Bacillus aneurinolyticus sp. nov., nom. rev.

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The taxonomic position of “*Bacillus aneurinolyticus*” was determined by numerical analyses based on phenotypic characteristics and whole-cell proteins profile, chemosystematic data, DNA base composition, and DNA relatedness. “*B. aneurinolyticus*” strains were separated into “*B. aneurinolyticus*” and *Bacillus migulanus* by DNA relatedness. This result correlated well with the clusters obtained from numerical analyses based on phenotypic characteristics and whole-cell proteins profile. “*B. aneurinolyticus*” was clearly distinct from other *Bacillus* species phenotypically and genetically. We propose the revival of the name *Bacillus aneurinolyticus*.

“*Bacillus aneurinolyticus*” was first described as a new thiamin-decomposing bacterium that was isolated from human feces by Aoyama (4) in 1952. “*B. aneurinolyticus*” was clearly distinct from *Bacillus thiaminolyticus* that was already known to be another thiamin-decomposing bacterium (10, 17). “*B. aneurinolyticus*” was shown to be related to the *Bacillus brevis* group phenotypically (5) and phylogenetically (7). Because taxonomical descriptions of “*B. aneurinolyticus*” were based on a few strains (4, 8), the name has not been validated or included in the *Approved Lists of Bacterial Names* (21) and consequently has lost its standing in bacteriological nomenclature.

The cell wall structure of “*B. aneurinolyticus*” consists of a peptidoglycan layer and a layer, the S layer, constructed of tetragonally regular arrayed protein (1, 3). Abe et al. (1) reported that the S layers of 20 “*B. aneurinolyticus*” strains were composed of two protein types that differed in molecular weight and in agglutination with the antiserum against the S layer protein of strain KA S232 (as mentioned below, this strain was identified as *Bacillus migulanus*). Therefore, an interesting question is raised concerning the taxonomic significance of the cell wall morphology of “*B. aneurinolyticus*.”

In order to clarify the taxonomic position of 21 “*B. aneurinolyticus*” strains including 16 strains isolated, identified, and maintained at the Department of Microbiology, Yamaguchi University School of Medicine (1), we examined their phenotypic characteristics, chemosystematic data, DNA base composition, DNA relatedness, and profile of whole-cell proteins.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are listed in Table 1. Working stocks were cultured on T2 agar plates (26) for 24 h at 30°C and stored at room temperature.

Phenotypic characterization and numerical analysis. Unless

indicated otherwise, phenotypic characterization and numerical analysis were carried out as described previously (24). The detection of the thiamin decomposition was carried out as described by Abe et al. (2). The relationship among 21 “*B. aneurinolyticus*” and 2 *B. migulanus* strains was determined by numerical analysis based on 22 differential phenotypic characteristics (Table 2).

DNA base composition and DNA relatedness. The procedures used for isolation and purification of chromosomal DNA and estimation of DNA base composition and DNA relatedness were those described previously (24).

Cellular fatty acid compositions and quinone systems. Cell cultivation was carried out as described previously (24). The preparation and determination of cellular fatty acids and isoprenoid quinones were carried out as described by Komagata and Suzuki (11).

Electrophoresis of whole-cell proteins and numerical analysis. Twenty milligrams (wet weight) of cells cultured on T2 agar plate for 24 h at 30°C were suspended in 200 µl of a treatment buffer (9). The suspension was boiled at 100°C for 10 min and then centrifuged (3 min; 15,000 rpm). The supernatant was designated as the whole-cell proteins. Sodium dodecyl sulfate–7.5% polyacrylamide gel electrophoresis (SDS–7.5% PAGE) was carried out as described by Laemmli (12). Densitometric scanning of electrophoresis gel was carried out with the Discovery Series system (PDI Inc., Huntington Station, N.Y.) for the numerical analysis. Similarity among the strains was estimated by using the simple matching coefficient, and clustering was based on the unweighted pair group arithmetic average algorithm (23). Computation was carried out with the NTSYS-pc program of Rohlf (19).

Immunological analysis. Western blot (immunoblot) analysis of whole-cell proteins was performed as described by Towbin et al. (25). Rabbit antiserum against the S layer protein of strain KA S232 (strain no. 21 in Table 1) was prepared as reported previously (1).

RESULTS

Phenotypic characterization. The 21 “*B. aneurinolyticus*” and 2 *B. migulanus* strains were tested for 130 phenotypic characteristics. Cells of all strains tested were rod shaped,

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TABLE 1. List of bacterial strains used in this study

No.	Strain ^a Designation	Source ^b	History ^c
1	" <i>B. aneurinolyticus</i> " ATCC 12856	ATCC	Y. Ito from R. Kimura (= IAM 1077 = JCM 9024 = IFO 15521)
2	ATCC 11376	ATCC	K. Arima, thiaminase-producing strain (= JCM 9023)
3	JCM 7539	JCM	Osaka University Medical School; (= IFO 3115)
4	KA IAM	Yamaguchi University	IAM 1077 from R. Kimura
5	KA E14	Yamaguchi University	NCIB 8698 from ATCC 11376 from K. Arima
6	KA 3	Yamaguchi University	Isolated from bovine feces
7	KA 4	Yamaguchi University	Isolated from dog feces
8	KA 5	Yamaguchi University	Isolated from rat feces
9	KA 6	Yamaguchi University	Isolated from chicken feces
10	KA 7	Yamaguchi University	Isolated from feces of a Japanese
11	KA 8	Yamaguchi University	Isolated from feces of a Japanese
12	KA 11	Yamaguchi University	Isolated from feces of a Japanese
13	KA 14	Yamaguchi University	R. L. Airth, isolated from feces of an American
14	KA 17	Yamaguchi University	R. L. Airth, isolated from feces of an American
15	KA 22	Yamaguchi University	R. L. Airth, isolated from feces of an American
16	KA 2	Yamaguchi University	Isolated from feces of a Japanese
17	KA 23	Yamaguchi University	Isolated from soil
18	KA 24	Yamaguchi University	Isolated from soil
19	KA 30	Yamaguchi University	Isolated from a turban shell in the Japan Sea
20	KA S23	Yamaguchi University	Isolated from feces of a Japanese
21	KA S232	Yamaguchi University	Isolated from feces of a Japanese
22	<i>B. migulanus</i> ATCC 9999 ^T	ATCC	NCTC 7096 from R. Synge from Moscow, gramicidin S-producing strain (= JCM 8504 = IFO 15520 = CIP 103841)
23	<i>B. migulanus</i> NRRL NRS-606	NRRL	J. R. Porter from G. Bredemann
24	<i>Bacillus</i> sp. strain HP926	Higeta Shoyu	H. Takagi et al., isolated from soil
25	<i>B. choshinensis</i> HPD52 ^T	Higeta Shoyu	H. Takagi et al., isolated from soil, protein-producing strain (= JCM 8505 = IFO 15518 = CIP 103838)
26	<i>B. centrosporus</i> NRRL NRS-664 ^T	NRRL	N. R. Smith strain 664 from B. S. Henry strain 120
27	<i>B. brevis</i> JCM 2503 ^T	JCM	DSM 30 from ATCC 8246 from N. R. Smith strain 604 from J. R. Porter from NCTC 2611 from W. W. Ford strain 27B
28	<i>B. parabrevis</i> IFO 12334 ^T	IFO	ATCC 10027 from N. R. Smith strain 605 from J. R. Porter from G. Bredemann (= JCM 8506 = CIP 103840)
29	<i>B. agri</i> NRRL NRS-1219 ^T	NRRL	N. R. Smith strain 1219 from C. Lamanna strain 13
30	<i>B. laterosporus</i> JCM 2496 ^T	JCM	CCM 2116 from R. E. Gordon
31	<i>B. subtilis</i> JCM 1465 ^T	JCM	IAM 12118 from ATCC 6051 from H. J. Cohn Marburg strain
32	<i>B. firmus</i> JCM 2512 ^T	JCM	CCM 2213 from NCIB 9366 from R. E. Gordon
33	<i>B. atrophaeus</i> DSM 5551 ^T	DSM	NRRL NRS-213 from N. R. Smith strain 213
34	<i>B. pumilus</i> JCM 2508 ^T	JCM	CCM 2144 from R. E. Gordon
35	<i>B.adius</i> ATCC 14574 ^T	ATCC	R. E. Gordon from N. R. Smith strain 663 from Henry strain 110 from M. Batchelor
36	<i>B. polymyxa</i> JCM 2507 ^T	JCM	CCM 1459 from BUCSAV 162
37	<i>B. alvei</i> IFO 3343 ^T	IFO	IMAB B-3-4 from ATCC 6344 from N. R. Smith strain 662 from A. G. Lochhead strain 127
38	<i>B. amyloliquefaciens</i> DSM 7 ^T	DSM	ATCC 23350 from L. L. Campbell strain F
39	<i>B. thiaminolyticus</i> JCM 8360 ^T	JCM	AHU 1393
40	<i>B. circulans</i> JCM 2504 ^T	JCM	CCM 2048 from R. E. Gordon
41	<i>B. sphaericus</i> JCM 2502 ^T	JCM	CCM 2120 from R. E. Gordon
42	" <i>B. freudenreichii</i> " ATCC 7053	ATCC	N. R. Smith strain 671 from T. Gordon strain 68

^a Names in quotation marks are not in the *Approved Lists of Bacterial Names* (21) or *Index of the Bacterial and Yeast Nomenclatural Changes* (15) and have not been validly published since 1 July 1993.

^b ATCC, American Type Culture Collection, Rockville, Md.; JCM, Japan Collection of Microorganisms, Saitama, Japan; NRRL, the Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Ill.; IFO, Institute for Fermentation, Osaka, Japan; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

^c IAM, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo, Japan; NCIB, National Collection of Industrial Bacteria, National Collections of Industrial and Marine Bacteria Ltd., Aberdeen, Scotland, United Kingdom; NCTC, National Collection of Type Cultures, Central Public Health Laboratory Service, London, United Kingdom; CIP, Collection des Bactéries de l'Institut Pasteur, Paris, France; CCM, Czech Collection of Microorganisms, Masaryk University, Bruno, The Czech Republic.; BUCSAV, Biologický Ústav, Československá Akademie Věd, Prague, The Czech Republic.; IMAB, Institute of Microbiology and Agropecuaria Industry, Castelar, B. A., Argentina; AHU, Department of Agricultural Chemistry, Hokkaido University, Hokkaido, Japan.

TABLE 2. Salient characteristics of "*B. aneurinolyticus*" and *B. migulanus* clusters^a

Characteristic	<i>"B. aneurinolyticus"</i>		<i>B. migulanus</i>	
	Cluster (%)	Type strain (ATCC 12856)	Cluster (%)	Type strain (ATCC 9999)
Catalase positive	100	w	100	+
Oxidase positive	100	+	88	+
Utilization of:				
Acetate	33	—	100	+
Fumarate	67	—	100	+
L-Malate	47	—	100	+
Lactate	20	—	100	+
Succinate	20	—	100	+
L-Glutamate	33	—	100	+
L-Asparatate	93	+	100	+
Ammonium	0	—	100	+
Hydrolysis of DNA	0	—	100	+
Growth at pH 5.0	100	+	0	—
Acid from:				
D-Fructose	13	—	25	+
Sucrose	7	—	0	—
Trehalose	7	—	0	—
Glycerol	100	+	50	+
D-Ribose	33	—	0	—
D-Sorbose	7	—	0	—
Resistant to:				
Sodium azide (0.02%)	80	+	100	+
Chloramphenicol (10 µg/ml)	0	—	100	+
Tetracycline (30 µg/ml)	0	—	100	+
Growth inhibition of <i>B. subtilis</i>	40	+	63	+

^a The number of strains in the cluster was 15 for "*B. aneurinolyticus*" and 8 for *B. migulanus*. Results are expressed as the percentage of positive strains for the clusters and as + (positive), w (weakly positive), or — (negative) for the type strains.

gram positive, and motile with peritrichous flagella and formed oval spores terminally in swollen sporangia. Colonies of these strains were flat, smooth, and yellowish gray. These strains grew at temperatures and pHs ranging from 20 to 50°C and from 5.5 to 9.0, respectively. All strains tested were positive for growth in the presence of 2% NaCl, 0.001% lysozyme, and 50 µg of gramicidin per ml; development of an alkaline pH in Voges-Proskauer broth (pH 7.3 to 8.1); alkaline reaction and reduction of litmus milk; reduction of nitrate; decomposition of tyrosine and thiamin by thiamin hydrolase; egg yolk reaction; and acid production from glycerol. They were negative for growth under anaerobic condition, in the presence of 5% NaCl, 10 µg of ampicillin per ml, 50 µg of neomycin per ml, and 20 µg of erythromycin per ml; Voges-Proskauer reaction; utilization of citrate, propionate, alginate, gluconate, malonate, tartrate, and nitrate; hydrolysis of casein, gelatin, starch, Tween 20, Tween 40, Tween 60, Tween 80, urea, and hippurate; deamination of phenylalanine; production of dihydroxyacetone, indole, and hydrogen sulfide; acid production from D-glucose, L-arabinose, D-galactose, maltose, lactose, D-xylose, D-mannitol, D-cellobiose, salicine, D-sorbose, D-mannose, melibiose, L-rhamnose, raffinose, inositol, erythritol, adonitol, and starch; gas production from all carbohydrates tested; and growth inhibition of *Escherichia coli*. Twenty-two variable characteristics were shown by the 23 strains (Table 2).

Numerical analysis based on the 22 differential characteristics shown in Table 2 separated the 21 "*B. aneurinolyticus*" and 2 *B. migulanus* strains into two clusters at a similarity level of 45% (Fig. 1). The "*B. aneurinolyticus*" cluster consisted of 15 strains, including the original strain, "*B. aneurinolyticus*" ATCC 12856; and the *B. migulanus* cluster consisted of 8 strains, including 2 strains of *B. migulanus* (ATCC 9999^T and NRRL NRS-606).

DNA base composition and hybridization. DNA base compositions (G+C content) of the 23 strains ranged from 41.1 to 43.4 mol%, as shown in Table 3.

The probes for the DNA-DNA hybridization were prepared from strains ATCC 12856, JCM 7539, ATCC 9999^T, and KA S232. DNA relatedness values among the 23 strains are shown in Table 3. These strains were separated into two independent groups on the basis of DNA relatedness higher than 72%, namely, the "*B. aneurinolyticus*" group and the *B. migulanus* group. This grouping correlated well with the phenotypic clusters. Reference strains of the known *Bacillus* species showed less than 25% DNA relatedness to "*B. aneurinolyticus*" (Table 4).

Cellular fatty acid compositions and quinone systems. Strains ATCC 12856, JCM 7539, KA S23, KA S232, ATCC 9999^T, and NRRL NRS-606 were analyzed for the cellular fatty acid compositions and quinone systems. All strains tested had iso-C_{15:0} acid in the range of 33 to 45% of total cellular fatty acid components. In addition to that, ATCC 12856 and JCM 7539 had iso-C_{16:0} acid (13 to 14%) and C_{16:0} (6 to 10%). In contrast, KA S23, KA S232, ATCC 9999^T, and NRRL NRS-606 had C_{16:0} acid (17 to 21%) and iso-C_{16:0} acid (3 to 10%).

All strains tested had menaquinone 7 (more than 99% of total menaquinones).

Numerical and immunological analyses of whole-cell proteins. The 23 strains were recovered in two clusters resulting from numerical analysis based on the SDS-PAGE pattern of whole-cell proteins at a similarity level of 35% (Fig. 2). This clustering correlated well with the grouping obtained by DNA relatedness.

The immunological relationships between the whole-cell proteins and the S layer protein of strain KA S232 (strain no. 21) are shown in Fig. 3. The antiserum against the S layer

FIG. 1. Phenotypic relationships among "*B. aneurinolyticus*" and *B. migulanus* strains. The dendrogram was produced by using the simple matching coefficient and the unweighted pair group arithmetic average algorithm.

protein cross-reacted with the whole-cell proteins from 23 "*B. aneurinolyticus*" and *B. migulanus* strains by Western blot analysis. Many strains of the "*B. aneurinolyticus*" group had two proteins that strongly cross-reacted, corresponding to type II protein (molecular weight, 105,000), and many strains of the *B. migulanus* group had one protein corresponding to type I protein (molecular weight, 115,000). The whole-cell proteins obtained from the other *Bacillus* species (strains no. 24 through 42) did not cross-react with the antiserum.

DISCUSSION

On the basis of numerical analyses based on phenotypic characteristics and whole-cell proteins profile, chemosystematic data, DNA base composition, and DNA relatedness, the 21 strains assigned to "*B. aneurinolyticus*" were separated into two independent species, which were identified as "*B. aneurinolyticus*" and *B. migulanus*. Although the two species had similar phenotypic characteristics and DNA base composition, they were clearly distinguishable by DNA relatedness and whole-cell proteins profile.

Bacillus firmus, *B. subtilis*, *B. atrophaeus*, *B. pumilus*, and *B. badius* are known strictly aerobic and oval-spore-

forming *Bacillus* species with G+C contents of 40 to 44 mol%. The *B. brevis* group (24) and *B. thiaminolyticus* show phenotypic characteristics similar to "*B. aneurinolyticus*." These species were genetically and phenotypically distinct from "*B. aneurinolyticus*," as shown in Tables 4 and 5. In addition, thiamin is decomposed by "*B. aneurinolyticus*" with thiamin hydrolase and by *B. thiaminolyticus* with thiamin pyridinylase (2, 10, this study). These results indicate distant relationships between "*B. aneurinolyticus*" and the above-mentioned *Bacillus* species.

Therefore, we propose that the name *Bacillus aneurinolyticus* should be revived and assigned to the same taxon to which they were originally applied, in accordance with Rules 27, 28a, 33a, and 33c of the *International Code of Nomenclature of Bacteria* (13).

S layer proteins have been found in many species of the genus *Bacillus* and other bacteria (14). Many eubacterial S layers were considered to be strain-specific features (16, 24). Takagi et al. (24) indicated that the characteristics of S layer protein could be an important marker for the taxonomy of the *B. brevis* group. All strains of *B. aneurinolyticus* and *B. migulanus* tested had the specific protein which cross-reacted with antiserum against S layer protein of *B. migulanus* KA S232 (identified in this study). These two species are not included in the *B. brevis* group on the basis of cellular fatty acid composition and immunological and genetical S layer characteristics (24).

Description of *Bacillus aneurinolyticus* sp. nov., nom. rev. *Bacillus aneurinolyticus* (an. eur. in. o. lytic. us. M. L. n. *aneurium*, thiamine; M. L. adj. *lyticus*, dissolving; M. L. adj. *aneurinolyticus*, decomposing thiamine).

Cells are rod shaped (0.7 to 0.9 by 3.0 to 5.0 μ m). Gram positive. Motile and peritrichous. Ellipsoidal spores are formed in swollen sporangia. Colonies are flat, smooth, and yellowish gray and do not produced soluble pigment on nutrient agar.

Strictly aerobic.

Catalase is weakly positive (the gas bubbles can be visible with a dissecting scope). Oxidase is positive.

The Voges-Proskauer reaction (production of acetylmethylcarbinol) is negative, and the pH in Voges-Proskauer broth is 7.3 to 8.1.

Dihydroxyacetone, hydrogen sulfide, and indole are not produced.

Nitrate is reduced to nitrite.

Casein, gelatin, starch, DNA, Tween 20, Tween 40, Tween 60, Tween 80, urea, and hippurate are not hydrolyzed.

Tyrosine is decomposed. Thiamin is decomposed by thiamin hydrolase.

Phenylalanine is deaminated.

Citrate, propionate, alginate, gluconate, malonate, and tartrate are not utilized; and acetate, fumarate, lactate, succinate, L-glutamate, L-asparatate, and α -ketoglutarate are utilized by some strains.

Nitrate and ammonium are not utilized.

Egg yolk reaction is positive.

Litmus milk becomes alkaline and is reduced.

Growth occurs at temperatures and pHs ranging from 20 to 50°C and 5.0 to 9.0, respectively. The optimum growth temperature and pH are 37°C and 7.0, respectively. Growth occurs in the presence of 2% NaCl, 0.001% lysozyme, and 50 μ g of gramicidin per ml, and some strains grow in the presence of 0.02% sodium azide. Growth is inhibited by the presence of 5% NaCl, 10 μ g of ampicillin per ml, 10 μ g of

TABLE 3. DNA base compositions and levels of DNA relatedness for "*B. aneurinolyticus*" and *B. migulanus* strains

No.	Strain Designation	G+C content (mol%)	% Reassociation with DNA from strain ^a			
			<i>"B. aneurinolyticus"</i>		<i>B. migulanus</i>	
			ATCC 12856	JCM 7539	KA S232	ATCC 9999 ^T
1	ATCC 12856	42.9	100	83	0	0
2	ATCC 11376	42.9	100	100	18	15
3	JCM 7539	43.0	100	100	0	23
4	KA IAM	42.6	100	NT ^b	NT	26
5	KA E14	41.8	83	NT	NT	9
6	KA 3	42.5	100	NT	NT	2
7	KA 4	43.0	100	100	15	8
8	KA 5	42.9	74	NT	0	1
9	KA 6	41.1	100	99	0	0
10	KA 7	42.9	100	100	15	15
11	KA 8	43.1	100	NT	NT	23
12	KA 11	41.3	100	100	0	7
13	KA 14	42.9	80	NT	NT	0
14	KA 17	43.4	100	NT	NT	33
15	KA 22	43.3	96	81	21	14
16	KA 2	43.2	0	NT	NT	92
17	KA 23	42.5	44	38	100	89
18	KA 24	42.4	16	13	93	80
19	KA 30	42.7	23	NT	NT	72
20	KA S23	41.7	0	19	100	95
21	KA S232	42.7	0	0	100	73
22	ATCC 9999 ^T	42.5	1	38	100	100
23	NRRL NRS-606	43.2	26	36	87	78

^a Reassociation values are the averages of two determinations. The maximum variation observed was 10%.^b NT, not tested.

chloramphenicol per ml, 50 µg of neomycin per ml, and 30 µg of tetracycline per ml.

Acid but no gas is produced from glycerol. Variable acid but no gas production occurs with D-fructose, sucrose, trehalose, D-ribose, and L-sorbose. Acid and gas are not produced from D-glucose, L-arabinose, D-galactose, maltose, lactose, D-xylose, D-mannitol, D-cellobiose, salicine, D-sorbitol, D-mannose, melibiose, L-rhamnose, raffinose, inositol, erythritol, adonitol, and starch.

Growth inhibition test against *E. coli* is negative, and growth of *B. subtilis* is inhibited by some strains.

Specific S layer proteins are present.

The major cellular fatty acid components are iso-C15:0, iso-C16:0, and C16:0 acids.

The major quinone is menaquinone 7.

The G+C content ranges from 41.1 to 43.4 mol%, with the G+C content of the type strain being 42.9 mol%. The type strain is ATCC 12856 (= IAM 1077), which was isolated

TABLE 4. DNA base compositions and levels of DNA relatedness for selected *Bacillus* strains

No.	Strain Designation	G+C content (mol%)	% Reassociation with DNA from " <i>B. aneurinolyticus</i> " ATCC 12856 ^a
1	" <i>B. aneurinolyticus</i> " ATCC 12856	42.9	100
25	<i>B. choshinensis</i> HPD52 ^T	48.2	2
26	<i>B. centrosporus</i> NRRL NRS-664 ^T	49.2	15
27	<i>B. brevis</i> JCM 2503 ^T	47.0	12
28	<i>B. parabrevis</i> IFO 12334 ^T	51.8	0
29	<i>B. agri</i> NRRL NRS-1219 ^T	53.2	25
30	<i>B. laterosporus</i> JCM 2496 ^T	40.4	0
31	<i>B. subtilis</i> JCM 1465 ^T	43.2	0
32	<i>B. firmus</i> JCM 2512 ^T	41.5	4
33	<i>B. atrophaeus</i> DSM 5551 ^T	43.1	9
34	<i>B. pumilus</i> JCM 2508 ^T	42.0	0
35	<i>B. badius</i> ATCC 14574 ^T	43.5	4
36	<i>B. polymyxa</i> JCM 2507 ^T	45.0	0
37	<i>B. alvei</i> IFO 3343 ^T	45.4	13
38	<i>B. amyloliquefaciens</i> DSM 7 ^T	45.8	13
39	<i>B. thiaminolyticus</i> JCM 8630 ^T	53.3	0
42	" <i>B. freudenreichii</i> " ATCC 7053	41.8	2

^a Reassociation values are the averages of two determinations. The maximum variation observed was 8%.

FIG. 2. Dendrogram showing whole-cell protein profiles of "*B. aneurinolyticus*" and *B. migulanus* strains, on the basis of simple matching coefficient and unweighted pair group arithmetic average algorithm. The proteins in an SDS-7.5% polyacrylamide gel were stained by Coomassie blue.

FIG. 3. Detection of S layer proteins of various *Bacillus* strains by Western blot analysis. The proteins in an SDS-7.5% polyacrylamide gel were transferred electrophoretically to nitrocellulose sheets. Immunoreactive protein bands were detected with an immunostaining HRP kit (Konica Co., Tokyo, Japan). The lane numbers correspond to the laboratory numbers for the strains (Table 1). Kd, kilodaltons.

TABLE 5. Characteristics that differentiate "*B. aneurinolyticus*", *B. migulanus*, and allied *Bacillus* species^a

Characteristic	" <i>B. aneurinolyticus</i> " ^b	<i>B. migulanus</i> ^b	<i>B. brevis</i> group ^c	<i>B. firmus</i> ^d	<i>B. atrophaeus</i> ^e	<i>B. pumilus</i> ^d	<i>B. subtilis</i> ^d	<i>B. thiaminolyticus</i> ^f
Swollen sporangia	+	+	+	+	—	—	—	+
Anaerobic growth	—	—	—	—	—	—	—	+
Catalase	w	+	+	+	+	+	+	+
Voges-Proskauer reaction	—	—	—	—	+	+	+	—
pH of Voges-Proskauer broth	>7.0	>7.0	>7.0	6 to 7	<6.0	<6.0	V	<6.0
Utilization of ammonium	—	+	+	NT	NT	NT	+	NT
Hydrolysis of:								
Starch	—	—	—	—	+	—	+	+
Casein	—	—	V	+	+	+	+	+
DNA	—	+	V	NT	NT	NT	NT	NT
Decomposition of thiamin	+ ^g	+ ^g	—	NT	NT	NT	NT	+ ^h
Growth in 5% NaCl	—	—	—	+	+	+	+	V
Growth at pH 5.0	+	—	—	—	NT	NT	NT	—
Resistance to:								
Chloramphenicol (10 µg/ml)	—	+	V	NT	NT	NT	+	NT
Tetracycline (30 µg/ml)	—	+	—	NT	NT	NT	—	NT
G+C content (mol%)	41–43	42–43	47–53	41–47	41–43	33–45	34–48	52–54

^a +, positive; w, weakly positive; —, negative; V, variable; NT, not tested.^b Data obtained in this study.^c Included *B. brevis*, *B. agri*, *B. centrosporus*, *B. choshinensis*, and *B. parabrevis*. Data are from references 18, 20, and 24.^d Physiological data are from reference 5. G+C content data are from reference 6.^e Data are from reference 16.^f Data are from reference 17.^g By thiamin hydrolase.^h By thiamin pyridinylase.

from human feces as a thiamin hydrolase producer. The type strain has been deposited at JCM and IFO as JCM 9024 and IFO 15521, respectively.

ACKNOWLEDGMENT

We thank Y. Sakaguchi for technical assistance.

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