Paenibacillus apiarius sp. nov.

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The name "*Bacillus apiarius*" Katznelson 1955 was not included on the Approved Lists of Bacterial Names and thus lost standing in bacterial nomenclature. The genetic homogeneity of "*B. apiarius*" strains was assessed by determining their G+C contents by the buoyant density method and by measuring the levels of DNA relatedness by spectrophotometric reassociation procedures. The G+C contents of the 15 strains examined, ranged from 52 to 54 mol%. DNA reassociation revealed the presence of two clusters, each with high levels of intragroup relatedness (60 to 100%). One cluster consisted of six strains highly related to *Bacillus thiaminolyticus*, and the other consisted of nine strains related to the designated type strain of "*B. apiarius*." The strains in the second cluster were not closely related genetically to the type strains of organisms frequently associated with honey bees (namely, *Paenibacillus alvei*, *Paenibacillus larvae*, *Bacillus laterosporus*, and *Paenibacillus pulvificiens*). The "*B. apiarius*" strains in the second cluster were also phenotypically homogeneous and distinguishable from the previously described species. Comparative analyses of the 16S rRNA gene DNA sequence showed that the proper phylogenetic position of the second cluster was in the genus *Paenibacillus*. These findings justify the proposal of a new species with the name *Paenibacillus apiarius*. The type strain is NRRL NRS-1438.

In 1955, Katznelson (9) described the species "*Bacillus apiarius*" based on two isolates obtained from honeybee larvae. In their studies, Gordon et al. (5) decided that characterizations based on two strains were not dependable and only provisionally recognized this species. "*B. apiarius*" was listed in *Bergey's Manual of Systematic Bacteriology* (3), with the reservation that more work was needed to establish its status as a distinct species. Presumably because the description of the species was based on only a few strains, the name "*B. apiarius*" was excluded from the Approved Lists of Bacterial Names (18) and, consequently, lost standing in bacterial nomenclature.

The unique habitat, biochemical characteristics, and spore morphology of "*B. apiarius*" suggest that it might be a distinct

species. This work was therefore undertaken to determine the taxonomic position of "*B. apiarius*" based on the results of DNA relatedness analyses, 16S rRNA gene DNA sequence determinations, and extensive phenotypic characterization experiments. On the basis of the findings of this study, the new species *Paenibacillus apiarius* is proposed.

MATERIALS AND METHODS

Organisms. Table 1 shows the "*B. apiarius*" strains used in this study. In addition, the following type strains were used: *Paenibacillus alvei* NRRL B-383, *Paenibacillus larvae* NRRL B-2605, *Bacillus laterosporus* NRRL NRS-314, *Paenibacillus pulvificiens* NRRL B-3685, and *Bacillus thiaminolyticus* NRRL B-4156.

TABLE 1. "*B. apiarius*" strains examined in this study

Strain	Designation as received	Source ^a	G+C content (mol%)	History
Cluster 1 strains				
NRRL NRS-1768	NRS-1768	1	54	M. Gilliam; isolated from honeybee larva; " <i>B. apiarius</i> "
NRRL NRS-1769	NRS-1769	1	53	M. Gilliam; isolated from honeybee larva; " <i>B. apiarius</i> "
NRRL NRS-1770	NRS-1770	1	54	M. Gilliam; isolated from honeybee larva; " <i>B. apiarius</i> "
NRRL NRS-1771	NRS-1771	1	53	M. Gilliam; isolated from honeybee larva; " <i>B. apiarius</i> "
NRRL NRS-1772	NRS-1772	1	53	M. Gilliam; isolated from honeybee larva; " <i>B. apiarius</i> "
NRRL NRS-1773	NRS-1773	1	54	M. Gilliam; isolated from honeybee larva; " <i>B. apiarius</i> "
Cluster 2 strains				
NRRL B-3678		2	54	H. Katznelson; " <i>B. apiarius</i> "
NRRL B-4187	8-20-62	3	54	H. Katznelson; isolated from dead honeybee; " <i>B. apiarius</i> "
NRRL B-4188	9-17-71	3	53	H. Katznelson; isolated from dead honeybee; " <i>B. apiarius</i> "
NRRL B-4299	1303	4	53	" <i>B. apiarius</i> "
NRRL B-4303	1304	4		" <i>B. apiarius</i> "
NRRL NRS-1438 ^T	NRS-1438	1	52	H. Katznelson BX3; isolated from honeybee larva; " <i>B. apiarius</i> "
NRRL NRS-1439	NRS-1439	1	52	H. Katznelson BX5; isolated from honeybee larva; " <i>B. apiarius</i> "
NRRL NRS-1577	NRS-1577	1	53	W. C. Haynes; " <i>B. apiarius</i> "
NRRL NRS-1578	NRS-1578	1	52	W. C. Haynes; " <i>B. apiarius</i> "

^a 1, N. R. Smith *Bacillus* collection maintained by R. E. Gordon, Rutgers University, New Brunswick, N.J.; 2, T. A. Gochbauer, Entomology Research Institute, Ottawa, Canada; 3, J. D. Hitchcock, U.S. Honeybee Research Station, Laramie, Wyo.; 4, R. J. Norris, University of Leeds, Leeds, England.

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The Agricultural Research Service Culture Collection (NRRL) at the National Center for Agricultural Utilization Research maintains these strains. Each NRRL designation includes the prefix B-, which designates strains acquired directly from individuals or strains isolated at the National Center for Agricultural Utilization Research, or the prefix NRS-, which designates strains obtained from the *Bacillus* collection of N. R. Smith deposited in the Agricultural Research Service Culture Collection by R. E. Gordon. Working stock cultures were incubated at 28°C on nutrient agar amended with 5 mg of $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ per liter until sporulation occurred and then were stored at 4°C.

DNA isolation, G+C contents, and DNA reassociation. The organisms used for DNA isolation were grown to the mid- to late-logarithmic phase (where microscopic examination showed an absence of sporulation) in TGY broth (6) at 28°C on a rotary shaker (200 rpm), and the cells were harvested by centrifugation at 5°C.

DNA was extracted and purified by a modification of the method of Marmur (13). The modification entailed the use of CsCl gradient ultracentrifugation to purify the DNA preparations (12). Consistent values of 1.8 to 1.9 and 2.0 to 2.3 for the ratios of A_{260} to A_{280} and the ratios of A_{260} to A_{230} , respectively, confirmed the high quality of each DNA preparation. Melting curves showing hyperchromicities ranging from 38 to 40% (14) corroborated the absorbance ratio observations.

In a previous publication (14), the protocol used for spectrophotometric estimation of the extent of DNA reassociation was described. The extent of reassociation was calculated by the equation of De Ley et al. (4).

The U.C. Cluster procedure of the PC-SAS version (SAS Institute, Inc., Cary, N.C.) facilitated computer-aided clustering of DNA relatedness data based on an unweighted pair group arithmetic average algorithm (19). SAS/GRAPH, in which the SAS macro GRAFTREE written and provided by Dan Jacobs, University of Maryland, was used, permitted computer-aided generation of dendrograms.

16S rRNA gene sequencing. A 16S rRNA gene DNA fragment of strain NRRL NRS-1438^T (T = type strain) that corresponds to positions 9 to 1510 of *Escherichia coli* 16S rRNA was amplified by PCR, using purified DNA and a primer combination consisting of 5'-AGAGTTTGATCCTGGCTCAG-3' (forward primer 27f [10]) and 5'-TACGG(CT)TACCTTGTACGACTT-3' (reverse primer 1492r [10]). The amplification products were purified with a GENE-CLEAN II kit (Bio 101, La Jolla, Calif.) and were sequenced with a Prism ABI dideoxy terminator cycle sequencing kit manufactured by Applied Biosystems, Ltd. The protocols used were those recommended by the manufacturer. Sequences were determined with the automated Applied Biosystems DNA sequencer. The following primers cited by Lane (10) were used for sequence analyses: primers 27f, 530f, 1114f, 1406f, 109rl, 342r, 685r3, 907r, 1100r, and 1492r. In addition, two other primers, designated as 304f (5'-GTAGCCGAC CTGAGAGG-3') and 801f (5'-AACAGGATTAGATACCC-3'), were designed to obtain unambiguous results. The CLUSTAL V (7) program was used to align the 16S rRNA gene DNA sequence generated with sequences of selected members of the family *Bacillaceae* obtained from GenBank (11). A similarity matrix was constructed from the aligned sequences. Pairwise evolutionary distances were computed from the similarity data by applying the Olsen correction parameter (15) of Jukes and Cantor (8). Preliminary relationships were determined by the neighbor-joining method of Saitou and Nei (16) and the DNA parsimony algorithm, using the DNAPARS program. The parsimony analysis revealed six equally parsimonious best trees. The robustness of the topologies was evaluated by bootstrap analysis (SEQBOOT program) through 100 bootstrap replications. The DNAML program was used to generate a maximum-likelihood tree (see Fig. 2) to which bootstrap values of more than 60% were applied. The DNAPARS, SEQBOOT, and DNAML programs are part of the PHYLIP, version 3.55c, software package (J. Felsenstein, University of Washington, Seattle).

Characterization. Strain characterization was carried out as described previously (5, 14). Whole-cell fatty acid profiles were determined by the MIDI system of Sasser (17).

Nucleotide sequence accession number. The GenBank accession number for the NRRL NRS-1438^T 16S rRNA gene DNA sequence is U49247.

RESULTS

The results of the DNA relatedness analyses segregated the "*B. apiarius*" strains into two clusters, as shown in Table 2. The members of one cluster, consisting of nine strains, showed intragroup levels of relatedness ranging from 93 to 100%, and the members of the second cluster, consisting of six strains, exhibited values ranging from 60 to 100%. In the cluster consisting of six strains, NRRL B-4156 is the type strain of *B. thiaminolyticus*. The dendrogram in Fig. 1 clearly shows the segregation of the "*B. apiarius*" strains into two clusters; cluster 1 contains organisms closely related to *B. thiaminolyticus*, and cluster 2 represents another distinct taxon. The G+C contents

TABLE 2. Levels of DNA relatedness of "*B. apiarius*" strains and type strain NRRL B-4156 of *B. thiaminolyticus*

Strain	% Reassociation with DNA from ^a														
	NRRL B-3678	NRRL B-4187	NRRL B-4188	NRRL B-4299	NRRL B-4303	NRRL NRS-1438 ^T	NRRL NRS-1439	NRRL NRS-1577	NRRL NRS-1578	NRRL NRS-1768	NRRL NRS-1769	NRRL NRS-1170	NRRL NRS-1771	NRRL NRS-1772	NRRL NRS-1773
NRRL B-3678	100														
NRRL B-4187	100	100													
NRRL B-4188	99	98	100												
NRRL B-4299	96	90	92	100											
NRRL B-4303	99	100	100	97	100										
NRRL NRS-1438 ^T	97	100	95	99	99	100									
NRRL NRS-1439	100	99	93	99	98	100	100								
NRRL NRS-1577	94	99	100	100	98	97	93	100							
NRRL NRS-1578	92	99	95	100	100	93	97	93	100						
NRRL NRS-1768	29	27	30	27	23	22	30	32	31	100					
NRRL NRS-1769	26	30	31	29	24	27	26	33	31	76	100				
NRRL NRS-1770	33	31	29	27	27	36	33	34	29	90	78	100			
NRRL NRS-1771	32	36	34	23	27	24	24	29	31	86	91	100	100		
NRRL NRS-1772	33	26	23	30	31	20	24	27	25	84	84	90	90	100	
NRRL NRS-1773	29	26	30	31	30	23	26	29	30	80	89	94	86	94	100
NRRL B-4156 ^T	30	28	29	25	23	25	26	24	28	61	69	64	75	71	60

^a The reassociation values are averages of two determinations; the maximum difference noted between two determinations was 6%.

FIG. 1. Dendrogram showing levels of DNA relatedness among "*B. apiarius*" strains and strain NRRL B-4156, the type strain of *B. thiaminolyticus*. Clustering was based on unweighted average-linkage clustering. All strain designations are NRRL designations.

of all of the strains based on buoyant density determinations ranged from 52 to 54 mol%.

As is shown in Table 3, low levels of DNA relatedness (range, 20 to 31%) demonstrated that the cluster 2 strains are only distantly related to species frequently associated with honeybees or their hives (namely, *P. alvei*, *P. larvae*, *B. laterosporus*, and *P. pulvifaciens*).

The 16S rRNA gene DNA of NRRL NRS-1438^T was sequenced to determine the phylogenetic position of this strain among the aerobic members of the *Bacillaceae*. A total of 12 primers were used, and a 1,489-base sequence was obtained. The extents of sequence similarity of the 16S rRNAs of *B. apiarius* NRRL NRS-1438^T and selected species belonging to the genera *Bacillus* (groups 1, 2, 4, and 5 as defined by Ash et al. [1]), *Paenibacillus* (formerly group 3 [1, 2]), *Alicyclobacillus*, *Saccharococcus*, *Sporolactobacillus*, and *Sporosarcina* are shown in Table 4. Similarity values of 90.5 to 95.5% indicated that NRRL NRS-1438^T was most closely related to members of the genus *Paenibacillus*. Furthermore, NRRL NRS-1438^T was equidistant from *Bacillus* groups 1, 2, and 4; the average similarity values were 87.1% for members of group 1 (*Bacillus cereus*, *Bacillus circulans*, *Bacillus coagulans*, *Bacillus smithii*, and *Bacillus subtilis*), 87.0% for group 2 members (*Bacillus globisporus*, *Bacillus insolitus*, *Bacillus sphaericus*, and *Sporosarcina ureae*), and 87.2% for group 4 members (*Bacillus brevis*, *Bacillus laterosporus*, and *Bacillus aneurinolyticus*). With similarity values of 85.6, 86.0, and 83.5%, respectively, members of group 5 (*Bacillus kaustophilus*, *Bacillus stearothermophilus*, *Bacillus thermoglucosidarius*, and *Saccharococcus thermophilus*), *Sporolactobacillus inulinus*, and *Alicyclobacillus cycloheptanicus* were more distantly related to NRRL NRS-1438^T.

On the basis of their sequences *Paenibacillus* species and NRRL NRS-1438^T were placed in a robust (bootstrap values, 100%) monophyletic group corresponding to the genus *Paenibacillus* (Fig. 2). NRRL NRS-1438^T formed a monophyletic group with *P. alvei*, as determined by all methods. The clustering of NRRL NRS-1438^T with *P. alvei* was supported by the results of the bootstrap analyses at a confidence level of 100%.

Phenotypically, the cluster 2 organisms are gram-variable, facultatively anaerobic, mesophilic, motile rods that produce ellipsoidal spores in swollen sporangia, hydrolyze starch and casein, and ferment a wide range of mono- and disaccharides. The phenotypic characteristics of cluster 2 strains, *P. alvei*, *P. larvae*, *B. laterosporus*, *P. pulvifaciens*, and *B. thiaminolyticus* are compared in Table 5. The cluster 2 strains were clearly distinct from the other recognized species.

Significant differences in the levels of several fatty acids allowed differentiation of cluster 2 strains from *P. alvei*, *P. larvae*, *B. laterosporus*, *P. pulvifaciens*, and *B. thiaminolyticus* (Table 6). Specifically, differences in the levels of the 14:0, 15:0 anteiso, and 16:0 fatty acids differentiated cluster 2 from *P. alvei*; the fatty acids that differentiated cluster 2 and *P. larvae* were the 14:0, 15:0 anteiso, 16:0 iso, and 16:0 fatty acids; the fatty acids that separated cluster 2 and *B. laterosporus* were the 14:0 iso, 15:0 iso, 16:0 iso, 16:1 ω 11*cis*, 17:1 iso ω 10*cis*, and 17:0 anteiso fatty acids; the fatty acids that separated cluster 2 and *P. pulvifaciens* were the 15:0 anteiso and 15:0 fatty acids; and the fatty acids that distinguished cluster 2 and *B. thiaminolyticus* were the 15:0 iso, 15:0 anteiso, 16:0 iso, 16:1 ω 11*cis*, 16:0, 17:0 iso, and 17:0 anteiso fatty acids.

The photomicrograph in Fig. 3 visually confirmed a previous written account (3) in which the spore coat of "*B. apiarius*" was described as being ridged, rectangular in outline, and unusually thick.

DISCUSSION

Because of their similar phenotypic characteristics, many *B. thiaminolyticus* strains have apparently been erroneously identified as "*B. apiarius*." The DNA relatedness data compiled in this study show that "*B. apiarius*" is a conglomerate of two genetically distinct taxa, one of which is *B. thiaminolyticus* and the other of which is designated cluster 2. Small-subunit rRNA sequence data revealed that cluster 2 is a member of the genus *Paenibacillus*, which includes species associated with honeybees or their hives (*P. alvei*, *P. larvae*, and *P. pulvifaciens*). The cluster 2 organisms have many of the phenotypic characteristics of the genus *Paenibacillus*, including a variable Gram reaction, production of ellipsoidal spores in swollen sporangia, facultatively anaerobic growth at mesophilic temperatures, hydrolysis of complex molecules, and fermentation of a wide range of sugars. Like other *Paenibacillus* species, the

TABLE 3. Levels of DNA relatedness of some cluster 2 strains ("*B. apiarius*"), *P. alvei*, *P. larvae*, *B. laterosporus*, and *P. pulvifaciens*

Strain	% Reassociation with DNA from ^a :		
	NRRL NRS-1438 ^T	NRRL NRS-1439	NRRL B-4299
<i>P. alvei</i> NRRL B-383 ^T	20	23	26
<i>P. larvae</i> NRRL B-2605 ^T	24	28	27
<i>B. laterosporus</i> NRRL NRS-314 ^T	30	29	31
<i>P. pulvifaciens</i> NRRL B-3685 ^T	24	24	27

^a The reassociation values are averages of two determinations; the maximum difference noted between determinations was 6%.

TABLE 4. Similarity matrix based on the 16S rRNA sequences of NRRL NRS-1438^T and related bacteria

Taxon	% Similarity to:																											
	NRRL NRS-1438 ^T	<i>Bacillus azotofixans</i>	<i>Bacillus cereus</i>	<i>Bacillus brevis</i>	<i>Bacillus circulans</i>	<i>Bacillus coagulans</i>	<i>Paenibacillus amylolyticus</i>	<i>Bacillus aneurinolyticus</i>	<i>Paenibacillus polymyxa</i>	<i>Bacillus smithii</i>	<i>Bacillus subtilis</i>	<i>Bacillus stearothermophilus</i>	<i>Paenibacillus pabuli</i>	<i>Bacillus laterosporus</i>	<i>Bacillus laevolacticus</i>	<i>Bacillus kaustophilus</i>	<i>Bacillus thermoglucosidasius</i>	<i>Sporolactobacillus inulinus</i>	<i>Alicyclobacillus cycloheptanicus</i>	<i>Bacillus globisporus</i>	<i>Bacillus insolitus</i>	<i>Bacillus sphaericus</i>	<i>Saccharococcus thermophilus</i>	<i>Sporosarcina ureae</i>	<i>Paenibacillus pulvificiens</i>	<i>Paenibacillus larvae</i>	<i>Paenibacillus alvei</i>	
NRRL NRS-1438 ^T	100																											
<i>Bacillus azotofixans</i>	91.4	100																										
<i>Bacillus cereus</i>	86.3	87.5	100																									
<i>Bacillus brevis</i>	87.4	86.5	87.2	100																								
<i>Bacillus circulans</i>	88.8	89.4	94.3	88.1	100																							
<i>Bacillus coagulans</i>	86.8	86.5	92.4	88.2	93.6	100																						
<i>Paenibacillus amylolyticus</i>	92.8	94.0	87.6	86.1	90.2	87.0	100																					
<i>Bacillus aneurinolyticus</i>	85.9	86.0	87.8	89.5	89.8	88.3	87.3	100																				
<i>Paenibacillus polymyxa</i>	90.5	93.8	86.6	86.0	88.7	87.0	92.8	85.9	100																			
<i>Bacillus smithii</i>	87.3	87.2	91.8	88.1	94.9	94.1	88.2	88.4	86.5	100																		
<i>Bacillus subtilis</i>	86.4	87.2	93.8	87.7	93.8	92.5	88.4	88.6	86.6	92.5	100																	
<i>Bacillus stearothermophilus</i>	85.4	86.9	90.4	87.7	91.0	90.0	86.5	88.9	85.5	92.2	89.8	100																
<i>Paenibacillus pabuli</i>	92.0	92.6	87.1	85.5	89.2	87.0	93.5	85.5	93.2	88.0	87.3	86.1	100															
<i>Bacillus laterosporus</i>	88.4	87.1	88.9	95.4	89.4	88.0	88.1	88.9	86.6	88.2	87.7	87.2	86.7	100														
<i>Bacillus laevolacticus</i>	86.2	87.0	89.9	85.8	91.3	90.7	87.0	86.7	86.3	89.0	90.5	87.3	87.1	86.4	100													
<i>Bacillus kaustophilus</i>	85.5	87.4	90.3	87.4	90.7	90.0	86.8	88.8	86.1	91.8	89.7	99.0	86.4	86.8	87.4	100												
<i>Bacillus thermoglucosidasius</i>	85.8	87.0	91.0	88.1	91.7	90.9	86.9	88.8	85.6	92.8	91.7	95.8	86.4	86.6	89.0	96.1	100											
<i>Sporolactobacillus inulinus</i>	86.2	87.8	89.6	85.7	91.2	87.5	86.6	87.3	89.8	90.7	88.5	87.2	86.3	87.5	88.7	89.2	89.2	100										
<i>Alicyclobacillus cycloheptanicus</i>	83.5	84.0	82.9	82.1	83.2	82.4	82.8	82.7	82.5	83.0	82.4	84.6	83.2	81.7	82.7	85.0	84.5	82.0	100									
<i>Bacillus globisporus</i>	87.0	87.4	90.7	87.7	93.3	90.5	87.0	88.1	87.1	91.9	91.2	89.1	89.5	87.4	89.4	88.7	90.2	89.3	82.3	95.6	100							
<i>Bacillus insolitus</i>	86.9	87.9	92.0	87.0	94.7	89.8	88.2	86.5	87.9	91.9	91.2	89.1	89.5	87.4	87.6	88.8	89.2	87.5	82.4	92.8	93.4	100						
<i>Bacillus sphaericus</i>	85.7	87.6	92.2	87.0	92.5	89.6	87.7	86.8	86.9	91.3	90.8	89.1	87.8	87.8	87.6	88.8	89.2	87.5	82.4	92.8	93.4	100						
<i>Saccharococcus thermophilus</i>	86.6	88.4	92.4	88.7	93.5	91.8	88.0	89.7	87.0	88.4	89.2	87.0	87.6	87.6	90.1	96.2	97.0	90.8	85.1	91.8	91.0	90.6	100					
<i>Sporosarcina ureae</i>	88.3	89.1	92.6	88.7	94.9	92.1	88.6	89.2	88.4	93.2	93.1	91.2	90.2	88.4	90.2	90.9	93.0	90.1	84.5	97.1	95.7	93.7	93.4	100				
<i>Paenibacillus pulvificiens</i>	91.4	92.0	86.1	86.8	89.3	87.0	93.0	86.8	92.6	88.2	87.5	86.3	92.6	87.5	87.0	86.5	86.0	87.2	85.1	87.7	88.0	86.8	87.9	89.0	100			
<i>Paenibacillus larvae</i>	90.6	91.2	85.3	86.3	88.3	86.2	92.3	85.9	91.3	87.3	86.2	85.3	91.6	87.0	85.9	85.4	85.0	86.4	84.0	86.7	86.9	85.8	87.1	88.2	99.4	100		
<i>Paenibacillus alvei</i>	95.5	93.3	88.5	87.9	90.2	87.9	93.0	86.7	92.8	88.7	88.0	86.7	92.5	88.7	87.9	86.9	87.2	87.6	84.7	87.9	88.7	87.5	88.4	89.3	92.4	92.0	100

FIG. 2. Tree showing the phylogenetic position of NRRL NRS-1438^T among selected bacteria belonging to the family Bacillaceae. The tree, which is rooted by using *E. coli* as the outgroup, was generated by maximum-likelihood method DNAML. Trees generated by the neighbor-joining method and by parsimony analysis had similar topologies. Bootstrap values (100 data sets) were determined by performing a parsimony analysis. Bar = 0.1 accumulated change per nucleotide.

predominant cellular fatty acid is the 15:0 anteiso fatty acid. The G+C contents of the cluster 2 strains fall within the range exhibited by members of the genus *Paenibacillus* (40 to 54 mol%). Although the results of the sequence analysis show that there is a close phylogenetic relationship between NRRL NRS-1438^T and *P. alvei*, the cluster 2 organisms are only distantly related genetically to *P. alvei*. The cluster 2 organisms and the other honeybee-associated *Paenibacillus* species are also only distantly related. Likewise, the cluster 2 strains and the honeybee-associated species *B. laterosporus* are not closely related genetically.

The cluster 2 organisms are also phenotypically distinct from the organisms obtained from honeybee environs mentioned above. The following characteristics are some outstanding characteristics that are not found in cluster 2 strains: *P. alvei* produces indole and dihydroxyacetone; *P. larvae* is pathogenic to honeybees and does not produce catalase; *B. laterosporus* has a distinct canoe-shaped sporangium; *P. pulvifaciens* does not utilize citrate or hydrolyze starch; and *B. thiaminolyticus* produces indole and decomposes thiamine. Dissimilar cellular fatty acid compositions also indicate that "*B. apiarius*" is distinct from the currently recognized honeybee-associated species.

All strains of "*B. apiarius*" produce thick-walled spores that are rectangular in outline and are unlike the ellipsoidal spores of *B. thiaminolyticus* and other species. This unusual morphology is clearly different from the canoe-shaped sporangia associated with *B. laterosporus*.

The data obtained in this study justify placing cluster 2 in the genus *Paenibacillus* and recognizing it as a phenotypically and genotypically distinct species, for which the name *Paenibacillus apiarius* is proposed. Strain NRRL NRS-1438 is the type strain of this species. A description of the type strain is given below.

Description of *Paenibacillus apiarius*. *Paenibacillus apiarius* (a.pi.a'ri.us. L. adj. *apiarius*, relating to bees).

Rods that are 0.7 to 0.8 by 3.0 to 5.0 µm, as determined from a photomicrograph. Gram staining is variable. Motile. Spores have thick walls, appear to be rectangular, and are produced in swollen sporangia.

Agar colonies are nonpigmented, translucent, thin, smooth, circular, and entire, and the average diameter is about 1.0 mm after 24 h of incubation on TGY agar at 28°C.

Catalase is produced. Oxidase negative.

Facultatively anaerobic.

Acetylmethylcarbinol, dihydroxyacetone, indole, and H₂S are not produced. The pH in Voges-Proskauer broth (test for acetylmethylcarbinol production) ranges from 4.4 to 5.4.

Nitrate is reduced to nitrite.

Starch, casein, tyrosine, and urea are hydrolyzed. Tween 80 and egg yolk lecithin are not decomposed. Lysine, ornithine, arginine, and phenylalanine are not decomposed.

Citrate is utilized; propionate is not utilized.

Growth occurs at pH 5.7, in the presence of 0.001% lysozyme, and in the presence of 5% NaCl; growth is inhibited by 7% NaCl.

The optimum growth temperature is 28°C; the maximum temperature is 40°C; and the minimum temperature is 15°C.

TABLE 5. Phenotypic comparison of cluster 2 ("*B. apiarius*"), *P. alvei*, *P. larvae*, *B. laterosporus*, *P. pulvifaciens*, and *B. thiaminolyticus*

Characteristic	Cluster 2	<i>P. alvei</i>	<i>P. larvae</i>	<i>B. laterosporus</i>	<i>P. pulvifaciens</i>	<i>B. thiaminolyticus</i>
Catalase	+	+	—	+	+	+
Acetylmethylcarbinol production	—	+	—	—	—	—
Dihydroxyacetone production	—	+	—	—	—	—
Indole production	—	+	—	v	—	+
Citrate utilization	+	—	—	—	—	+
Nitrate reduction to nitrite	+	—	v	+	+	+
Tyrosine decomposition	+	v	—	+	—	+
Casein hydrolysis	+	+	+	+	+	+
Starch hydrolysis	+	+	—	—	—	+
Acid production from arabinose	—	—	—	—	—	v
Acid production from mannitol	—	—	v	+	+	v
Growth at pH 5.6	—	+	—	—	—	—
Growth at 50°C	—	—	—	+	—	—
G+C content (mol%)	52–54	44–47	50	40–42	44–46	52–54

^a +, positive reaction; —, negative reaction; v, variable reaction.

TABLE 6. Cellular fatty acid compositions of cluster 2 ("*B. apiarius*") and selected *Bacillus* and *Paenibacillus* species

Fatty acid	% in ^a :					
	Cluster 2	<i>P. alvei</i>	<i>P. larvae</i>	<i>B. laterosporus</i>	<i>P. pulvifaciens</i>	<i>B. thiaminolyticus</i>
14:0 iso	0.35 ± 0.50 ^b	0.58 ± 0.82	0.69 ± 0.71	7.24 ± 1.82^c	ND ^d	0.12 ± 0.29
14:0	1.11 ± 0.62	2.28 ± 0.13	3.31 ± 0.50	1.43 ± 0.11	1.18 ± 0.16	1.93 ± 0.62
15:0 iso	14.15 ± 3.18	11.09 ± 0.97	10.51 ± 1.61	34.27 ± 12.71	15.90 ± 1.11	6.12 ± 0.68
15:0 anteiso	51.89 ± 0.01	57.29 ± 1.97	28.21 ± 0.87	48.04 ± 12.60	39.13 ± 2.73	47.73 ± 2.52
15:0	0.53 ± 0.74	0.58 ± 0.81	1.58 ± 0.35	ND	6.71 ± 2.43	0.25 ± 0.39
16:1 ω ₉ alcohol	0.50 ± 0.70	ND	ND	0.70 ± 0.50	ND	ND
16:0 iso	5.02 ± 0.72	4.51 ± 2.47	3.20 ± 0.59	2.89 ± 0.79	4.13 ± 0.73	3.47 ± 0.69
16:1 ω ₁₁ cis	1.96 ± 0.38	2.97 ± 1.73	ND	0.77 ± 0.54	ND	5.23 ± 2.17
16:0	3.76 ± 1.62	8.46 ± 1.17	27.97 ± 2.30	ND	8.12 ± 2.66	11.55 ± 4.60
17:1 iso ω ₁₀ cis	1.84 ± 0.33	ND	ND	0.62 ± 0.44	ND	0.82 ± 0.66
17:0 iso	6.39 ± 0.57	4.35 ± 1.46	6.91 ± 0.24	1.05 ± 0.90	6.77 ± 0.71	4.32 ± 0.77
17:0 anteiso	10.13 ± 2.06	7.48 ± 1.97	9.46 ± 1.10	1.28 ± 0.17	12.79 ± 2.02	16.31 ± 2.73

^a Six strains of each organism were analyzed.^b Mean ± standard deviation.^c Values in boldface type are significantly different (*P* < 0.05) from the cluster 2 values.^d ND, not detected.

Acid but no gas is produced from cellobiose, D-galactose, D-glucose, maltose, D-mannose (weak), melibiose, D-ribose (weak), salicin, sucrose, and trehalose. L-Arabinose, D-fructose, lactose, mannitol, L-rhamnose, sorbitol, and D-xylose are not fermented.

The DNA buoyant density ranges from 1.7055 to 1.7075 g/cm³; the G+C content is 52 to 54 mol%.

Isolated from honeybees, their larvae, and their hives.

Type strain NRRL NRS-1438 has been deposited in the Agricultural Research Service Culture Collection at the National Center for Agricultural Utilization Research in Peoria, Ill.

FIG. 3. Phase-contrast photograph of "*B. apiarius*" NRRL NRS-1438^T. Magnification, ×1,400.

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