Comparative Sequence Analyses on the 16S rRNA (rDNA) of Bacillus acidocaldarius, Bacillus acidoterrestris, and Bacillus cycloheptanicus and Proposal for Creation of a New Genus, Alicyclobacillus gen. nov.

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Comparative 16S rRNA (rDNA) sequence analyses performed on the thermophilic Bacillus species Bacillus acidocaldarius, Bacillus acidoterrestris, and Bacillus cycloheptanicus revealed that these organisms are sufficiently different from the traditional Bacillus species to warrant reclassification in a new genus, Alicyclobacillus gen. nov. An analysis of 16S rRNA sequences established that these three thermoacidophiles cluster in a group that differs markedly from both the obligately thermophilic organism Bacillus steaethermophilus and the facultatively thermophilic organism Bacillus coagulans, as well as many other common mesophilic and thermophilic Bacillus species. The thermoacidophilic Bacillus species B. acidocaldarius, B. acidoterrestris, and B. cycloheptanicus also are unique in that they possess ω-alicyclic fatty acid as the major natural membranous lipid component, which is a rare phenotype that has not been found in any other Bacillus species characterized to date. This phenotype, along with the 16S rRNA sequence data, suggests that these thermoacidophiles are biochemically and genetically unique and supports the proposal that they should be reclassified in the new genus Alicyclobacillus.

Phenotypically, the genus Bacillus is a large and heterogeneous collection of aerobic, rod-shaped, gram-positive (to gram-variable), endospore-forming bacteria (19, 20, 34). The diversity that exists in this genus is demonstrated by the enormous range of genomic guanine-plus-cytosine contents (32 to 69 mol%), as well as the variety of interesting phenotypes, that are found in the various Bacillus species (7, 19, 27, 30). These phenotypes include (but are not limited to) the ability to fix molecular nitrogen and growth under extreme conditions, including growth in thermophilic, psychrophilic, acidophilic, alkalophilic, and halophilic environments, (i.e., hot environments, cold environments, acidic environments, alkaline environments, and environments containing high salt concentrations, respectively) (5, 9, 10, 43). Many Bacillus species also utilize a wide assortment of carbon sources for heterotrophic growth, ranging from methanol to complex natural polymers, such as like chitosan and chitin (34). Even facultatively autotrophic hydrogen-oxidizing sporeformers have been isolated and studied (5). Extensive multiphenotypic and molecular analyses have been used to identify species in this group, as well as to establish the taxonomic relationships among Bacillus species (27, 30–32). However, even the resulting data have not simplified the task of organizing the members of this group into meaningful taxa, and the diversity found among the species is great enough that the members of the genus no doubt should be subdivided into several genera (2, 33, 36). 16S rRNA catalog and subsequent 16S rRNA and rDNA sequence analyses have clearly revealed the presence of at least three main clusters (Fig. 1) in the genus Bacillus (2, 33). In addition, a significant number of Bacillus species are known to fall outside these three clusters. Thus, it is apparent (2) that at least two and probably more additional well-defined groups will eventually be established. Among those bacilli that are not members of the main groups, 16S rRNA catalog data (36) have indicated that one of the taxa that branch most deeply is Bacillus acidocaldarius. The proper taxonomic placement of this obligately acidophilic thermophile, along with two other phenotypically related species (Bacillus acidoterrestris and Bacillus cycloheptanicus), is the focus of this paper. More than 1,400 nucleotides in the 16S rRNA genes of each of these three species were determined. Our data indicate that these thermoacidophilic species should be grouped together in a distinct cluster; these organisms appear to be related, and they are distinctly different from the other species that make up the genus Bacillus. All three species in this cluster, B. acidocaldarius, B. acidoterrestris, and B. cycloheptanicus, contain a unique type of lipid (ω-alicyclic fatty acids) as the major membrane fatty acid component in their cells (11, 12, 23). This lipid has not been found in any other Bacillus species examined to date. Therefore, we propose that this group of three related organisms should be placed in a new genus, Alicyclobacillus gen. nov., in the family Bacillaceae.

A preliminary report of our findings has been published elsewhere (39).

MATERIALS AND METHODS

Bacterial strains and culture conditions. The Bacillus strains used in this study are shown in Table 1. Three strains of B. acidocaldarius (strains DSM 446T [T = type strain], ATCC 43034, and ATCC 43035) were grown and maintained on American Type Culture Collection medium 573 at 65°C. B. acidoterrestris DSM 2923 and B. cycloheptanicus DSM 4006T were grown as described previously (10, 11, 25) and were lyophilized before their nucleic acids were extracted (39).

Amplification and cloning. Chromosomal DNA was pre-
B. acidocaldarius was carried out as previously described (35). The two positions of 16s rRNA sequence data by using the UPGMA method. A very detailed tree showing the highly divergent position of B. cycloheptanicus (and hence by inference the highly divergent positions of B. acidocaldarius and B. acidoterrestris) has been published elsewhere (2).

Table 1. Bacillus strains and sequencing procedures used in this study

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>G+C content of genome (mol%)</th>
<th>G+C content of rRNA (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. acidocaldarius</td>
<td>DSM 446T</td>
<td>60.3</td>
<td>60.8</td>
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<tr>
<td>B. acidocaldarius</td>
<td>ATCC 43034</td>
<td>59.4</td>
<td>59</td>
</tr>
<tr>
<td>B. acidoterrestris</td>
<td>DSM 3923</td>
<td>52.2</td>
<td>60</td>
</tr>
<tr>
<td>B. cycloheptanicus</td>
<td>DSM 4006T</td>
<td>55.6</td>
<td>59</td>
</tr>
</tbody>
</table>

* In addition, B. acidocaldarius ATCC 43035 was also used in this study.
* G+C content as determined by Tm measurements (10).
* 16S rDNA was sequenced directly by using the reverse transcriptase-mediated, primer-directed, deoxyribonucleotide chain-terminating sequencing technique (26).

**RESULTS**

The sequences of 16S rRNA segments totalling more than 1,400 nucleotides each were determined for B. acidocaldarius ATCC 43034 and DSM 446T, B. acidoterrestris DSM 3923, and B. cycloheptanicus DSM 4006T. In addition, several hundred nucleotides of the B. acidocaldarius ATCC 43035 sequence were found to be effectively identical (>99.6%) to the nucleotides of the sequence of the type strain, strain DSM 446T. A comparison of the 16S rRNA sequences of two thermoacidophiles, B. acidocaldarius and B. acidoterrestris (Table 2), showed that they were almost identical (98.8%), whereas the levels of relatedness of these organisms to B. cycloheptanicus were lower (similarity values, 93.2 and 92.7%, respectively). This finding suggests that all three of these organisms are unique species and that B. acidocaldarius and B. acidoterrestris clearly belong to the same genus.

The results described above had to be evaluated in the context of other 16S rRNA sequence data, especially data for the members of the genus Bacillus and other related genera belonging to the family Bacillaceae. To do this, all of the available sequences for the organisms were examined and aligned, and a distance matrix was assembled. The unweighted pair-group method with arithmetic mean, UPGMA, was used to construct dendrograms. A representative subset of the matrix is shown in Table 2, and the associated tree is shown in Fig. 1. Alternative tree-making programs available
TABLE 2. 16S rRNA sequence similarity values for B. acidocaldarius, B. acidoterrestris, B. cycloheptanicus, other Bacillus species, and related species

<table>
<thead>
<tr>
<th>Species</th>
<th>B. subtilis</th>
<th>B. acidocaldarius</th>
<th>B. acidoterrestris</th>
<th>B. cycloheptanicus</th>
<th>B. coagulans</th>
<th>B. steatorrhophilus</th>
<th>B. alvei</th>
<th>B. brevis</th>
<th>Streptococcus cecorum</th>
<th>Lactobacillus lactis</th>
<th>Leuconostoc mesenteroides</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. acidocaldarius</td>
<td>84.4</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>B. acidoterrestris</td>
<td>84.3</td>
<td>98.8</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td>B. cycloheptanicus</td>
<td>85.3</td>
<td>93.2</td>
<td>92.7</td>
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<tr>
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<td>93.4</td>
<td>85.2</td>
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<td></td>
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<tr>
<td>B. steatorrhophilus</td>
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<td>91.3</td>
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<tr>
<td>Streptococcus cecorum</td>
<td>89.6</td>
<td>84.8</td>
<td>84.5</td>
<td>84.5</td>
<td>88.9</td>
<td>88.3</td>
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<tr>
<td>Lactobacillus lactis</td>
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<td>81.8</td>
<td>81.6</td>
<td>82.6</td>
<td>86.0</td>
<td>86.0</td>
<td>86.3</td>
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<tr>
<td>Leuconostoc mesenteroides</td>
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<td>78.9</td>
<td>79.3</td>
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<td>83.5</td>
<td>82.3</td>
<td>84.9</td>
<td>85.8</td>
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<tr>
<td>Clostridium innocuum</td>
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<td>83.0</td>
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<td>81.4</td>
<td>81.3</td>
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</table>

in the PHYLIP package (16) produced trees that were topologically similar to the tree shown in Fig. 1.

The purpose of the dendrogram in Fig. 1 is to illustrate the phylogenetic position of B. acidocaldarius and its thermoa
idophilic relatives compared with three previously established clusters of Bacillus species and other genera in the Bacillaceae. A great deal of data is summarized in Fig. 1. In actuality, partial 16S RNA sequences have been determined for at least 85 Bacillus strains representing almost every recognized species. A comprehensive tree that summarizes much of this information has been published elsewhere (2). At the time that Ash et al. (2) published their tree, B. cycloheptanicus was the single most atypical isolate available. None of the numerous Bacillus strains that were included in the study of Ash et al. was specifically related to B. cycloheptanicus. Indeed, as Fig. 1 shows and the results obtained by examining the secondary structure of 16S rRNAs. A comparative analysis of the secondary structures of B. acidocaldarius, B. acidoterrestris, and B. cycloheptanicus 16S rRNAs revealed several helical regions that differed from the helical regions in other members of the genus Bacillus. Among closely related organisms, it is common to observe no significant differences in secondary structure. Indeed, the structures are typically identical in terms of numbers of pairs in each helix and number of nucleotides in each loop. Even between major taxonomic groups, such as the archaebacteria and the eubacteria, surprisingly few changes occur. Therefore, secondary structure variation suggests considerable phylogenetic diversity. In the case of organisms belonging to the genus Bacillus, no structural variation has been observed in any of the members of the B. subtilis cluster examined to date (1, 22, 33). Thus, it is noteworthy that we observed significant differences compared with B. subtilis in four helical regions in B. acidocaldarius and its relatives. Moreover, in these structurally different areas of their 16S rRNAs the thermoa
idophiles were either identical to one another in structure or at least very similar.

The most drastic structural difference occurred in the helix at positions 65 to 104 (position numbers refer to the equiv-
alent region in the Escherichia coli sequence; helix 6 in the nomenclature of Brimacombe et al. [6]). The obvious change in this region was the loss of the apex helical element (Fig. 2). In both B. cycloheptanicus and B. acidoterrestris (the sequence of this region was not completely determined in B. acidocaldarius) this was the consequence of a 12- to 14-nucleotide deletion event. The abbreviated version of the
Although the complete sequence of *Bacillus* was not determined, our gels suggested that this sequence also too has an abbreviated structure in this region. The remaining changes were more subtle and therefore probably are of primary interest only to connoisseurs of 16S rRNA structure. Examples were found in the region from position 829 to position 857 (helix 26), in the region from position 997 to position 1044 (helix 33a,b,c), and in the region from position 1128 to position 1144 (helix 39b). Helix 26 was fairly typical. This helix was characteristically shorter in the three thermoacidophiles than it was in other Bacillus groups. *B. cycloheptanicus* had 11 pairs in the helix, with an apex loop of six bases, while *B. acidocaldarius* and *B. acidoterrestris* had 10 base pairs and a seven-base loop. In contrast, this helix had 12 base pairs in *B. subtilis*.

These structural considerations confirmed the essential conclusions of the primary sequence analysis. The 16S rRNAs of *B. acidocaldarius*, *B. acidoterrestris*, and *B. cycloheptanicus* specifically resemble one another but are quite distinct from the 16S rRNAs of the typical members of the genus *Bacillus*.

**DISCUSSION**

Over the past two decades several unusual thermoacidophilic *Bacillus* species have been isolated and characterized (namely, *B. acidocaldarius* [9], *B. acidoterrestris* [10], and *B. cycloheptanicus* [11]). Other thermoacidophiles, such as *Bacillus tusciae* (5), have also been isolated, but unlike the other three thermoacidophiles, *B. tusciae* is only moderately acidophilic and does not contain ω-acyclic fatty acids, hopanoids, or saponolipids (29). Instead, the membranous lipids found in *B. tusciae* are branched-chain iso and anteiso fatty acids. In an effort to determine the evolutionary and taxonomic position of *B. acidocaldarius*, *B. acidoterrestris*, and *B. cycloheptanicus*, the sequences of their 16S rRNA genes were determined and compared. This appears to be the most appropriate method for determining taxonomic relationships (17, 41). This is especially true for the classification of organisms that are as physiologically diverse as the organisms in the genus *Bacillus*. While specific growth requirements, such as extreme pH conditions and high (or low) growth temperatures, may interfere with traditional physiological characterizations (20), the 16S rRNA sequencing method is not affected by such environmental influences.

The classically broad criteria used for identifying organisms in the genus *Bacillus* (gram-positive, aerobic, endospore-forming rods) yield a heterogeneous collection of species that are not necessarily related to each other at the genus level (2, 33, 36). An analysis of the 16S rRNA sequences (Fig. 1) clearly demonstrated that *B. acidocaldarius*, *B. acidoterrestris*, and *B. cycloheptanicus* form a cluster that is distinct from other members of the genus *Bacillus*. Several types of distance matrix and tree-generating programs (16) were used to analyze the sequence data, and all yielded similar results in that the *B. acidocaldarius* cluster remained coherent in all cases and always branched more deeply than any of the other groups in the genus *Bacillus*.

The phylogenetic relationship of these three thermoacidophiles to each other is in basic agreement with the phenotypic data reported previously (9–11). As noted previously, two of these thermoacidophiles, *B. acidocaldarius* and *B. acidoterrestris*, seem to be very closely related, and both contain rare fatty acids (namely, ω-cyclohexyl fatty acids), as well as hopanoids. *B. cycloheptanicus* contains an ω-cyclohexyl type of fatty acid, which appears to be a phenotypically related characteristic, yet this fatty acid is chemically distinct from the fatty acids found in the other two thermoacidophiles. The presence of naturally occurring ω-acyclic fatty acids in these three thermoacidophiles makes them unique among the known *Bacillus* species (23). This phenotype has been induced and studied in a *B. subtilis* mutant, strain bfm 49. When this auxotroph was grown in the presence of cyclic fatty acid precursors (14), the formation of ω-acyclic fatty acids was induced so that the fatty acid composition of strain bfm 49 resembled the fatty acid composition found in *B. acidocaldarius* (25). However, strain bfm 49 did not grow thermophilically at 47°C or under acidicophilic conditions at pH 5.0. A similar phenomenon of nutritional induction has been observed in certain hydrocarbon-utilizing coryneform bacteria (namely, the mesophilic *Mycobacterium* and *Nocardia* species). ω-Cyclohexyl fatty acid is formed in these organisms when they are grown in the presence of n-alkyl-substituted cycloparaffins, although normally such lipids are not found in these bacteria. The presence of a naturally occurring ω-cyclohexyl type of fatty acid has been observed in an actinomycete, the nonacidophilic mesophile *Curtobacterium pusillum* (37). By using model membrane systems it has been shown that ω-cyclohexyl fatty acid-containing lipids pack densely, resulting in low diffusion at high temperatures (24). Presumably, this property provides an advantage when cultures are grown at a high temperature and a low pH.

The patterns of variation in the secondary structures of some of the hypervariable regions of the 16S rRNAs offer more support for the hypothesis that *B. acidocaldarius*, *B. acidoterrestris*, and *B. cycloheptanicus* should be differentiated and separated from other members of the genus *Bacil- lus*. 16S rRNA helices 6, 26, and 39 exhibit secondary structure variations that appear to be characteristic of the three thermoacidophiles (Fig. 2). Perhaps most compelling, however (Fig. 1 and Table 2), is the fact that the three thermoacidophiles are no more closely related to other traditional species belonging to the genus *Bacillus* than organisms like *Streptococcus cecorum* and *Lactobacillus lactis* are.

An important phylogenetic question that must be addressed in a study of organisms that exist in extreme or unusual environments is whether these organisms possess an evolutionary “fast clock.” While the constancy of the molecular clock (i.e., the rate of evolution) is fundamental to
the study of evolution at the molecular level, it has been demonstrated that not all organisms do in fact evolve at the same rate (41, 42, 44). Rapidly evolving organisms typically have three basic characteristics. First, fast-clock organisms share relatively low levels of DNA-DNA homology with other members of the same group. Taken alone, this is an arbitrary trait because of the subjective definition of what defines a group. Second, fast-clock organisms have an unusual phenotype (or phenotypes) that may be relevant to some environmental or physiological stress placed upon the organisms. Third, fast-clock organisms typically have a tendency to change genotypically (on a global level) in chronometric molecules, such as 16S rRNA (44). This tendency can be measured by examining the rate of change in the regions of 16S rRNA that consist of highly conserved sequences. It is expected that highly variable regions of 16S rRNA exhibit high degrees of sequence divergence in even closely related organisms. A genotypic indication of a fast-clock organism is an elevated rate of change in more conserved regions compared with the rate of change in the more variable regions of the same molecule (44). The extremely acidophilic nature of and the DNA-DNA homology data (9–11) for the thermoacidophiles *B. acidocaldarius, B. acidotewestris, and B. cycloheptanicus* satisfy the first two criteria, and this suggests that these bacteria may indeed be fast-clock organisms. In an effort to determine whether these three thermoacidophiles satisfy all of the properties of fast-clock organisms, we performed sequence analyses similar to those used by Yang and Woese (44). We found that the thermoacidophiles did not evolve any faster than the other members of the genus *Bacillus* as it is currently recognized. Therefore, the distinct and distant 16S rRNA cluster of thermoacidophiles apparently was not entirely due to major differences in the rate of evolution of their 16S rRNAs.

As is typical in bacterial taxonomy, it has been difficult to identify a single phenotypic trait, such as a particular biochemical pathway or the presence of a specific isoprenoid quinone type, that is present exclusively in all members of one *Bacillus* group and absent in members of other groups. Even polygenomic traits, such as spore morphology (i.e., shape, size, position, and swelling), are not satisfactory although they are useful for identification purposes. While most *Bacillus* strains have definite phenotypic characteristics that are useful for species identification, such characteristics tend to invariably cross group boundaries, and to be useful, they have to be considered in conjunction with other taxonomic characteristics. It is entirely possible, but not certain, that eventually workers will identify other phenotypic determinants (or a defined set of phenotypes) that accurately reflect the genotypic relationships indicated by the analysis of 16S rRNA data (2, 33, 36). For the present, it is satisfying that one phenotype (the presence of α-alicyclic fatty acids) supports the separation of the obligate aicyclic thermophilic *Bacillus* species from all other *Bacillus* species.

However, the specific relationship among *B. acidocaldarius, B. acidotewestris*, and *B. cycloheptanicus* appears to be challenged by the previously published observation (2) that *B. cycloheptanicus* and *B. acidotewestris* do not cluster together. The *B. acidotewestris* sequence which we determined differs in more than 250 positions from the sequence published previously by Ash et al. (2), who from ostensibly used the same strain. Such extensive differences cannot be the result of experimental error but must reflect a strain mix-up. It has been communicated to us (8) that the strain maintained by the Deutsche Sammlung von Mikroorganis-

men and subsequently supplied to Ash et al. (2) was indeed incorrect. Therefore, the sequence reported previously (2) as being from *B. acidotewestris* has no bearing on our study. It also might be argued that although our data set was large, it was deficient in its representation of thermophiles, which therefore might also be related to *B. acidocaldarius*. This is in fact not true. The previously published sequences include sequences for strains of *B. coagulans, Bacillus kaustophilus, Bacillus smithii, B. steathermophilus, and Bacillus thermogluscosidase*. Unpublished sequence data available to use included data for additional strains of *B. coagulans, B. smithii*, and *B. steathermophilus*, as well as data for the type strains of *Bacillus caldolyticus, Bacillus thermoleovorans*, and *Thermoactinomyces candidus*.

In view of the evidence presented in this paper, we formally propose that the three thermoacidophilic species previously designated *B. acidocaldarius* (9), *B. acidotewestris* (10), and *B. cycloheptanicus* (11), should be reclassified in a new genus, *Alicyclobacillus* gen. nov., as *Alicyclobacillus acidocaldarius* comb. nov., *Alicyclobacillus acidotewestris* comb. nov., and *Alicyclobacillus cycloheptanicus* comb. nov., respectively.

**Description of Alicyclobacillus gen. nov. **Alicyclobacillus (Al.i.cy.clo.ba.cil’lus. G. adj. aliphos, fat; G.n.kyklo, circle; L. adj. alicyclo, referring to circular fatty acids; G. n. bacillus, small rod; *Alicyclobacillus*, small rods containing α-alicyclic fatty acids). The description below is based on our own observations, as well as on previous descriptions of the obligately acidophilic belonging to this genus species (9–11). Rod-shaped cells that are straight or nearly straight (0.3 to 0.8 by 2.0 to 4.5 μm). Aerobic or facultatively anaerobic. Gram positive or gram variable. Endospores are formed under adverse environmental or nutritional conditions. One endospore per cell. Sporulation is tolerant of oxygen. Growth is obligately acidophilic and occurs at pH 2 to 6. Growth factors may or may not be required. The predominant membrane fatty acids are α-alicyclic fatty acids that contain six- or seven-carbon rings. The main isoprenoid quinone is menaquinone with seven isoprene units (MK-7). Hopanoids may be present; sulfonolipids are present. Growth temperatures range from less than 40 to 70°C. The 16S rRNA molecules of the species in this genus exhibit more than 92% sequence homology to each other. The G+C content of the DNA ranges from 51.6 to 60.3 mol% (as determined by the thermal denaturation method). The type species is *Alicyclobacillus acidocaldarius*; the type strain of this species is strain 104-1A (= ATCC 27009 = DSM 446).

**Description of Alicyclobacillus acidocaldarius** (Darland and Brock 1971) comb. nov. *Alicyclobacillus acidocaldarius* (a.ci.do.cal.dar’i.lus. M.L. n. acidum, acid; L. adj. cal-dar’ius, pertaining to warm or hot; M.L. adj. acidocaldarius, pertaining to acid thermal [habitats]). The description below is based on data from previous studies (9), as well as our own analyses. Aerobic, gram-positive, spore-forming rods that often occur in short chains containing five or six cells; the rods are 2 to 3 μm long and 0.7 to 0.8 μm wide. The sporangia are not swollen by endospores, which are ellipsoidal and located terminally to subterminally. Spores have relatively weak heat resistance (half-time for death at 86°C, 10 to 12 min). Colonies are not pigmented, are typically flat, and have irregular margins. The predominant membrane fatty acids are the ω-cyclohexyl acids; ω-cyclohexylundecanoic and ω-cyclohexyltridecanoic acids are the major components. Hopanoids and sulfonolipids are present (13). The carbon and energy sources utilized include glucose, galactose, glycerol, and Casamino Acids. No growth occurs
when succinate, acetate, sorbitol, citrate, or ethanol is the sole carbon source. Only aerobic growth occurs on media containing nitrate. Growth occurs with ammonia but not with nitrate as a sole nitrogen source. The pH range for growth is 2 to 6. The temperature range for growth is 45 to 70°C. No growth factors are required. 16S rRNA similarity data indicate a close relationship to *Alicyclobacillus acidoterrestris*. The main menaquinone is MK-7.

Sources: acidic thermal terrestrial and aqueous environments.

The G+C contents of DNAs range from 61.2 to 62.2 mol% (as determined by the buoyant density method) for three strains. The type strain is strain 104-1A (= ATCC 27009 = DSM 446). The G+C content of the DNA of the type strain is 60.3 mol% (as determined by the thermal denaturation method) or 62.3 mol% (as determined by the buoyant density method).

Description of *Alicyclobacillus acidoterrestris* (Deinhard et al. 1987) comb. nov. *Alicyclobacillus acidoterrestris* (a.ci.do.ter.rest'ris. L.n. acido, acid; L. adj. terrestris, from the earth; M.L. masc. adj. acidoterrestris, acid loving and isolated from soil). The description below is based on data from previous studies (10) and our own analyses. Aerobic, gram-positive, endospore-forming rods that are 2.9 to 4.5 μm long and 0.6 to 0.8 μm wide. The sporangia are slightly swollen or not swollen; the spores are oval, subterminal to terminal, 1.5 to 1.8 μm long, and 0.9 to 1.0 μm wide. Colonies are round, creamy white, translucent to opaque, and 3 to 5 mm in diameter after 6 days of growth at pH 4.0 and 50°C. No growth factors are required. The temperature range for growth is less than 35°C to more than 55°C; the optimal temperature ranges from 42 to 53°C. The pH range for growth is 4.0 and 5.5. Growth occurs at pH values between 3.0 and 5.5. Growth occurs on media containing fermentable sugars. Acid is formed from the following carbohydrates: α-d-arabinose, ribose, α-d-xylose, galactose, glucose, fructose, mannose, sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, amygdalin, α-d-lyxose, l-fucose, d-arabitol, and 5 keto-gluconate. Esculin is hydrolyzed.

A total of 90% of the fatty acids of the cell membrane are accounted for by ω-cycloheptyldecanoic acid, ω-cycloheptyltridecanoic acid, and ω-cyclohexyl-α-hydroxyundecanoic acid. A sulfonolipid is present. MK-7 is the main menaquinone component, while MK-6 and MK-9 are minor components. 16S rRNA similarity data indicate a moderate relationship to *A. acidocaldarius* and *A. acidoterrestris*. The G+C content of the DNA is 54.0 to 56.9 (as determined by the thermal denaturation method). The type strain is strain SCH (= DSM 4006).

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