Characterization of alkaliphilic *Bacillus* strains used in industry: proposal of five novel species

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Twenty alkaliphilic bacterial strains from industrial applications or enzyme studies were subjected to a polyphasic taxonomic investigation, including 16S rRNA gene sequencing, determination of genomic DNA G + C content, DNA–DNA hybridization, fatty acid analysis and standard bacteriological characterization. By comparing the groupings obtained based on the genomic DNA G + C content and the construction of a phylogenetic tree based on the 16S rRNA gene sequence, 12 clusters of similar strains were recognized. DNA–DNA hybridization revealed that these clusters represented five novel genospecies. Further analysis supported the proposal of five novel species in the genus *Bacillus*: *Bacillus wakoensis* sp. nov. (type strain N-1^T = JCM 9140^T = DSM 2521^T), *Bacillus hemicellulosilyticus* sp. nov. (type strain C-11^T = JCM 9152^T = DSM 16731^T), *Bacillus cellulosilyticus* sp. nov. (type strain N-4^T = JCM 9156^T = DSM 2522^T), *Bacillus akibai* sp. nov. (type strain 1139^T = JCM 9157^T = ATCC 43226^T) and *Bacillus mannanylyticus* sp. nov. (type strain AM-001^T = JCM 10596^T = DSM 16130^T).

INTRODUCTION

Alkaliphilic *Bacillus* species have important industrial applications due to their ability to produce alkaline enzymes such as protease (Horikoshi, 1971), cyclomaltodextrin glucanotransferase (CGTase) (Yamamoto *et al.*, 1972) and cellulase (Horikoshi *et al.*, 1971). These species produce extracellular enzymes that are resistant to high pH and/or high temperature conditions (Nielsen *et al.*, 1995; Horikoshi, 1999). Since Vedder (1934) first isolated the aerobic, endospore-forming, obligate alkaliphilic bacterium *Bacillus alcalophilus*, many obligately or facultatively alkaliphilic *Bacillus* strains have been isolated for use in industry, biotechnology and physiology (Horikoshi, 1999). However, the majority of these strains have not been identified at the species level. The aim of this study was to classify a collection of 20 industrially important alkaliphilic *Bacillus* strains at the species level.

Abbreviation: CGTase, cyclomaltodextrin glucanotransferase.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of *Bacillus wakoensis* JCM 9140^T, *Bacillus hemicellulosilyticus* JCM 9152^T, *Bacillus cellulosilyticus* JCM 9156^T, *Bacillus akibai* JCM 9157^T and *Bacillus mannanylyticus* JCM 10596^T are AB043851, AB043846, AB043852, AB043858 and AB043864, respectively.

Tables detailing the growth characters, the utilization of carbohydrate substrates and fatty acid profiles of the five novel species are available as supplementary material in IJSEM Online.

METHODS

Bacterial strains and culture conditions. The alkaliphilic bacterial strains investigated in this study are listed in Table 1. The strains were stored as a working collection at 5 °C on Horikoshi II agar slopes (10 g glucose, 5 g polypeptone, 5 g yeast extract, 10 g NaCl, 1 g K₂HPO₄, 0.2 g MgSO₄·7H₂O, 15 g agar, 900 ml distilled water, pH 9, to which 100 ml 10 % NaHCO₃ solution was added aseptically after autoclaving).

Standard bacteriological characterization. Growth experiments at pH 6–11 were performed on Horikoshi II broth adjusted to various pH values: pH 6 (adjusted by adding HCl), pH 7–9 (adjusted by adding NaHCO₃) and pH 10–11 (adjusted by adding Na₂CO₃). Growth at various NaCl concentrations (0–15 %) and at various temperatures (10–60 °C) was investigated in Horikoshi II broth (pH 9). Utilization of carbohydrates was determined with the Biolog automatic identification system, according to the manufacturer's recommendations. Other standard tests were performed by following the general procedures described in Cowan & Steel's manual (Barrow & Feltham, 1993).

Cellular fatty acids and respiratory lipoquinones. Alkaliphilic *Bacillus* strains were cultured in Horikoshi II broth at pH 9 and 30 °C for 2 days, under which conditions all strains exhibited growth. Cells were washed twice with 0.7 % NaCl at 4 °C followed by centrifugation at 8000 g and freeze-drying. Dried cells (20 mg) were placed in Teflon-lined, screw-capped tubes containing 2 ml anhydrous methanolic HCl and heated to 100 °C for 3 h. After cooling, 1 ml water was added and the fatty acid methyl esters were extracted with n-hexane. Samples were analysed for cellular fatty acids using a gas-liquid chromatography/mass spectrometer (Komagata & Suzuki, 1987).

Table 1. Alkaliphilic *Bacillus* strains investigated in this study

CGTase, Cyclomaltodextrin glucanotransferase; CMCase, carboxymethyl cellulase. *Bsp*O4I is a restriction enzyme.

Strain	Product	Reference
O-4 (=JCM 9137=DSM 2514)	Protease, <i>Bsp</i> O4I	Horikoshi & Ikeda (1970)
N-1 ^T (=JCM 9140 ^T =DSM 2521 ^T)	Cellulase	Horikoshi <i>et al.</i> (1971)
17-1 (=JCM 9142=DSM 2524)	Amylase, CGTase	Yamamoto <i>et al.</i> (1972)
27-1 (=JCM 9144=DSM 2520)	Amylase	Yamamoto <i>et al.</i> (1972)
13 (=JCM 9145=DSM 2523)	Amylase, CGTase	Yamamoto <i>et al.</i> (1972)
K-12-5 (=JCM 9149)	β -1,3-Glucanase	Horikoshi & Atsukawa (1973)
202-1 (=JCM 9151)	Pullulanase	Nakamura <i>et al.</i> (1975)
C-11 ^T (=JCM 9152 ^T =DSM 16731 ^T)	Hemicellulase	Ikura & Horikoshi (1977)
D-6 (=JCM 9154)	Protease	Horikoshi & Akiba (1982)
2b-2 (=JCM 9155)		Kudo & Horikoshi (1983)
N-4 ^T (=JCM 9156 ^T =DSM 2522 ^T)	Cellulase	Horikoshi <i>et al.</i> (1971)
1139 ^T (=JCM 9157 ^T =ATCC 43226 ^T)	CMCase	Fukumori <i>et al.</i> (1985)
IC (=JCM 9158)	Enolase	Kitada <i>et al.</i> (1987)
KX-6 (=JCM 9159)	D-Xylose isomerase	Kwon <i>et al.</i> (1987)
H-167 (=JCM 9160)	Amylase	Hayashi <i>et al.</i> (1988)
199 (=JCM 9163)	Cyclomaltodextrinase	Yoshida <i>et al.</i> (1991)
C-3 (=JCM 9164)	5'-Nucleotidase	Ikura & Horikoshi (1989)
S-2 (=JCM 9166)	Galactanase	Tsumura <i>et al.</i> (1991)
AM-001 ^T (=JCM 10596 ^T = DSM 16130 ^T)	β -Mannosidase, β -mannanase	Akino <i>et al.</i> (1987)
8-1 (=JCM 10598)	Protease	Kitada & Horikoshi (1976)

Isoprenoid quinones were extracted with chloroform/methanol (2:1) from dried cells (200 mg) and purified on TLC. The purified isoprenoid quinones were analysed using reverse-phase HPLC (Komagata & Suzuki, 1987).

Genotypic characterization. Chromosomal DNA was purified using a standard method (Saito & Miura, 1963). The DNA G+C content was determined using reverse-phase HPLC (Tamaoka & Komagata, 1984). For analysis of relatedness, DNA–DNA hybridization was carried out at 40 °C for 4 h and measured fluorometrically using the method of Ezaki *et al.* (1989).

16S rRNA gene sequences were obtained by direct sequencing of PCR-amplified DNA as described previously (Kato *et al.*, 1998). Nucleotide substitution rates (K_{nuc}) (Kimura, 1980) were determined and a distance matrix tree was constructed with the neighbour-joining method (Saitou & Nei, 1987) using the CLUSTAL_X program (Thompson *et al.*, 1997). Alignment gaps and unidentified base positions were not taken into consideration for the calculations. The topology of the phylogenetic tree was evaluated by performing a bootstrap analysis with 1000 replicates. The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of the isolates are shown in Fig. 1. Other reference sequences were obtained from the GenBank database.

RESULTS AND DISCUSSION

16S rRNA-based phylogeny and DNA–DNA relatedness

To investigate the taxonomic position of the 20 alkali-philic *Bacillus* strains, 16S rRNA gene sequence analysis and

genomic DNA–DNA hybridization studies were performed. The generally recommended and accepted criteria for delineating bacterial species state that strains with a DNA–DNA relatedness of less than 70 % as measured by hybridization or with a 16S rRNA gene sequence dissimilarity greater than 3 % are considered to belong to separate species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994; Stackebrandt *et al.*, 2002). However, bacterial strains with a difference in 16S rRNA gene sequence of less than 3 % cannot be allocated to the same species without support from DNA–DNA relatedness studies. In the 16S rRNA gene sequence clustering, 12 groups of strains that showed a high degree of internal sequence similarity (98.3–99.7 %) could be delineated. In the neighbour-joining tree, the sequences form a distinct lineage, with alkaliphilic *Bacillus* species as the closest relatives (Fig. 1).

The DNA–DNA hybridization values between *Bacillus clausii* DSM 8716^T and strains O-4, H-167 and IC were greater than 89 % and therefore these three strains were identified as *B. clausii*. DNA–DNA relatedness values between *Bacillus pseudofirmus* DSM 8715^T and strains 8-1, 2b-2 and 27-1 were greater than 80 % and therefore these three strains were identified as *B. pseudofirmus*. For *Bacillus halodurans* DSM 497^T and strains C-3 and 202-1, DNA–DNA relatedness was greater than 93 % and therefore these two strains were identified as *B. halodurans*. Between *Bacillus cohnii* DSM 6307^T and strains D-6 and 199, DNA–DNA relatedness was greater than 78 % and therefore

Fig. 1. Phylogenetic tree showing the relationships of the identified alkaliphilic bacterial strains and the type strains of closely related *Bacillus* species, constructed using the neighbour-joining method and based on 16S rRNA gene sequences. GenBank accession numbers are given in parentheses. Bootstrap values were calculated from multiple resamplings of the sequence dataset, which are the basis for multiple tree topologies. Bar, 0.01 nucleotide substitutions per site.

these two strains were identified as *B. cohnii*. The DNA–DNA hybridization value of 98 % indicated that *Bacillus agaradhaerens* DSM 8721^T and strain S-2 represent the same species and a value of 70 % indicated that *Bacillus horikoshii* DSM 8719^T and strain KX-6 also represent the same species.

The 16S rRNA gene sequence analysis showed that strains 13, 17-1, K-12-5 and DSM 8717 are closely related to each other (99.1–99.7 %). The DNA–DNA reassociation values among strains 13, 17-1, K-12-5 and DSM 8717 were greater than 80 % and therefore these four strains were identified as representatives of the same species. According to the

sequence similarity values and results of the phylogenetic analysis based on these 16S rRNA gene sequences, these strains are most closely related to *Bacillus oshimensis* JCM 12663^T (98.4–98.5 %). The DNA–DNA reassociation value between strain DSM 8717 and *B. oshimensis* JCM 12663^T was 67.6 % (Yumoto *et al.*, 2005). Yumoto *et al.* (2005) found seven characteristics that differentiated *B. oshimensis* JCM 12663^T from DSM 8717. In this study, three of these characteristics, hydrolysis of Tweens 20, 40 and 60, were found to be the same for *B. oshimensis* JCM 12663^T and DSM 8717 and another of these characteristics, lower temperature for growth, was found to be variable for each strain. On the basis of these observations, these strains were

identified as representatives of the same species. However, according to the results of the 16S rRNA gene sequence analysis, strains N-1^T and 1139^T were closely related to *Bacillus krulwichiae* JCM 11691^T (97.4 and 96.1 %, respectively, and between strain N-1^T and 1139^T the sequence similarity value was 97.1 %), but the DNA–DNA relatedness values between strains N-1^T and 1139^T and *B. krulwichiae* JCM 11691^T were less than 30 %, and therefore these strains were identified as representing a novel species.

In the 16S rRNA gene sequence analysis, strain C-11^T was closely related to *Bacillus alcalophilus* DSM 485^T (96.3 %), *B. halodurans* ATCC 27557^T (96.1 %), *Bacillus okuhidensis* JCM 10945^T (96.1 %) and strain 1139^T (96.3 %), but the respective DNA–DNA reassociation values between strain C-11^T and these type strains were less than 25 %, and therefore this strain was identified as representing a novel species. The 16S rRNA gene sequence analysis showed that strain N-4^T was closely related to *Bacillus vedderi* DSM 9768^T (97.4 %), but the DNA–DNA reassociation value between strain N-4^T and this type strain was less than 27 % and therefore this strain was identified as representing a novel species. In the 16S rRNA gene sequence analysis of strain AM-001^T, the highest similarity was observed with *Bacillus horti* JCM 9943^T (94.1 %). The DNA–DNA relatedness value between strain AM-001^T and this type strain was less than 20 % and therefore this strain was identified as representing a novel species.

Physiological and biochemical characteristics

Phenotypic characteristics of the 20 alkaliphilic *Bacillus* strains and alkaliphilic *Bacillus* type strains are shown in Table 2 and in Supplementary Table S1 in IJSEM Online, respectively. The utilization of carbohydrate substrates for the novel species, as deduced from the Biolog tests, is shown in Supplementary Table S2 in IJSEM Online. A comparison of the physiological and biochemical characteristics of the five groups showed some differences when compared with the type strains and with each other. Strain N-1^T and *B. pseudoalcalophilus* grew at similar temperatures (10–40 °C), pH (8–10) and NaCl concentration (0–10 %), but showed varying results for the hydrolysis of casein and gelatin and for the reduction of nitrate. Strains C-11^T and N-1^T grew at similar temperatures, pH and NaCl concentrations (C-11^T: 10–40 °C, pH 8–11, 0–12 % NaCl; N-1^T: 20–40 °C, pH 8–10, 0–12 % NaCl) as *B. clarkii* (20–45 °C, pH 8–11, 0–12 % NaCl) and *B. krulwichiae* (temperature range not determined, pH 8–10, 0–12 % NaCl), although their hydrolysis of casein and gelatin and their menaquinone content were different. Strain C-11^T also showed characteristic utilization of the carbohydrate substrates D-melibiose, D-raffinose and stachyose. Strain 1139^T and *B. cohnii* displayed growth at similar temperatures, pH and NaCl concentrations (20–45 °C, pH 8–11, 0–7 % NaCl), although they are phylogenetically separate and their hydrolysis of casein and gelatin is different. Strain 1139^T utilized various carbohydrates. Strain AM-001^T showed characteristics unlike those of any other strain and utilized a limited range of carbohydrates.

Fatty acid analysis

Almost all of the whole-cell fatty acid profiles of the alkaliphilic *Bacillus* strains grown at pH 9 consisted of the major fatty acids iso-C_{15:0} and anteiso-C_{15:0}, except for the profile of strain AM-001^T (see Supplementary Table S3 in IJSEM Online). Most of the whole-cell fatty acid content of strain AM-001^T was C_{16:0} and C_{16:1}, which differed markedly from those of the other strains. Strains N-4^T, C-11^T, N-1^T and 1139^T also contained slightly more C_{16:0} and slightly less iso-C_{15:0} compared with other strains (Supplementary Table S3 in IJSEM Online). Strain 1139^T had more fatty acids of comparatively short chain length, such as iso-C_{14:0} or C_{14:0}, compared with other strains.

On the basis of these chemotaxonomic and phylogenetic studies, five novel alkaliphilic *Bacillus* species are proposed.

Description of *Bacillus wakoensis* sp. nov.

Bacillus wakoensis (wa.ko.en'sis. N.L. masc. adj. *wakoensis* of Wako, a city in Japan).

Cells are rod-shaped, 1.5–2.0 µm in length and 0.5–0.8 µm in width, Gram-positive. Cells are motile by peritrichous flagella and produce spores that are ellipsoidal and located terminally in the sporangium, which is swollen. Colonies are circular and yellowish. Grows between 10 and 40 °C, with the optimum at around 37 °C. The range of pH for growth is from 8 to 10, with the optimum at pH 9–10. Tests positive for catalase, for hydrolysis of starch and cellulose and for nitrate reduction. It is negative for hydrolysis of Tweens 20, 40 and 60, casein, gelatin and oxidase and for the production of indole and H₂S. Acid is produced from arbutin, cellobiose, D-fructose, glucose, D-maltose, D-mannitol, D-mannose, D-psicose, salicin, D-sorbitol, sucrose, D-trehalose, turanose and D-xylose; no gas evolution occurs from these carbohydrates. The organism can grow at an NaCl concentration of 10 % (w/v). The major isoprenoid quinone types are MK-6 and MK-7. Major fatty acids are iso-C_{15:0}, anteiso-C_{15:0} and C_{16:0}. The genomic DNA G + C content is 38.1 mol%, as determined by HPLC.

The type strain is N-1^T (= JCM 9140^T = DSM 2521^T).

Description of *Bacillus hemicellulosilyticus* sp. nov.

Bacillus hemicellulosilyticus (hem.i.cell.u.lo.si.ly'ti.cus. N.L. neut. n. *hemicellulosum* hemicellulose; Gr. adj. *lutikos* able to loosen, able to dissolve; N.L. masc. adj. *hemicellulosilyticus* hemicellulose-dissolving).

Cells are rod-shaped, 2.0–6.0 µm in length and 0.3–0.5 µm in width, Gram-variable. Cells are motile by peritrichous flagella and produce spores that are ellipsoidal and located terminally in the sporangium, which is swollen. Colonies are circular and white. It grows at between 10 and 40 °C, with the optimum at around 37 °C. The range of pH for growth is from 8 to 11, with the optimum at around pH 10. Tests

Table 2. Phenotypic characteristics of the novel and other alkaliphilic *Bacillus* species

One strain per species was examined unless indicated otherwise. Species: 1, *B. wakoensis* sp. nov.; 2, *B. hemicellulosilyticus* sp. nov.; 3, *B. cellulosilyticus* sp. nov.; 4, *B. akibai* sp. nov.; 5, *B. mannanylyticus* sp. nov.; 6, *B. agaradhaerens* (two strains); 7, *B. alcalophilus*; 8, *B. clarkii*; 9, *B. clausii* (four strains); 10, *B. cohnii* (three strains); 11, *B. gibsonii*; 12, *B. halmapalus*; 13, *B. halodurans* (three strains); 14, *B. horikoshii* (two strains); 15, *B. horti*; 16, *B. krulwichiae*; 17, *B. okuhidensis*; 18, *B. oshimensis* (five strains); 19, *B. pseudoalcalophilus*; 20, *B. pseudofirmus* (four strains); 21, *B. vedderi*. +, Positive; –, negative; $\pm$, slightly swollen to not swollen; v, variable; ND, no data. Data for species 7, 8, 11, 12 15–17, 19 are from Nielsen *et al.* (1995), Yumoto *et al.* 1998, 2003, Li *et al.* (2002) and Agnew *et al.* (1995). Data for species 18 are from Yumoto *et al.* (2005) and this study.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Spore location*	T	T	ST	ST	T	ST	ST	ST	STC	T	STC	STC	ST	ST	ST	ST	T	STT	STC	STC	T
Swollen sporangia	+	+	+	$\pm$	+	+	–	+	$\pm$	+	–	–	$\pm$	$\pm$	ND	–	–	$\pm$	+	–	+
Hydrolysis of:																					
Casein	–	–	–	–	+	–	+	+	+	V	+	+	+	+	+	V	+	+	+	+	–
Gelatin	–	–	–	–	+	–	+	+	+	+	+	+	+	+	+	V	+	+	+	+	+
Starch	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Tween 20	–	+	+	+	–	–	V	–	–	V	–	–	v	–	–	+	–	+	–	–	ND
Tween 40	–	+	+	+	+	+	+	+	–	V	V	–	+	V	–	+	–	+	V	+	ND
Tween 60	–	+	+	+	+	+	+	+	–	+	V	–	+	V	–	+	–	+	V	+	ND
Reduction of nitrate	+	–	+	+	+	+	V	+	+	+	V	–	V	–	+	+	+	–	–	–	ND
Menaquinone content (%):																					
MK-5																3					
MK-6	5		1	5		1	21		13	1	93	3	11		4	28		2	13	55	
MK-7	95	100	99	95	100	99	79	100	87	99	7	97	89	99	96	69	ND	98	87	45	100
MK-8														1							
DNA G+C content (mol%)	38·1	36·8	39·6	34·4	37·4	36·5–36·8	36·2–38·4	42·4–43·0	41·7–43·5	33·8–35·0	40·6–41·7	38·6	41·5–41·9	39·8–40·7	40·2–40·9	40·6–41·5	41·0–41·1	39·1–40·8	38·2–39·0	38·7–40·9	38·3

*ST, subterminal; STC, subterminal to central; STT, subterminal to terminal; T, terminal.

positive for catalase and for the hydrolysis of Tweens 20, 40 and 60 and hemicellulose. It is negative for oxidase, hydrolysis of soluble starch, gelatin and casein, for the production of indole and H₂S and for nitrate reduction. Acid is produced from arbutin, cellobiose, D-fructose, D-glucose, D-lactose, D-maltose, maltotriose, D-mannose, D-mannitol, D-melibiose, palatinose, D-raffinose, salicin, D-sorbitol, stachyose, sucrose, D-trehalose and turanose; no gas evolution occurs from these carbohydrates. The organism can grow at an NaCl concentration of 12 % (w/v), but not at 15 %. The major isoprenoid quinone type is MK-7. Major fatty acids are iso-C_{15:0}, anteiso-C_{15:0}, C_{16:0} and anteiso-C_{17:0}. The genomic DNA G+C content is 36.8 mol%, as determined by HPLC.

The type strain is C-11^T (=JCM 9152^T=DSM 16731^T).

Description of *Bacillus cellulosityticus* sp. nov.

Bacillus cellulosityticus (cell.u.lo.si.ly'ti.cus. N.L. neut. n. *cellulosum* cellulose; Gr. adj. *lutikos* able to loosen, able to dissolve; N.L. masc. adj. *cellulosityticus* cellulose-dissolving).

Cells are rod-shaped, 2.0–3.0 µm in length and 0.3–0.4 µm in width, Gram-positive. Cells are motile by peritrichous flagella and produce spores that are ellipsoidal and located subterminally in the sporangium, which is swollen. Colonies are circular to slightly irregular and cream-coloured. Grows at between 20 and 40 °C, with the optimum at around 37 °C. The range of pH for growth is from 8 to 10, with the optimum at around pH 9–10. Tests positive for catalase and nitrate reduction and for the hydrolysis of soluble starch, Tweens 20, 40 and 60 and cellulose. It is negative for oxidase, for hydrolysis of gelatin and casein and for the production of indole and H₂S. Acid is produced from arbutin, cellobiose, D-fructose, D-glucose, D-lactose, D-maltose, maltotriose, D-mannose, salicin, sucrose, D-trehalose and turanose; no gas evolution occurs from these carbohydrates. The organism can grow at an NaCl concentration of 12 % (w/v) NaCl, but not at 15 %. The major isoprenoid quinone type is MK-7. Major fatty acids are iso-C_{15:0}, anteiso-C_{15:0} and C_{16:0}. The genomic DNA G+C content is 39.6 mol%, as determined by HPLC.

The type strain is N-4^T (=JCM 9156^T=DSM 2522^T).

Description of *Bacillus akibai* sp. nov.

Bacillus akibai (a.ki.ba'i. N.L. gen. n. *akibai* of Akiba, named after the Japanese microbiologist Teruhiko Akiba, who made fundamental contributions to the study of alkaliphilic bacteria).

Cells are rod-shaped, 3.0–4.0 µm in length and 0.6–0.8 µm in width, Gram-positive. Cells are motile by peritrichous flagella and produce spores that are ellipsoidal and located subterminally in the sporangium, which is slightly swollen. Colonies are circular and yellowish. It grows at between 20 and 45 °C, with the optimum at around 37 °C. The range of pH for growth is from 8 to 10, with the optimum at around

pH 9–10. Tests positive for catalase, for nitrate reduction and for the hydrolysis of soluble starch, Tweens 20, 40 and 60 and cellulose. It is negative for oxidase, for hydrolysis of gelatin and casein and for the production of indole and H₂S. Acid is produced from L-arabinose, arbutin, cellobiose, D-fructose, D-galactose, D-glucose, D-maltose, maltotriose, D-mannose, D-mannitol, D-melezitose, palatinose, D-psicose, salicin, D-sorbitol, sucrose, D-trehalose and turanose; no gas evolution occurs from these carbohydrates. The organism can grow at an NaCl concentration of 7 % (w/v), but not at 10 %. The major isoprenoid quinone types are MK-6 and MK-7. Major fatty acids are iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0} and C_{16:0}. The genomic DNA G+C content is 34.4 mol%, as determined by HPLC.

The type strain is 1139^T (=JCM 9157^T=ATCC 43226^T).

Description of *Bacillus mannanilyticus* sp. nov.

Bacillus mannanilyticus (mann.an.i.ly'ti.cus. N.L. neut. n. *mannanum* mannan; Gr. adj. *lutikos* able to loosen, able to dissolve; N.L. masc. adj. *mannanilyticus* mannan-dissolving).

Cells are rod-shaped, 3.0–6.0 µm in length and 0.6–0.8 µm in width, Gram-variable. Cells are motile by peritrichous flagella and produce spores that are ellipsoidal and located terminally in the sporangium, which is swollen. Colonies are circular and yellow. It grows at between 20 and 45 °C, with the optimum at around 37 °C. The range of pH for growth is from 8 to 10, with the optimum at around pH 9. The bacterium tests positive for catalase and for the hydrolysis of Tweens 40 and 60, soluble starch, gelatin, casein and mannan and is negative for oxidase, for the production of indole and H₂S and for nitrate reduction. Acid is produced from D-fructose, glucose, D-maltose, mannose, D-galactose, D-xylose, D-mannitol, sorbitol, D-trehalose, raffinose, sucrose, D-lactose, glycerol, inulin and starch; no gas evolution occurs from these carbohydrates. The organism can grow at an NaCl concentration of 3 % (w/v), but not at 5 %. The major isoprenoid quinone type is MK-7. Major fatty acids are C_{16:0} and C_{16:1}. The genomic DNA G+C content is 37.4 mol%, as determined by HPLC.

The type strain is AM-001^T (=JCM 10596^T=DSM 16130^T).

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