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Bacillus amyloliquefaciens sp. nov., nom. rev.

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The name "*Bacillus amyloliquefaciens*" Fukomoto 1943 was not included on the Approved Lists of Bacterial Names and has not been validly published since 1 January 1980; hence, it has lost standing in bacterial nomenclature. The taxon to which this name is applied is a distinct entity, and it can be distinguished from other named species of *Bacillus*. Consequently, the name *Bacillus amyloliquefaciens* is revived for the same organism to which the name originally referred. The type strain of *Bacillus amyloliquefaciens* is strain ATCC 23350.

The name "*Bacillus amyloliquefaciens*" Fukomoto 1943 (3, 4) does not appear on the Approved Lists of Bacterial Names (15) and has not been validly published since 1 January 1980; thus, it has no standing in bacterial nomenclature. In accordance with Rules 24a and 28a of the *International Code of Nomenclature of Bacteria* (6) the name is hereby revived.

"*Bacillus amyloliquefaciens*" is responsible for much of the world production of α-amylase and protease. Its close affinity with *Bacillus subtilis* has long been recognized, and the organism has been given subspecies status as "*B. subtilis* subsp. *amyloliquefaciens*" (16) or has been included in *B. subtilis* as a variant that produces copious quantities of extracellular enzymes (5). Thus, "*Bacillus amyloliquefaciens*" is closely related to *B. subtilis* and the other two species which compose the *B. subtilis* group, *Bacillus licheniformis* and *Bacillus pumilus*. These organisms share many common properties, and few characteristics have been found by which they can be discriminated (5). Indeed, "*B. amyloliquefaciens*" is phenotypically so similar to *B. subtilis* that it is not possible to separate these organisms solely on the basis of classical tests (5, 7, 9, 12), and it is for this reason that "*B. amyloliquefaciens*" was not included as a separate species on the Approved Lists (15). However, there is now a body of evidence that suggests that the name "*B. amyloliquefaciens*" should be revived. It has been shown that "*B. amyloliquefaciens*" and *B. subtilis* can be differentiated by using a number of techniques. Moreover, there is a need for this name in the enzyme industry to avoid confusion with *B. subtilis*, which differs metabolically and secretes different enzymes (10).

Deoxyribonucleic acids (DNAs) from strains of "*B. amyloliquefaciens*" have consistently been found to share less than 25, 13, and 5% homology with DNAs from strains of *B. subtilis*, *B. licheniformis*, and *B. pumilus*, respectively, under optimal conditions (60 to 65°C) (9, 11, 13, 17; L. A. Shute, Ph.D. thesis, University of Bristol, Bristol, United Kingdom, 1986). Although there is no universally accepted level of DNA homology which delineates a bacterial species, most workers agree that strains within a species should share at least 50 to 60% homology. Thus, although "*B. amyloliquefaciens*" is related to *B. subtilis* on the basis of molecular genetic data, the level of DNA homology is not high enough for these two groups of organisms to be considered a single species.

In addition to the molecular genetic evidence, it has been shown that numerical analysis of phenotypic features enables discrimination of "*B. amyloliquefaciens*" from *B. subtilis* (9). A study of these organisms with API tests also discriminated "*B. amyloliquefaciens*" from *B. subtilis* (9); in this study eight strains of each species were examined. However, a more comprehensive study conducted later, in which 52 "*B. amyloliquefaciens*" strains and 131 *B. subtilis* strains were used (8), revealed intermediate strains which tended to obscure the distinction seen earlier (9). Nevertheless, the species can be separated by using probabilistic identification methods based on API tests (2).

All four species can also be differentiated by using pyrolysis gas-liquid chromatography (9) and pyrolysis mass spectrometry (14). In previous physiological-biochemical studies, Welker and Campbell (17, 18) were also able to separate *B. subtilis* and "*B. amyloliquefaciens*," and Baptist et al. (1) were able to discriminate all four species by their enzyme electrophoresis profiles.

Thus, the combined evidence suggests that "*B. amyloliquefaciens*" is indeed a species separate from *B. subtilis*, even though it may be difficult to differentiate the two species on the basis of classical phenotypic tests alone. The data in Table 1 were derived from the studies of O'Donnell et al. (9) and Logan and Berkeley (8), in which the API system was used, and from our own unpublished data. The latter were obtained from a study of 17 strains of *B. subtilis*, 19 strains of *B. pumilus*, 17 strains of *B. licheniformis*, and 9 strains of "*B. amyloliquefaciens*," including the type strain of each taxon. "*B. amyloliquefaciens*" strains can be distinguished from *B. subtilis* strains by the inability of most strains to hydrolyze DNA and pectin, the failure of the organisms to produce acid from inulin (8), and the formation in most "*B. amyloliquefaciens*" strains of long chains of cells. Where these tests fail to produce a clear separation, DNA homology determinations should produce a definite identification.

The description of "*B. amyloliquefaciens*" given below is based on the data of Welker and Campbell (17) and Gordon et al. (5) and our own unpublished data.

***Bacillus amyloliquefaciens* sp. nov., nom. rev.** *Bacillus amyloliquefaciens* (am. yl. o. li. que. fac' i. ens. L.n. *amylum* starch; M.L. part adj. *amyloliquefaciens* starch digesting). Gram positive rods, 0.7 to 0.9 by 1.8 to 3.0 μm.

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TABLE 1. Characteristics of diagnostic value in distinguishing *B. amyloliquefaciens* from *B. subtilis* and other closely related *Bacillus* species

Characteristic	% Of strains positive			
	B. amyloliquefaciens[a]	*B. subtilis*	*B. licheniformis*	*B. pumilus*
API tests[b]				
Acid produced from:				
Galactose	44	30	100	100
Sorbitol	88	88	95	17
Inulin[c]	11	83	68	1
Arginine dihydrolase	0	0	95	0
α-L-Arabinosidase	0	75	100	100
N-benzoyl-L-leucine aminopeptidase	0	0	100	12
α-D-Glucosidase	12	100	100	0
β-D-Glucosidase	0	87	75	100
L-Pyrrolidone aminopeptidase	100	100	100	0
L-Tryptophan aminopeptidase	100	0	100	87
Degradation tests				
DNA	33	93	100	100
$NO_3 \rightarrow NO_2$	78	100	100	0
Starch	100	100	100	0
Pectin	0	27	95	85
Physiological tests				
Anaerobic growth	0	0	100	0
Phosphatase	100	67	100	15
Propionate utilization	0	0	100	0
Chain formation[c]	84	22	80	5
Guanine-plus-cytosine content (mol%)	44–46	42–48	43–47	42–47

[a] The type strain conforms to the pattern of characteristics of the majority of the strains.
[b] Data from reference 9.
[c] Data from reference 8.

Cells often form chains and are motile, with peritrichous flagella. Oval spores (0.6 to 0.8 by 1.0 to 1.4 μm) are central or paracentral in sporangia which are not swollen. Optimal temperature for growth is 30 to 40°C. No growth occurs below 15°C or above 50°C. Casein, elastin, gelatin, starch, tributyrin, Tween 20, Tween 40, and Tween 60 are degraded, but adenine, cellulose, guanine, hypoxanthine, pectin, testosterone, tyrosine, and xanthine are not. (Only a small proportion of strains is able to degrade DNA.) Acetoin and phosphatase are produced, nitrate is reduced to nitrite, esculin and arbutin are hydrolyzed, citrate is used as a sole carbon source, and growth occurs in the presence of 5% (wt/vol) NaCl, and for most strains 10% NaCl; but neither allantoin nor urea is hydrolyzed. Acid is produced from cellobiose, fructose, glucose, glycerol, lactose, maltose, mannose, mannitol, raffinose, salicin, sorbitol, sucrose, and trehalose when the medium of Gordon et al. (5) is used.

The guanine-plus-cytosine content of the DNA is 44.35 ± 0.38 mol% (mean ± standard deviation) for eight strains as determined by the thermal denaturation method and 44.2 ± 0.7 mol% for the same eight strains as determined by the bouyant density method (17). Extensive DNA pairing studies have shown that *B. amyloliquefaciens* DNA shares less than 25, 13, and 5% homology with DNAs from *B. subtilis*, *B. licheniformis*, and *B. pumilus*, respectively, under optimal conditions (11; Shute, Ph.D. thesis).

B. amyloliquefaciens has been isolated from soil and industrial amylase fermentations. The type strain, strain ATCC 23350 (= Fukomoto strain F), has a DNA base composition of 44.6 mol% guanine plus cytosine.

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