Bacillus peoriae sp. nov.

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The taxonomy of an apparently genetically distinct gas-producing group of *Bacillus* strains formerly classified as *Bacillus polymyxa* was studied. Multilocus enzyme electrophoresis analysis confirmed the distinctiveness of the unknown taxon. Low DNA relatedness values also demonstrated that the unknown was not closely related genetically to any of the presently recognized species that have guanine-plus-cytosine values of 45 to 47 mol% or produce gas by fermentation of sugars. The unknown group was also a phenotypically distinct taxon. The data suggest that the unknown group merits recognition as a new species, for which the name *Bacillus peoriae* is proposed.

The species *Bacillus polymyxa* was validly proposed as a species by Mace in 1889 (6). Because of their rather rare ability to form gas, their unique spore morphology, and their apparent phenotypic homogeneity, *B. polymyxa* strains were easily identified. Until 1984, when Seldin et al. (13) named the gas-forming, nitrogen-fixing organism *B. azotofixans*, *B. polymyxa* and *B. macerans* were the only gas-forming *Bacillus* species known.

In contrast to Gordon et al. (4), who reported the phenotypic homogeneity of the species, other researchers have found that *B. polymyxa* was variable for citrate utilization and glycerol fermentation (8), nitrogen-fixing activity (5, 13), and polymyxin production (4) and in the ratio of pullulanase and β -amylase produced (3). Recently, DNA reassociation analyses have shown that *B. polymyxa* consists of two genetically distinct taxa, one being the species sensu stricto and the other being a yet-unnamed species (8). Differences in several characteristics distinguished *B. polymyxa* sensu stricto from the other taxon. This intruding group was also distinguishable by a number of phenotypic characteristics from species with similar guanine-plus-cytosine (G+C) contents of 44 to 47 mol% (*B. alvei*, *B. badius*, *B. brevis*, *B. coagulans*, and *B. licheniformis*) and from species that produce gas, namely, *B. azotofixans* and *B. macerans* (8).

In the present study, distinction among *B. azotofixans*, *B. polymyxa*, and the unknown taxon was confirmed by multilocus enzyme electrophoresis analyses described by Selander et al. (12). Because experience showed that *B. macerans* was not a very active gas producer and was therefore not likely to be related to the unknown taxon, only the type strain was included in this study. The 19 enzymes assayed included alanine dehydrogenase (EC 1.4.1.1), alcohol dehydrogenase (EC 1.1.1.1), aspartate dehydrogenase (EC 1.4.3.x), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glutamate dehydrogenase (EC 1.4.1.2), 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30), superoxide:superoxide oxidoreductase (EC 1.15.1.1), isocitrate dehydrogenase (EC 1.1.1.42), L-lactate dehydrogenase (EC 1.1.1.27), leucine dehydrogenase (EC 1.4.3.2), lysine dehydrogenase (EC 1.4.3.x), mannitol-1-phosphate dehydrogenase (EC 1.1.1.17), 6-phosphogluconate dehydrogenase (EC 1.1.1.44), xanthine dehydrogenase (EC 1.2.3.2), mannose phosphate isomerase (EC 5.3.1.8), phosphoglucose isomerase (EC 5.3.1.9), hexokinase (EC 2.7.1.1), esterase (EC 3.1.1.1), and

leucine aminopeptidase (EC 3.4.1.1). The procedure for determining the relative mobilities of alternative forms of each enzyme was described previously (9). Levels of similarity among strains were estimated on the basis of the Jaccard coefficient, and clustering was based on unweighted pair-group arithmetic average algorithm (14). Computation was carried out with a DTK 386 computer with the SAS TAXAN 3.0, created by David Swartz, University of Maryland, College Park, Md.

The dendrogram in Fig. 1 shows that the organisms studied segregated into four distinct phena, designated 1, 2, 3, and 4. *B. polymyxa* (phenon 2), *B. azotofixans* (phenon 3), and the unknown taxon (phenon 4) consist of 23, 3, and 18 electrophoretic types, respectively. Phenon 4 separates from phenon 2 and 3 and the type strain of *B. macerans* (phenon 1) at about the 0.30, 0.38, and 0.22 similarity levels, respectively.

Spectrophotometrically determined (1, 10) DNA relatedness values showed that the unnamed taxon is not closely related genetically to *B. alginolyticus*, *B. alvei*, *B. azotofixans*, *B. badius*, *B. brevis*, *B. chondroitinus*, *B. coagulans*, *B. licheniformis*, or *B. macerans* (Table 1). *B. alginolyticus* and

TABLE 1. DNA relatedness of three strains of the unnamed taxon and selected *Bacillus* type strains

Type strain	G+C (mol%) ^a	% Reassociation with DNA from ^b :		
		NRRL BD-57 ^c	NRRL NRS-1312 ^c	NRRL B-14474 ^c
<i>B. badius</i> NRRL NRS-663	44	22	18	25
<i>B. coagulans</i> NRRL NRS-609	45	33	30	28
<i>B. polymyxa</i> NRRL B-4317	45	29	33	29
<i>B. licheniformis</i> NRRL NRS-1264	46	29	25	26
<i>B. alvei</i> NRRL B-383	46	17	20	16
<i>B. chondroitinus</i> NRRL NRS-1351	47 ^d	26	25	19
<i>B. alginolyticus</i> NRRL NRS-1347	48 ^d	30	27	20
<i>B. brevis</i> NRRL NRS-604	48	14	15	17
<i>B. azotofixans</i> NRRL B-14372	51 ^e	30	28	26
<i>B. macerans</i> NRRL B-4267	53	19	27	24

^a Data taken from reference 2.

^b Reassociation values are averages of two determinations; the maximum difference between determination was 5%.

^c DNA reassociation values among the three strains range from 98 to 100% (8).

^d Data taken from reference 7.

^e Data taken from reference 13.

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FIG. 1. Relationship among the unknown taxon, *B. azotofixans*, and *B. polymyxa*. The dendrogram was generated by unweighted pair-group arithmetic average linkage clustering from a matrix of Jaccard coefficients based on 19 enzyme loci.

B. chondroitinus are relatively newly described species with G+C values of 48 and 47 mol%, respectively (7, 11).

A collation of data from previous studies (Table 2) also shows that the unnamed taxon is phenotypically distinct from the other gas-forming species, namely, *B. polymyxa*, *B. azotofixans*, and *B. macerans*. Positive citrate and succinate utilization and negative results for glycerol fermentation and production of dihydroxyacetone by the unknown taxon differentiate it from *B. polymyxa*. Differences in G+C contents and in the ability to ferment arabinose and xylose, to hydrolyze casein and starch, to utilize citrate, and to reduce nitrate to nitrite distinguish the unknown group from *B. azotofixans*. The unknown taxon and *B. macerans* can be distinguished by tests for acetylmethylcarbinol production, growth in 3% NaCl, crystalline dextrin production, and G+C contents. These data support the conclusion derived from DNA relatedness studies that the unnamed group is a new species.

On the basis of observations from previous (8) and present studies, the unnamed group merits designation as members

of a new species, for which we proposed the name *Bacillus peoriae*. A description of the species is given below.

***Bacillus peoriae* sp. nov.** *Bacillus peoriae* (pe.o'.riae L. gen. n. *peoriae*, named after Peoria, where the study on the organism was done) motile vegetative cells are 0.5 to 1.0 μ m wide and 3.0 to 6.0 μ m long (as determined by phase microscopy) and occur singly and in short chains. They produce ellipsoidal spores in definitely swollen sporangia and are gram positive.

Agar colonies are translucent, thin, smooth, circular, and entire and measure about 2 mm in diameter after 2 days at 28°C.

Catalase is produced. Oxidase is not produced. Facultatively anaerobic. Acetylmethylcarbinol (Voges-Proskauer test) is produced. The pHs in Voges-Proskauer broth range from 5.0 to 6.5. Hydrogen sulfide, indole, and dihydroxyacetone are not formed. Nitrate is reduced to nitrite. Citrate and succinate, but not propionate, are utilized. Casein, pectin, starch, and xylan are hydrolyzed. Egg yolk lecithin,

TABLE 2. Differentiation of the unknown taxon from other gas-producing *Bacillus* spp.^a

Characteristic	Result for			
	Unknown ^b	<i>B. polymyxa</i> ^b	<i>B. azotofixans</i> ^c	<i>B. macerans</i> ^d
Swelling sporangium	+	+	+	+
Catalase	+	+	+	+
Anaerobic growth	+	+	+	+
Acetylmethylcarbinol produced	+	+	+	—
pH in Voges-Proskauer broth	5.5–6.5	4.5–6.8	ND	5.0–68
Growth at 45°C	+	+	—	—
Growth in 0.001% lysozyme	+	v	—	—
Growth at pH 5.7	+	+	+	+
Growth in 3% NaCl	—	—	—	+
Acid from:				
Arabinose	+	+	—	+
Glucose	+	+	+	+
Mannitol	+	+	+	+
Xylose	+	+	—	+
Glycerol	—	+	—	+
Trehalose	—	+	ND	ND
Gas from carbohydrate	+	+	+	v
Starch hydrolysis	+	+	—	+
Citrate utilization	+	—	—	v
Succinate utilization	+	—	ND	ND
Nitrate reduced to nitrite	+	+	—	+
Dihydroxyacetone produced	—	+	—	—
Crystalline dextrin produced	—	—	—	+
Indole produced	—	—	—	—
Casein hydrolysis	+	+	—	—
Tyrosine decomposition	—	—	—	—
G+C content (mol%)	45–47	43–46	48–53	52–53 ^e

^a +, positive reaction; —, negative reaction; v, variable reaction; ND, not done.^b Data taken from reference 7.^c Data taken from reference 11.^d Data taken from reference 4.^e Data taken from reference 2.

Tween 80, and urea are not decomposed. Litmus milk is acidified and curdled.

Arginine, lysine, ornithine, phenylalanine, and tyrosine are not decomposed.

The optimum, maximum, and minimum growth temperatures range from 28 to 30°C, from 35 to 45°C, and from 5 to 10°C, respectively. Grows at pH 5.6 or 5.7, variably in 0.001% lysozyme, and not in 3% NaCl. Acid and gas are produced from arabinose, glucose, mannitol, and xylose. Other carbohydrates fermented include cellobiose, galactose, fructose, lactose, maltose, mannitol, mannose, melibiose, ribose, rhamnose, salicin, and sucrose. Glycerol and trehalose are not fermented.

The DNA buoyant densities for 28 strains ranged from 1.6986 to 1.7005 g/cm³, and the G+C values determined from these values ranged from 45 to 47 mol%.

Isolated mainly from soil and rotting vegetative materials.

The type strain is BD-57, which has been deposited as NRRL B-14750 in the Agricultural Research Service Culture Collection, Peoria, Ill.

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