description of *Exiguobacterium acetylicum* is given in reference 20. The type strain of *Exiguobacterium acetylicum* is ATCC 953.

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Robert Anderson of the Institute of Food Research devised the computer program used for automating the invariance analysis.

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