Bacillus amylolyticus sp. nov., nom. rev., *Bacillus lautus* sp. nov., nom. rev., *Bacillus pabuli* sp. nov., nom. rev., and *Bacillus validus* sp. nov., nom. rev.

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The names "*Bacillus amylolyticus*" Choukévitch 1911, "*Bacillus lautus*" Batchelor 1919, "*Bacillus pabuli*" Schiebllich 1923, and "*Bacillus validus*" Bredemann and Heigener 1935 were not included on the Approved Lists of Bacterial Names. The names *Bacillus amylolyticus*, *Bacillus lautus*, *Bacillus pabuli*, and *Bacillus validus* are here revived; all of these names except *Bacillus amylolyticus* are used for the same taxa to which they were originally applied. The name *Bacillus amylolyticus* is applied to the taxon described by Kellerman and McBeth. The type strains of *B. amylolyticus*, *B. lautus*, *B. pabuli*, and *B. validus* are strains NRRL NRS-290, NRRL NRS-666, NRRL NRS-924, and NRRL NRS-1000, respectively.

Choukévitch (4), Batchelor (1), Schiebllich (11), and Bredemann and Heigener (3) validly published descriptions of the new species "*Bacillus amylolyticus*," "*Bacillus lautus*," "*Bacillus pabuli*," and "*Bacillus validus*," respectively. Because the name *B. amylolyticus* was validly published in 1911, its use for a different species by Kellerman and McBeth in 1912 (7) was illegitimate. These four species were listed in *Bergey's Manual of Determinative Bacteriology*, 5th ed. (2). Because of an apparent oversight, "*B. amylolyticus*" Kellerman and McBeth, not "*B. amylolyticus*" Choukévitch, was listed. However, since they were poorly described, these species were excluded from subsequent editions of *Bergey's Manual*. Smith et al. (14) and Gordon et al. (6) reclassified "*B. amylolyticus*" Kellerman and McBeth and the three other species as *Bacillus circulans* strains. Except in the originally published description, the name "*B. amylolyticus*" Choukévitch has not appeared in the literature, and the locations of strains of this species are not known. The loss of the taxonomic status of the names was reinforced by exclusion from the Approved Lists of Bacterial Names published in 1980 (13).

Recent taxonomic studies have revealed that the guanine-plus-cytosine (G+C) contents of the deoxyribonucleic acids (DNAs) of *B. circulans* sensu stricto ranged from 37 to 39 mol% (10). Because those *B. circulans* strains that were originally classified as "*B. amylolyticus*," "*B. lautus*," "*B. pabuli*," and "*B. validus*" had G+C contents ranging from 47 to 54 mol%, they were considered to be not related genetically to the type strain (10). In the present study, spectrophotometrically determined (5) DNA relatedness values confirmed that "*B. amylolyticus*," "*B. lautus*," "*B. pabuli*," and "*B. validus*" are not genetically related to each other, to *B. circulans* NRRL NRS-726^T (T = type strain), or to the type strains of established species having similar G+C contents, such as *Bacillus coagulans* NRRL NRS-609^T, *Bacillus macerans* NRRL NRS-888^T, *Bacillus polymyxa* NRRL NRS-1105^T, and *Bacillus stearothermophilus* NRRL NRS-18^T (Table 1). Furthermore, each of the tentative species was shown to be represented by at least four genetically related strains (Table 1). Extensive characterization by the method of Gordon et al. (6) showed that each group is phenotypically distinct from the other groups and from *B. circulans* sensu stricto (10). However, all four groups and *B. circulans* shared the following characteristics:

they were motile, catalase positive, gram positive, and facultatively anaerobic; they produced oval spores in swollen sporangia; they hydrolyzed starch; they acidified litmus milk; they fermented L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannitol, D-mannose, melibiose, D-ribose, sucrose, trehalose, and D-xylose; they did not produce acetylmethylcarbinol, H₂S, oxidases, or indole; and they did not degrade filter paper (cellulose) in nutrient broth, egg yolk lecithine, tyrosine, arginine, lysine, or ornithine. Furthermore, all five species formed agar colonies that were grayish white, translucent, smooth, circular, entire, and 1.0 to 2.0 mm in diameter after 3 days at 30°C. The salient characteristics for differentiating the four groups and *B. circulans* are given in Table 2.

Based on the data described above, the names *Bacillus amylolyticus*, *Bacillus lautus*, *Bacillus pabuli*, and *Bacillus validus* are revived, and each name except *B. amylolyticus* is used for the same taxon to which it was originally applied in accordance with Rules 27 and 28a and Provisional Rules B₂ and B₃ of the *International Code of Nomenclature of Bacteria* (8). Because the revival is based on an examination of the original strain of Kellerman and McBeth, the name *B. amylolyticus* is applied to the taxon originally described by these authors.

The following descriptions include only the salient characteristic of the new species; the commonly shared characteristics are listed above.

Bacillus amylolyticus (am.y.lo.ly'ti.cus. L. n. *amylum* starch; Gr. adj. *lyticus* dissolving; M.L. masc. adj. *amylolyticus* starch dissolving) cells are rods that are 0.5 to 1.0 µm wide by 3.0 to 6.0 µm long.

Growth is not inhibited by 3% NaCl, by 0.001% lysozyme, or at pH 5.6. Most strains, including the type strain, are inhibited by 5% NaCl.

The optimum growth temperature is 28 to 30°C, the maximum temperature is 40 to 45°C, and the minimum temperature is 10 to 15°C.

Urea is seldom hydrolyzed.

Nitrate is reduced to nitrite.

Acetate is utilized by most strains; citrate, fumarate, malate, and succinate are not utilized.

L-Rhamnose is seldom fermented.

The average G+C content of the DNA is 53 mol%.

Habitat: soil (7).

TABLE 1. DNA relatedness of "*B. amylolyticus*," "*B. lautus*," "*B. pabuli*," and "*B. validus*" strains

Strain	% Reassociation with DNA from: ^a			
	" <i>B. amylolyticus</i> " NRRL NRS-290 ^T	" <i>B. lautus</i> " NRRL NRS-666 ^T	" <i>B. pabuli</i> " NRRL NRS-924 ^T	" <i>B. validus</i> " NRRL NRS-1000 ^T
<i>"B. amylolyticus"</i> ^b				
NRRL B-142	89	20	23	20
NRRL NRS-290 ^T	(100) ^c	18	3	8
NRRL NRS-359	100	11	11	15
NRRL NRS-1136	100	29	6	23
<i>"B. lautus"</i> ^b				
NRRL NRS-666 ^T	18	(100)	3	13
NRRL NRS-676	12	75	19	18
NRRL NRS-678	3	70	3	23
NRRL NRS-679	5	84	13	17
NRRL NRS-1171	19	100	23	20
<i>"B. pabuli"</i> ^b				
NRRL B-745	6	2	75	8
NRRL B-1373	18	5	82	19
NRRL B-1488	7	2	80	26
NRRL BD-536	15	3	72	19
NRRL BD-537	10	4	70	16
NRRL NRS-924 ^T	3	3	(100)	16
<i>"B. validus"</i> ^b				
NRRL NRS-625	7	16	12	97
NRRL NRS-639	23	10	20	97
NRRL NRS-838a	13	20	17	100
NRRL NRS-839a	25	23	10	100
NRRL NRS-1000 ^T	8	13	16	(100)
<i>B. circulans</i> NRRL NRS-726 ^T	10	2	14	3
<i>B. coagulans</i> NRRL NRS-609 ^T	20	16	21	29
<i>B. macerans</i> NRRL NRS-888 ^T	14	15	5	8
<i>B. polymyxa</i> NRRL NRS-1105 ^T	26	29	18	9
<i>B. stearothermophilus</i> NRRL NRS-18 ^T	29	25	14	22

^a Reassociation values are averages of two determinations.^b Histories of these strains are given in reference 9.^c The values in parentheses indicate that by definition the reassociation value was 100%.

The type strain is strain NRRL NRS-290. Except for its inability to utilize acetate, the type strain is phenotypically identical to the species. It does not hydrolyze urea or ferment L-rhamnose.

Bacillus lautus (lau'tus. L. part. adj. *lautus* washed, splendid) cells are rods that are 0.5 to 1.0 µm wide by 4.0 to 7.0 µm long.

Growth is not inhibited by 5% NaCl or by 0.001% lysozyme. No growth occurs at pH 5.6

The optimum growth temperature is 28 to 30°C, the maximum temperature is 35 to 40°C, and the minimum temperature is 5 to 10°C.

Urea is hydrolyzed.

Nitrate is reduced to nitrite.

Acetate is utilized; citrate, fumarate, malate, and succinate are not utilized.

L-Rhamnose is fermented in addition to other carbohydrates.

The average G+C content of the DNA is 51 mol%.

Habitat: human intestinal tracts (1) and soil (9).

The type strain is strain NRRL NRS-666. The description of this strain is identical to that of the species.

Bacillus pabuli (pa'bu.li. L. gen. n. *pabuli* of fodder) cells are rods that are 0.5 to 1.0 µm wide by 2.0 to 4.0 µm long.

Growth is not inhibited by 3% NaCl; not all strains are inhibited by 5% NaCl. Growth occurs at pH 5.6. Growth is inhibited by 0.001% lysozyme.

The optimum growth temperature is 28 to 30°C, the maximum temperature is 35 to 40°C, and the minimum temperature is 5 to 10°C.

Casein is hydrolyzed by most strains, including the type strain.

Nitrate is not reduced to nitrite.

Acetate is utilized; citrate, fumarate, malate, and succinate are not utilized.

L-Rhamnose is fermented in addition to other carbohydrates.

The average G+C content of the DNA is 49 mol%.

Habitat: fodder (11) and soil (9).

The type strain is strain NRRL NRS-924. The description of this strain is essentially identical to that of the species; it is inhibited by 5% NaCl.

Bacillus validus (val'i.dus. L. adj. *validus* strong, vigorous) cells are rods that are 0.5 to 1.0 µm wide by 5.0 to 7.0 µm long.

Growth is inhibited by 3% NaCl and 0.001% lysozyme. Growth takes place at pH 5.6

The optimum growth temperature is 28 to 30°C, the maximum temperature is 45 to 50°C, and the minimum temperature is 5 to 10°C.

Tween 80 and urea are hydrolyzed.

Nitrate is not reduced to nitrite.

Acetate, citrate, fumarate, malate, and succinate are utilized.

L-Rhamnose and D-sorbitol are fermented in addition to other carbohydrates.

The average G+C content of the DNA is 54 mol%.

Habitat: soil (3).

The type strain is strain NRRL NRS-1000; its description is identical to that of the species.

TABLE 2. Characteristics for differentiating "*B. amylolyticus*," *B. circulans*, "*B. lautus*," "*B. pabuli*," and "*B. validus*"^a

Characteristic	<i>B. circulans</i>	" <i>B. amylolyticus</i> "	" <i>B. lautus</i> "	" <i>B. pabuli</i> "	" <i>B. validus</i> "
Growth at or in:					
3% NaCl	+	+	+	+	—
5% NaCl	+	v	+	v	—
pH 5.6	+	+	—	+	+
0.001% Lysozyme	—	+	+	—	—
Hydrolysis of:					
Casein	—	—	—	v	—
Tween 80	+	—	—	—	+
Urea	—	v	+	—	+
Nitrate reduced to nitrite	—	+	+	—	—
pH < 5.5 in Voges-Proskauer broth ^c	+	+	+	+	—
Utilization of:					
Acetate	+	v	+	+	+
Citrate	—	—	—	—	+
Fumarate	+	—	—	—	+
Malate	+	—	—	—	+
Succinate	—	—	—	—	+
Fermentation of:					
D-Sorbitol	+	—	—	—	+
L-Rhamnose	+	v	+	+	+
G+C content (mol%) ^d	37–39	53	50–52	48–50	53–54

^a Data taken from reference 10.^b +, Positive; —, negative; v, variable with strain.^c pH after 7 days at 30°C in Voges-Proskauer broth.^d Determined by buoyant density centrifugation (12).

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