Bacillus curdlanolyticus sp. nov. and *Bacillus kobensis* sp. nov., Which Hydrolyze Resistant Curdlan

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Taxonomic characteristics of seven bacterial strains which were isolated from soil and hydrolyze resistant curdlan were studied. These bacteria were aerobic, spore-forming rods, contained menaquinone 7 as a major quinone, contained anteiso-C_{15:0} and iso-C_{16:0} as major cellular fatty acids, had guanine-plus-cytosine contents of 50 to 52 mol%, and could be divided into two groups on the basis of physiological and chemotaxonomic characteristics and DNA-DNA hybridization data. We propose the following two new species: *Bacillus curdlanolyticus* for strains YK9, YK121, YK161, YK201, and YK203, with type strain YK9 (= IFO 15724); and *Bacillus kobensis* for strains YK205 and YK207, with type strain YK205 (= IFO 15729).

Many microorganisms are able to produce (1→3)-β-D-glucanase (17). Some bacteria produce endo-(1→3)-β-D-glucanase, whereas certain fungi and yeasts produce exo-(1→3)-β-D-glucanase to yield α-glucose. Curdlan is composed entirely of (1→3)-β-D-glucosidic linkages (6) and is utilized as a substrate by (1→3)-β-D-glucanase. When glucan is heated to more than 95°C in water, it becomes resistant to the actions of Zymolyase, a crude preparation of (1→3)-β-D-glucanase (19), and to 32% sulfuric acid at 32°C (8). The resistant curdlan forms pseudocrystals which are mainly composed of triple helices, as shown by X-ray diffraction analysis and electron microscopy (5). We have isolated many bacterial strains that are able to hydrolyze resistant curdlan from soil (7). Seven of these strains were found to belong to the genus *Bacillus*, and strain YK9^T (T = type strain) produced a high level of exo-(1→3)-β-D-glucanase to yield α-laminaribiose (9).

In this study, we examined physiological and chemotaxonomic properties of these strains, the abilities of the strains to hydrolyze many polysaccharides, including curdlan, and the G+C contents of the DNAs of the strains, and we determined the levels of DNA relatedness between our isolates and related species. We propose that five and 2 of these strains belong to the new species *Bacillus curdlanolyticus* and *Bacillus kobensis*, respectively.

MATERIALS AND METHODS

Bacterial strains. The strains used in this study are listed in Table 1. All of the strains were cultured at 30°C on medium containing 0.5% yeast extract, 0.5% peptone, and 1.5% agar (pH 7.2) and were stored at room temperature.

Phenotypic characteristics. Cell morphology was examined by using cells grown on nutrient agar (18). Unless otherwise stated, all tests were carried out at 30°C. Catalase activity was determined by examining bubbling in a 3% hydrogen peroxide solution. Oxidase activity was determined by examining the oxidation of 1% tetramethyl-*p*-phenylenediamine on filter paper. Acid production from carbohydrates (0.5%) after 5 days of incubation was determined by using media containing 0.15% (NH₄)₂HPO₄, 0.1% KH₂PO₄, 0.05% MgSO₄·7H₂O, and 1.5% agar; the pH of each medium was adjusted to 7.0 before a solution of bromocresol purple was added.

Hydrolysis of polysaccharides. A synthetic medium containing each polysac-

charide at a concentration of 0.2%, the inorganic salts described above, and 1.5% agar was used to determine hydrolysis of polysaccharides. Hydrolysis was observed after 5 days of incubation. Hydrolysis of curdlan (regenerated and resistant) was determined by the method described previously by Kanzawa et al. (7). Hydrolysis of pustulan was determined by the method of Alexander and Priest (1). Hydrolysis of β-1,2-Glucan and carboxymethyl cellulose was determined by using a method similar to the method used to determine pustulan hydrolysis.

DNA base compositions. DNA was obtained by the method of Saito and Miura (16). The G+C contents of DNAs were determined by the method of Mesbah et al. (12).

DNA hybridization. DNA-DNA hybridization was carried out at 45°C for 2 h fluorometrically in microdilution wells by using biotinylated DNA (3, 4).

Cellular fatty acid compositions and quinone systems. Cellular fatty acids and isoprenoid quinones were prepared and studied as described by Komagata and Suzuki (10).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole-cell proteins. Wet cells (0.1 g) were suspended in 1.0 ml of 0.0625M Tris-HCl buffer (pH 6.8) containing 2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, and 10% glycerol and heated at 100°C for 10 min. After centrifugation at 13,000 × *g* for 10 min, the supernatant was subjected to electrophoresis by the method of Laemmli (11).

RESULTS AND DISCUSSION

Strains YK9^T, YK122, YK161, YK201, YK203, YK205^T, and YK207 were isolated from soil as organisms that were able to attack resistant curdlan (7). YK9^T colonies growing on medium containing glucose or curdlan and inorganic salts or on peptone-yeast extract medium with or without glucose are shown in Fig. 1. Clear motile colonies grew on synthetic medium containing curdlan or glucose, whereas round colonies usually grew on natural medium with or without glucose. Growth of the organism was poor without glucose or curdlan as a carbon source. We compared the taxonomic properties of our isolates with those of related *Bacillus* species. All of our strains (YK9^T, YK122, YK161, YK201, YK203, YK205^T, and YK207) were gram-positive, motile rods with cell diameters of >1.0 μm. Ellipsoidal spores were formed in swollen sporangia. These organisms were catalase positive, but oxidase negative. Gas was not formed from glucose. The major cellular fatty acid components were anteiso-C_{15:0} and iso-C_{16:0}. The isoprenoid quinone was menaquinone 7. On the basis of the similar properties of our strains and members of the *Bacillus circulans* complex, we assumed that our strains belong to the *B. circulans* complex (2); our strains belong to a sepa-

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TABLE 1. Bacterial strains used in this study

Strain or species	IFO no. ^a	Origin or other designation(s) ^a
YK9 ^T	15724 ^T	Soil from Kobe City
YK122	15725	Soil from Kobe City
YK161	15726	Soil from Kobe City
YK201	15727	Soil from Kobe City
YK203	15728	Soil from Kobe City
YK205 ^T	15729 ^T	Soil from Kobe City
YK207	15730	Soil from Kobe City
<i>Bacillus circulans</i> WL-12		
<i>Bacillus circulans</i>	3329	ATCC 9966
<i>Bacillus circulans</i>	3967	
<i>Bacillus circulans</i>	13157	NRRL B-3312
<i>Bacillus circulans</i>	13294	
<i>Bacillus circulans</i>	13579	ATCC 8384
<i>Bacillus circulans</i>	13626 ^T	ATCC 4513 ^T , NCTC 2610 ^T , NCIB 9374 ^T
<i>Bacillus circulans</i>	13627	ATCC 4515
<i>Bacillus circulans</i>	13628	ATCC 4516
<i>Bacillus circulans</i>	13629	ATCC 4530
<i>Bacillus circulans</i>	13630	ATCC 8241
<i>Bacillus circulans</i>	13632	ATCC 14176
<i>Bacillus circulans</i>	13633	ATCC 14175
<i>Bacillus circulans</i>	13634	NRRL NRS-826
<i>Bacillus circulans</i>	13637	NRRL NRS-841
<i>Bacillus circulans</i>	13639	NRRL NRS-977
<i>Bacillus circulans</i>	13640	NRRL NRS-1108
<i>Bacillus circulans</i>	15377	NRRL NRS-381
<i>Bacillus circulans</i>	15378	NRRL NRS-1434
<i>Bacillus circulans</i>	15379	NRRL NRS-1173
<i>Bacillus validus</i>	13635	NRRL NRS-838a
<i>Bacillus validus</i>	13636	NRRL NRS-839a
<i>Bacillus validus</i>	15382 ^T	NRRL NRS-1000 ^T , DSM 3037 ^T
<i>Bacillus pabuli</i>	13638 ^T	NRRL NRS-924 ^T
<i>Bacillus glucanolyticus</i>	15330 ^T	DSM 5162 ^T , NCB 12809 ^T
<i>Bacillus amylolyticus</i>	13625 ^T	ATCC 9995 ^T
<i>Bacillus alginolyticus</i>	15375 ^T	NRRL NRS-1347 ^T , DSM 5050 ^T
<i>Bacillus chondroitinus</i>	15376 ^T	NRRL NRS-1351 ^T , DSM 5051 ^T
<i>Bacillus lautus</i>	15380 ^T	NRRL NRS-666 ^T , DSM 3035 ^T
<i>Bacillus parabrevis</i>	12334 ^T	ATCC 10027 ^T
<i>Bacillus choshinensis</i>	15518 ^T	JCM 8505 ^T
<i>Bacillus centrosporus</i>	15540 ^T	NRRL NRS-664 ^T
<i>Bacillus agri</i>	15538 ^T	NRRL NRS-1219 ^T
<i>Bacillus macerans</i>	15307 ^T	ATCC 8244 ^T , JCM 2500 ^T , CCM 2012 ^T
<i>Bacillus thiaminolyticus</i>	15656 ^T	JCM 8360 ^T

^a Abbreviations for culture collections: IFO, Institute for Fermentation, Osaka, Osaka, Japan; ATCC, American Type Culture Collection, Rockville, Md.; NRRL, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Ill.; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; NCIB, National Collection of Industrial and Marine Bacteria, Ltd., Aberdeen, Scotland; JCM, Japan Collection of Microorganisms, RIKEN, Wako, Saitama, Japan; CCM, Czech Collection of Microorganisms, Masarik University, Brno, Czech Republic.

rate group in the complex since the DNA G+C contents of our strains were 50.1 to 51.8 mol% and the G+C contents of other members of the *B. circulans* complex were 37 to 54 mol% (14).

The ability to hydrolyze polysaccharides is thought to be important in the classification of members of the *B. circulans* complex. For example, the names *Bacillus amylolyticus* (12), *Bacillus alginolyticus* (13) *Bacillus chondroitinus* (13), and *Bacillus glucanolyticus* (1) were proposed on the basis of the abilities of the organisms to hydrolyze starch, alginic acid, chondroitin, and β -glucans (such as pustulan), respectively. We examined the abilities of our strains and related members of

the *B. circulans* complex to hydrolyze β -glucans containing curdlan, cyclic β -1,2-glucan, carboxymethyl cellulose, and pustulan (β -1,6-glucan) (Table 2). Our isolates hydrolyzed resistant curdlan, as well as regenerated curdlan and pustulan. Strain YK207 also hydrolyzed β -1,2-glucan. Strain WL-12, which had a lower DNA G+C content than our strains, hydrolyzed regenerated curdlan, but not resistant curdlan. There are many strains which hydrolyze regenerated curdlan among the organisms which have G+C contents similar to or slightly lower than the DNA G+C contents of our strains. We selected *B. glucanolyticus* IFO 15330^T, *Bacillus lautus* IFO 15380^T, and *B. circulans* IFO 13157 as strains that are able to hydrolyze regenerated curdlan and pustulan and have DNA G+C contents similar to the G+C contents of our strains. We also selected a strain of the G form of the *B. circulans* complex, IFO 15377, which had a G+C content similar to the G+C contents of our strains, although this strain was not able to hydrolyze curdlan.

Table 3 shows characteristics which distinguish our organisms from related species. There are many differences between our strains and related organisms. One significant difference is the inability of our organisms to utilize the five organic acids tested. Acetate is utilized by all of the other organisms tested. Our organisms are not able to form acids from galactose, mannose, and ribose, but the other organisms which we studied produce acids from those sugars. Strains YK205^T and YK207 cannot produce acids from glycerol and raffinose, which distinguishes them from the other new strains.

Table 4 shows the levels of DNA relatedness between members of the *B. circulans* complex and related strains that hydrolyze curdlan. The group I strains (YK9^T, YK122, YK161, YK201, YK203) exhibited high levels of DNA relatedness (more than 87%) to strain YK9^T, and the group II strains (YK205^T, YK207) exhibited high levels of relatedness (more than 94%) to strain YK205^T. The group I and II strains exhibited low levels of DNA relatedness to the reference strains of the other species (less than 22 and 31%, respectively).

Strains IFO 13157 (*B. circulans*), IFO 15380^T (*B. lautus*; group H of the *B. circulans* complex), and IFO 15330^T (*B. glucanolyticus*), which have DNA G+C contents of 51.4, 50 to 52, and 48 to 50 mol%, respectively, and are able to hydrolyze resistant curdlan, exhibited low levels of DNA relatedness strains YK9^T and YK205^T; the levels of relatedness between these strains and YK9^T were 12, 8, and 1%, respectively, and the levels of relatedness between these strains and YK205^T were 16, 11, and 3%, respectively. Strain IFO 15377 (group G of the *B. circulans* complex), which had a G+C content of 51 to 52 mol% and was not able to hydrolyze curdlan, also exhibited low levels of DNA relatedness to strains YK9^T and YK205^T (12 and 13%, respectively).

In addition to the DNA relatedness of strains belonging to the *B. circulans* complex, we also examined the DNA relatedness of six *Bacillus* strains that had DNA G+C contents of 49 to 54 mol% (Table 4). One of these strains, strain IFO 15307 (*Bacillus macerans*), is able to hydrolyze curdlan. All of these strains exhibited less than 20% DNA relatedness to strains YK9^T and YK205^T. Thus, all of the *Bacillus* strains which we tested exhibited low levels of DNA relatedness to the new isolates.

Next, we examined the whole-cell protein patterns of our isolates by performing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 2). The patterns of all of the group I strains (YK9^T, YK122, YK161, YK201, YK203) were very similar to the YK9^T pattern, and the patterns of the two group II strains (YK205^T, YK207) were very similar to each other; the

FIG. 1. Colonies of strain YK9^T grown on agar medium containing 0.5% glucose (A) or 0.5% curdlan (B) and inorganic salts (pH 7.2) and on agar medium containing 0.5% peptone and 0.5% yeast extract with (C) or without (D) 0.5% glucose (pH 7.2). The photographs were taken after 10 days of incubation.

group I and II patterns were similar in many respects, but differed significantly in some bands.

One of the characteristics of the isolates which we studied is the ability to hydrolyze regenerated and resistant curdlans. The principal enzyme which hydrolyzes curdlan in YK9^T was purified, and its properties have been investigated previously by us (9). We found that the molecular mass of the protein of the enzyme is about 70 kDa, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and that the β -1,3-glucanase band is a major band in the pattern of YK9^T proteins obtained from a culture filtrate grown on curdlan-inorganic salts medium. This enzyme was found to be an exohydrolase which liberates α -laminaribiose.

On the basis of the results described above, we propose two new species, *Bacillus curdlanolyticus* and *Bacillus kobensis*, for the organisms which we studied. Descriptions of these species are given below.

Description of *Bacillus curdlanolyticus* sp. nov. *Bacillus curdlanolyticus* (curd.lan. o. ly'ti. cus. Engl. n. curdlan, a polysaccharide produced by bacteria; Gr. adj. lyticus, dissolving; N.L. adj. curdlanolyticus, hydrolyzing curdlan). Cells are rod shaped (0.5 to 1.0 by 2.0 to 6.0 μ m). Gram positive. Motile by means of peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia. Colonies are flat, smooth, and opaque on nutrient agar plates and are motile during growth in synthetic medium containing glucose or curdlan and inorganic salts.

Colonies grow poorly in the absence of glucose or a carbon source. Aerobic. Catalase positive, but oxidase negative. The Voges-Proskauer reaction is negative, and the pH in Voges-Proskauer broth is 5.3 to 5.4. Hydrogen sulfide and indole are not produced. Nitrate is reduced to nitrite. Casein is not hydrolyzed, but urea is hydrolyzed. Curdlan, including resistant curdlan, pustulan, starch, and pullulan are hydrolyzed, but carboxymethyl cellulose and cyclic 1,2- β -glucan are not hydrolyzed. Growth occurs in the presence of 0.001% lysozyme, but does not occur in the presence of 5% NaCl. No growth occurs at 50°C. Acid but no gas is produced from glucose, fructose, xylose, L-arabinose, glycerol, sucrose, maltose, cellobiose, lactose, salicin, trehalose, and raffinose. Acid is not produced from galactose, mannose, rhamnose, ribose, melibiose, sorbitol, and mannitol. The major cellular fatty acid components are anteiso-C_{15:0} and iso-C_{16:0}. The major quinone is menaquinone 7. The G+C content ranges from 50 to 52 mol%. Habitat: isolated from soil.

The type strain is IFO 15724 (= YK9).

Description of *Bacillus kobensis* sp. nov. *Bacillus kobensis* (ko. ben'sis. M. L. adj. kobensis, referring to Kobe City, Hyogo Prefecture, Japan, the source of the soil from which the organisms were isolated.) Cells are rod shaped (0.5 to 1.0 by 2.0 to 6.0 μ m). Gram positive. Motile by means of peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia. Colonies are flat, smooth, and opaque on nutrient agar plates and

TABLE 2. Hydrolysis of polysaccharides by *Bacillus* isolates that are able to hydrolyze resistant curdian and related *Bacillus* strains

Strain	<i>B. circulans</i> complex group ^a	G+C content (mol%) ^b	Hydrolysis of:				
			Regenerated curdian	Resistant curdian	β -1,2-Glucan	Carboxymethyl cellulose	Pustulan
YK9 ^T		51.5	+++ ^c	++	—	—	+
YK122		51.2	+++	++	—	—	+
YK161		50.3	+++	++	—	—	+
YK201		51.2	++	+	—	—	++
YK203		51.1	++	+	—	—	++
YK205 ^T		51.0	++	+	—	—	++
YK207		51.8	++	+	+	—	++
<i>B. alginolyticus</i> IFO 15375 ^T	C	47-49	—	—	+	+	—
<i>B. amylohyticus</i> IFO 13625 ^T	K	53	+	—	—	—	+
<i>B. circulans</i>							
IFO 3967		35.2	—	—	—	—	—
IFO 13626 ^T	A	37-39	—	—	—	—	—
IFO 13627		37-39	—	—	—	—	—
IFO 13628		37-39	—	—	—	—	—
IFO 13629		37-39	—	—	—	—	—
IFO 13634		37-39	—	—	—	—	—
IFO 13640		37-39	—	—	—	—	—
IFO 15378	B	45-47	—	—	—	—	—
WL-12		45.8	+++	—	+	+	+
IFO 15379	F	48-49	+	—	—	+	+
IFO 13633		49.0	—	—	+	—	+
IFO 13294		49.9	+	—	+	—	+
IFO 13630		49-50	++	—	—	—	—
IFO 13632		50.9	—	—	+	—	—
IFO 13639		50.5	+	—	+	+	+
IFO 13157		51.4	+++	++	—	—	+
IFO 15377	G	51-52	—	—	—	—	—
IFO 3329		53.0	—	—	—	+	—
IFO 13637		53-54	++	—	+	+	—
IFO 13579		55.5	++	++	+	+	+
<i>B. chondroitinus</i> IFO 15376 ^T	D	49.0	—	—	—	+	—
<i>B. glucanolyticus</i> IFO 15330 ^T		48-50	+++	—	+	+	+
<i>B. lautus</i> IFO 15380 ^T	H	50-52	++	—	+	+	+
<i>B. pabuli</i> IFO 13638 ^T	E	48-50	+	—	—	+	—
<i>B. validus</i>							
IFO 15382 ^T	I	53-54	—	—	+	+	+
IFO 13635		53-54	—	—	+	+	—
IFO 13636		53-54	—	—	—	+	—

^a See reference 2.^b Data from this study and references 2 and 15.^c +++, lytic zone more than 1 cm wide; ++, lytic zone 0.5 to 1 cm wide; +, lytic zone 0.1 to 0.5 cm wide; —, no lytic zone. All data were obtained after incubation for 5 days.

TABLE 3. Characteristics that distinguish the new soil isolates from related *Bacillus* species

Characteristic	Strains YK9 ^T , YK122, YK161, YK201, and YK203	Strains YK205 ^T and YK207	<i>B. glucanolyticus</i> IFO 15330 ^T	<i>B. lautus</i> IFO 15380 ^T	<i>B. circulans</i> IFO 15377	<i>B. circulans</i> IFO 13157
G+C content (mol%)	50.1-51.8	50.1-51.8	48-49	50-52	51-52	51.4
Anaerobic growth	—	—	+	+	—	ND ^a
Growth in the presence of 5% NaCl	—	—	+	+	—	ND
Growth with 0.001% lysozyme	+	+	ND	+	+	ND
Nitrate reduction	+	+	+	+	—	ND
pH in Voges-Proskauer broth of <5.5	+	+	+	+	—	ND
Hydrolysis of:						
Curdlan	+	+	+	+	—	+
Carboxymethyl cellulose	—	—	+	+	—	—
Pustulan	+	+	+	+	—	+
Starch	+	+	+	+	—	+
Casein	—	—	ND	—	ND	ND
Urea	+	+	—	+	—	—
β -1,2-Glucan	—	—(+) ^b	+	+	—	—
Production of:						
Indole	—	—	—	—	—	—
H ₂ S	—	—	—	—	—	—
Utilization of:						
Acetate	—	—	+	+	+	+
Succinate	—	—	+	—	—	—
Malate	—	—	+	—	—	+
Fumarate	—	—	+	—	+	+
Citrate	—	—	+	+	—	—
Acid production from:						
Glucose	+	+	+	+	+	+
Fructose	+	+	+	+	—	—
Galactose	—	—	+	+	+	+
Mannose	—	—	+	+	+	+
Rhamnose	—	—	+	+	+	—
Xylose	+	+	+	+	+	—
L-Arabinose	+	+	+	+	+	—
Ribose	—	—	+	+	+	+
Glycerol	+	—	+	—	—	+
Sucrose	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Cellobiose	+	+	+	+	—	+
Lactose	+	+	+	+	—	—
Salicin	+	+	+	—	—	+
Trehalose	+	+	+	+	+	+
Melibiose	—	—	+	+	—	+
Raffinose	+	—	+	+	—	—
Sorbitol	—	—	—	—	—	—
Mannitol	—	—	+	+	—	+

^a ND, not determined.^b Strain YK207 hydrolyzed β -1,2-glucan.

TABLE 4. Levels of DNA relatedness for members of the *B. circulans* complex that hydrolyze resistant curdlan

Strain	G+C content (mol %)	Curdlan hydrolysis	% Reassociation with DNA from ^a :	
			YK9 ^T	YK205 ^T
<i>B. curdlano-lyticus</i>				
YK9 ^T	51.5	+	100	31
YK122	51.2	+	89	25
YK161	50.3	+	97	29
YK201	51.2	+	97	27
YK203	51.1	+	87	26
<i>B. kobensis</i>				
YK205 ^T	51.0	+	22	100
YK207	51.8	+	15	94
<i>B. circulans</i> complex				
WL-12	45.8	+	9	12
IFO 13630	49-50	+	7	7
IFO 13639	50.5	+	7	4
IFO 13632	50.9	—	9	9
IFO 13157	51.4	+	12	16
<i>B. pabuli</i> IFO 13638 ^{Tb}	48-50	+	10	11
<i>B. circulans</i> IFO 15379 ^c	48-49	+	11	13
<i>B. circulans</i> IFO 15377 ^d	51-52	—	12	13
<i>B. lautus</i> IFO 15380 ^{Te}	50-52	+	8	11
<i>B. validus</i> IFO 15382 ^{Tf}	53-54	—	13	15
<i>B. amylolyticus</i> IFO 13625 ^{Tg}	53	+	10	12
<i>B. glucanolyticus</i> IFO 15330 ^T	48-50	+	1	3
<i>B. chondroitinus</i> IFO 15376 ^T	49.0	—	12	8
<i>B. circulans</i> IFO 13579 ^T	55.5	+	15	14
<i>B. parabravis</i> IFO 12334 ^T	50.0	—	8	8
<i>B. choshinensis</i> IFO 15518 ^T	49.1	—	8	7
<i>B. centrosporus</i> IFO 15540 ^T	50.3	—	19	13
<i>B. agri</i> IFO 15538 ^T	52.6	—	4	7
<i>B. macerans</i> IFO 15307 ^T	53.4	+	15	13
<i>B. thiaminolyticus</i> IFO 15656 ^T	52.6	—	15	11

^a The data are averages of values obtained from three determinations. The values from the three determinations differed by no more than 10%.

^b *B. circulans* complex group E (2).

^c *B. circulans* complex group F (2).

^d *B. circulans* complex group G (2).

^e *B. circulans* complex group H (2).

^f *B. circulans* complex group I (2).

^g *B. circulans* complex group K (2).

are motile during growth in synthetic medium containing glucose or curdlan and inorganic salts. Colonies grow poorly in the absence of glucose or a carbon source. Aerobic. Catalase positive, but oxidase negative. The Voges-Proskauer reaction is negative, and the pH in Voges-Proskauer broth is 5.3 to 5.4. Hydrogen sulfide and indole are not produced. Nitrate is reduced to nitrite. Casein is not hydrolyzed, but urea is hydrolyzed. Curdlan, including resistant curdlan, pustulan, starch, and pullulan are hydrolyzed, but carboxymethyl cellulose is not hydrolyzed. Hydrolysis of cyclic 1,2- β -glucan is variable. Growth occurs in the presence of 0.001% lysozyme but does not occur in the presence of 5% NaCl. No growth occurs at 50°C. Acid but no gas is produced from glucose, fructose, xylose, L-arabinose, sucrose, maltose, cellobiose, lactose, salicin, and trehalose. Acid is not produced from galactose, mannose, rhamnose, ribose, glycerol, melibiose, raffinose, sorbitol, and mannitol. The major cellular fatty acid components are anteiso-C_{15:0} and iso-C_{16:0}. The major quinone is menaquinone 7. The G+C content ranges from 50 to 52 mol%. Habitat: isolated from soil.

The type strain is IFO 15729 (= YK205).

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the proteins in cells grown on medium containing 0.5% glucose and 0.5% yeast extract (pH 7.2). Lane A contained marker proteins (94-kDa phosphorylase b, 67-kDa albumin, 43-kDa ovalbumin, 30-kDa carbonic anhydrase, 20.1-kDa trypsin inhibitor, and 14.7-kDa α -lactalbumin). Electrophoresis was carried out in a 10 to 20% polyacrylamide gel at pH 8.4. The proteins were stained with Coomassie blue R-250.

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