Proposal of *Bacillus reuszeri* sp. nov., *Bacillus formosus* sp. nov., nom. rev., and *Bacillus borstelensis* sp. nov., nom. rev.

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Taxonomic studies of 26 putative *Bacillus brevis* strains (12 strains formerly assigned to groups 2, 4 through 7, and B and 14 ungrouped strains) were carried out. These organisms were placed in five distinct groups on the basis of phenotypic characteristics, the results of DNA base composition and reassociation analyses, and the results of cellular fatty acid and isoprenoid quinone composition analyses. Group 2, which contained three strains, was identified as *Bacillus choshinensis*, and group 4, which contained one strain, was identified as *Bacillus parabrevis*. The three remaining unidentified groups were clearly distinguishable phenotypically and genetically from the type strains of *B. brevis* and the recently described species *Bacillus agri*, *Bacillus centrosporus*, *Bacillus migulanus*, *Bacillus choshinensis*, and *Bacillus parabrevis*. Therefore, the names *Bacillus reuszeri* sp. nov., *Bacillus formosus* sp. nov., nom. rev., and *Bacillus borstelensis* sp. nov., nom. rev. are proposed for groups 5 (3 strains), 6 (3 strains), and 7 (16 strains), respectively. The S-layer proteins found in the three new species were immunologically and genetically similar to the S-layer proteins found previously in *B. brevis*, *B. agri*, *B. centrosporus*, *B. choshinensis*, and *B. parabrevis*. The G+C content of NRRL NRS-1206, the type strain of *B. reuszeri* sp. nov., was 46.5 mol%; the G+C content of NRRL NRS-863, the type strain of *B. formosus* sp. nov., nom. rev., was 47.2 mol%; and the G+C content of NRRL NRS-818, the type strain of *B. borstelensis* sp. nov., nom. rev., was 51.3 mol%.

Previously, Nakamura (6, 7) and Takagi et al. (11) independently studied the taxonomy of *Bacillus brevis* and, on the basis of phenotypic, chemosystematic, and DNA relatedness data, separated the organisms in this species into seven species (*B. brevis sensu stricto*, *Bacillus agri*, *Bacillus centrosporus*, *Bacillus migulanus*, *Bacillus choshinensis*, *Bacillus parabrevis*, and *Bacillus galactophilus*) and six unidentified groups. Recently, Shida et al. (8) studied the six species described by Nakamura (7) and Takagi et al. (11) and established that *B. galactophilus* is a later subjective synonym of *B. agri*. The taxonomic status of the five unidentified groups of Nakamura (7) (groups 2 and 4 through 7) and one strain belonging to group B of Takagi et al. (11) was not resolved by these authors. We performed this study to establish the taxonomic positions of these organisms, as well as 14 other *B. brevis*-like strains; to do this, we examined the phenotypic characteristics of these bacteria, as well as chemosystematic data, DNA base compositions, and levels of DNA relatedness.

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 (12) for 24 h at 30°C and were stored at room temperature.

Phenotypic characterization. Phenotypic characteristics of 22 strains (strains 5 through 26 in Table 1) were determined as described previously (6, 7, 11).

DNA base composition and DNA relatedness. The procedures used to isolate and purify chromosomal DNA and to estimate DNA base composition have been described previously (11). The G+C contents of 26 strains (strains 1 through 26 in Table 1) were determined. DNA relatedness values were determined by using probes labeled with photobiotin (Vector Laboratories, Inc., Burlingame, Calif.) in microplate wells and the fluorometric method described by Ezaki et al. (2). Probes for DNA-DNA hybridization experiments were prepared from *B. brevis*

JCM 2503^T (T = type strain), *B. agri* NRRL NRS-1219^T, *B. centrosporus* NRRL NRS-664^T, *B. migulanus* ATCC 9999^T, *B. choshinensis* HPD52^T, *B. parabrevis* IFO 12334^T, and the following *Bacillus* sp. strains: NRRL NRS-1206^T, NRRL NRS-1208, NRRL NRS-863^T, NRRL NRS-1210, NRRL NRS-818^T, NRRL NRS-1029b, NRRL NRS-948, and HP926.

Cellular fatty acid and isoprenoid quinone compositions. Cells were cultured as described previously (11). Cellular fatty acids and isoprenoid quinones were prepared and cellular fatty acid and isoprenoid quinone compositions were determined as described by Komagata and Suzuki (3).

Immunological analysis. The procedures used for electrophoretic analysis and Western blot (immunoblot) analysis of whole-cell proteins have been described previously (8, 9, 11). Rabbit antiserum against the S-layer protein (protein HWP) of *B. choshinensis* HPD31 was prepared as described elsewhere (1, 11).

Southern blot analysis. Chromosomal DNAs were digested completely with *Eco*RI, and then the digested DNAs were separated by electrophoresis on 0.8% agarose gels. A Southern blot analysis of chromosomal DNA restriction fragments was performed as described by Takagi et al. (11). The hybridization probe used was a 1.0-kb *Hpa*I-*Bgl*II fragment of ϕ -SK10 containing the HWP gene of *B. choshinensis* HPD31 (1, 11). Hybridization of the probe to the digested DNA was detected with an enhanced chemiluminescence gene detection system (Amersham International plc, Amersham, United Kingdom) by using the manufacturer's protocol.

RESULTS

DNA base composition and DNA relatedness. The DNA base compositions (G+C contents) of the 26 strains studied ranged from 46.4 to 51.8 mol% (Table 2).

Table 2 shows that the levels of DNA relatedness between *B. choshinensis* HPD52^T and group 2 strains NRRL NRS-376, NRRL NRS-378, and NRRL NRS-380 and between *B. parabrevis* IFO 12334^T and representative group 4 strain NRRL NRS-815 were greater than 70%. Furthermore, we observed low levels of DNA reassociation between the type strains of the species tested and members of *B. brevis*-like groups 5 through 7 and B and 14 ungrouped strains (strains 10 and 14 through 26 in Table 1).

Table 3 shows levels of DNA relatedness between previously unclassified *B. brevis sensu lato* strains. Intragroup levels of

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TABLE 1. Bacterial strains used in this study

Strain		Source ^a	History ^b
Laboratory no.	Designation		
Group 2 strains ^c			
1	NRRL NRS-376	1	N. R. Smith, from soil
2	NRRL NRS-378	1	N. R. Smith, from soil
3	NRRL NRS-380	1	N. R. Smith, from soil
Group 4 strain ^c			
4	NRRL NRS-815	1	J. R. Porter, " <i>Bacillus bellus</i> " B1, from G. Bredemann
Group 5 strains ^c			
5	NRRL NRS-1206 ^T	1	H. W. Reuszer Army strain 39 ^T (= JCM 9170 ^T = IFO 15719 ^T)
6	NRRL NRS-1207	1	H. W. Reuszer Army strain 723
7	NRRL NRS-1208	1	H. W. Reuszer Army strain 842
Group 6 strains ^c			
8	NRRL NRS-863 ^T	1	J. R. Porter, " <i>Bacillus formosus</i> " F12 ^T , from G. Bredemann (= JCM 9169 ^T = IFO 15716 ^T)
9	NRRL NRS-1210	1	H. W. Reuszer Army strain 1035
Group 7 strains ^c			
11	NRRL NRS-818 ^T	1	J. R. Porter, " <i>Bacillus borstelensis</i> " (= JCM 9022 ^T = IFO 15714 ^T)
12	NRRL NRS-1029b	1	G. M. Eisenberg, " <i>Bacillus schuykilliensis</i> "
Group B strain ^d			
13	HP926	2	H. Takagi et al., from soil
Ungrouped strains			
10	NRRL NRS-910	1	J. R. Porter, " <i>Bacillus mycoides</i> " M26, from G. Bredemann from O. Gottheil
14	NRRL NRS-948	1	J. R. Porter, " <i>Bacillus rufescens</i> " R8, from G. Bredemann
15	NRRL NRS-1373	1	W. G. Walter strain 141, from a hot spring
16	CCM 1613	3	H. Proom (= DSM 298 = CN 2918)
17	NCFB 1133	4	Wellcome collection, through J. G. Franklin, NIRD 1957 (= CN 2904)
18	NCFB 1158	4	Wellcome collection, through J. G. Franklin, NIRD 1957 (= CN 3010)
19	NCFB 1165	4	Wellcome collection, through J. G. Franklin, NIRD 1957 (= CN 3005)
20	S038	5	NCIB 8803
21	S042	5	WR 2904, from soil
22	S043	5	No information available
23	S044	5	WR 2932, from soil
24	S047	5	WR 3005, from soil
25	E15	6	No information available
26	E16	6	No information available
Other strains			
27	<i>B. brevis</i> JCM 2503 ^T	7	DSM 30 ^T from ATCC 8246 ^T from N. R. Smith strain 604 ^T from J. R. Porter from NCTC 2611 ^T from W. W. Ford strain 27B ^T
28	<i>B. agri</i> NRRL NRS-1219 ^T	1	N. R. Smith strain 1219 ^T from C. Lamanna strain 13 ^T
29	<i>B. centrosporus</i> NRRL NRS-664 ^T	1	N. R. Smith strain 664 ^T from B. S. Henry strain 120 ^T
30	<i>B. migulanus</i> ATCC 9999 ^T	8	NCTC 7096 ^T from R. Synge from Moscow, gramicidin S-producing strain (= JCM 8504 ^T = IFO 15520 ^T = CIP 103841 ^T)
31	<i>B. choshinensis</i> HPD52 ^T	2	H. Takagi et al., from soil, protein-producing strain (= JCM 8505 ^T = IFO 15518 ^T = CIP 103838 ^T = DSM 8552 ^T)
32	<i>B. choshinensis</i> HPD31	2	H. Takagi et al., from soil, protein-producing strain
33	<i>B. parabrevis</i> IFO 12334 ^T	9	ATCC 10027 ^T from N. R. Smith strain 605 ^T from J. R. Porter from G. Bredemann (= JCM 8506 ^T = CIP 103840 ^T)

^a 1, NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Ill.; 2, Research Laboratory, Higeta Shoyu Co., Ltd., Chiba, Japan; 3, CCM, Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic; 4, NCFB, National Collection of Food Bacteria, Shinfield, Reading, United Kingdom; 5, Heriot-Watt University, Edinburgh, Scotland, United Kingdom; 6, Yamaguchi University School of Medicine, Yamaguchi, Japan; 7, JCM, Japan Collection of Microorganisms, Saitama, Japan; 8, ATCC, American Type Culture Collection, Rockville, Md.; 9, IFO, Institute for Fermentation, Osaka, Japan.

^b Names in quotation marks are not on the Approved Lists of Bacterial Names (10) or the Index of the Bacterial and Yeast Nomenclatural Changes (5) and have not been validly published since 1 July 1994. NIRD, National Institute for Research in Dairying, Shinfield, Reading, United Kingdom; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; CN, Burroughs Wellcome Research Laboratories, Kew, Surrey, United Kingdom; NCIB, National Collection of Industrial and Marine Bacteria, Ltd., Aberdeen, Scotland, United Kingdom; WR, Queensland Wheat Research Institute, Toowoomba, Australia; NCTC, National Collection of Type Cultures, Central Public Health Laboratory Service, London, United Kingdom; CIP, Collection des Bactéries de l'Institut Pasteur, Paris, France; JCM, Japan Collection of Microorganisms, Saitama, Japan; IFO, Institute for Fermentation, Osaka, Japan; ATCC, American Type Culture Collection, Rockville, Md.

^c Group designation used by Nakamura (7).

^d Group designation used by Takagi et al. (11).

DNA relatedness higher than 71% and intergroup levels of relatedness lower than 26% indicated that Nakamura groups 5 through 7 were genetically distinct groups. Moreover, the DNA relatedness study revealed that HP926, a strain previously assigned to group B of Takagi et al. (11), and all 13

ungrouped strains (strains 14 through 26 in Table 1) were closely related genetically to the group 7 organisms. In his 1991 study (6), Nakamura showed that NRRL NRS-910 (strain 10 in Table 1) was not a *B. brevis* sensu stricto strain, but this strain was not included in his subsequent study (7). High levels of

TABLE 2. DNA base compositions and levels of DNA relatedness for *B. brevis* sensu stricto, *B. agri*, *B. centrosporus*, *B. migulanus*, *B. choshinensis*, and *B. parabrevis* strains and previously ungrouped *B. brevis*-like organisms

Strain		G+C content (mol%)	% Reassociation with DNA from ^a :					
Laboratory no.	Designation		<i>B. brevis</i> JCM 2503 ^T	<i>B. agri</i> NRRL NRS-1219 ^T	<i>B. centrosporus</i> NRRL NRS-664 ^T	<i>B. migulanus</i> ATCC 9999 ^T	<i>B. choshinensis</i> HPD52 ^T	<i>B. parabrevis</i> IFO 12334 ^T
1	NRRL NRS-376	47.9	28	12	25	7	80	20
2	NRRL NRS-378	48.1	21	12	23	6	88	14
3	NRRL NRS-380	48.3	20	15	21	9	85	10
4	NRRL NRS-815	51.8	21	21	14	5	13	70
5	NRRL NRS-1206 ^T	46.5	20	18	10	7	14	17
6	NRRL NRS-1207	46.7	21	11	14	7	15	15
7	NRRL NRS-1208	46.4	29	24	24	17	14	15
8	NRRL NRS-863 ^T	47.2	6	23	13	4	16	3
9	NRRL NRS-1210	46.8	20	23	30	27	14	16
10	NRRL NRS-910	47.1	0	15	9	0	11	0
11	NRRL NRS-818 ^T	51.3	20	27	33	17	15	19
12	NRRL NRS-1029b	51.3	15	11	10	5	11	12
13	HP926	50.4	NT ^b	10	12	NT	NT	NT
14	NRRL NRS-948	51.5	12	10	11	5	10	10
15	NRRL NRS-1373	50.9	10	11	8	5	9	8
16	CCM 1613	51.4	16	12	15	37	11	11
17	NCFB 1133	51.4	34	24	35	NT	17	27
18	NCFB 1158	51.4	18	15	17	NT	14	16
19	NCFB 1165	51.4	16	14	16	45	10	20
20	S038	51.7	31	25	26	NT	19	26
21	S042	51.7	28	17	22	NT	15	22
22	S043	51.2	25	17	16	12	17	16
23	S044	51.6	28	20	18	12	18	15
24	S047	51.6	26	19	22	11	17	20
25	E15	51.7	24	19	16	11	13	16
26	E16	51.8	24	18	16	15	14	16

^a Reassociation values are the averages of two determinations. The maximum variation observed was 9%.^b NT, not tested.

DNA relatedness showed that this strain was closely related genetically to the group 6 strains.

Cellular fatty acid and quinone compositions. The cellular fatty acid and quinone compositions of strains NRRL NRS-1206^T, NRRL NRS-1208, NRRL NRS-863^T, NRRL NRS-910, NRRL NRS-818^T, and NRRL NRS-948 were determined. Table 4 shows that all of these strains contained anteiso-C_{15:0} (32 to 66% of the total cellular fatty acids) and iso-C_{15:0} (8 to 42%). All strains contained menaquinone 7 (more than 90% of the total menaquinone).

Immunological and genetic analyses of cell wall proteins. Figure 1A shows the banding patterns of the electrophoretically treated whole-cell proteins of all of the strains belonging to groups 2 and 4 through 7. The Western blot analysis revealed that the antiserum against HWP cross-reacted with a number of bands from all of the strains tested, confirming the presence of the characteristic S-layer proteins (Fig. 1B).

Figure 2 shows that the DNAs from the group 2 and 4 through 7 strains examined hybridized with the *HpaI*-*Bgl*II probe, indicating that these organisms contained DNA base sequences very similar to the sequence of the HWP gene of strain HPD31. These results demonstrated that the group 2 and 4 through 7 strains contained S-layer proteins that were immunologically and genetically similar to those found in members of the *B. brevis* group (8, 11).

Phenotypic characterization. The 22 group 5 through 7 strains examined formed peritrichous, motile, rod-shaped cells which were strictly aerobic and produced oval spores in swollen sporangia. Their colonies were flat, smooth, circular, and entire.

The members of these three groups had a number of

characteristics in common. Positive reactions were observed for catalase activity, development of an alkaline pH in Voges-Proskauer broth, and acid production from fructose. Negative results were obtained for the oxidase test, the Voges-Proskauer reaction, the egg yolk reaction, hydrolysis of starch and urea, production of hydrogen sulfide and indole, utilization of propionate, DL-lactate, alginate, malonate, and tartrate, and acid production from L-arabinose, D-galactose, lactose, sucrose, D-xylose, trehalose, salicin, D-sorbitol, L-sorbose, D-mannose, melibiose, L-rhamnose, inositol, erythritol, and adonitol.

The differentiating characteristics of the three groups are shown in Table 5.

DISCUSSION

On the basis of DNA relatedness values, the groups formerly designated groups 2 and 4 by Nakamura (7) were identified as *B. choshinensis* and *B. parabrevis*, respectively. DNA relatedness values of less than 35% indicated that the group 5 through 7 strains and 15 ungrouped *B. brevis*-like strains (including strain HP926 belonging to group B of Takagi et al.) were not members of previously described *B. brevis*-like species. Intra-group DNA relatedness values of more than 70% and inter-group DNA relatedness values of less than 26% suggested strongly that groups 5 through 7 were genetically distinct taxa. High levels of DNA relatedness also indicated that the 13 ungrouped strains (strains 14 through 26 in Table 1) and group B strain HP926 were members of group 7 and that ungrouped strain NRRL NRS-910 was a member of group 6.

Table 6 shows that groups 5 through 7 could be distinguished

TABLE 3. DNA relatedness values for *B. brevis* sensu lato strains

Strain		% Reassociation with DNA from ^a :							
Laboratory no.	Designation	NRRL NRS-1206 ^T	NRRL NRS-1208	NRRL NRS-863 ^T	NRRL NRS-1210	NRRL NRS-818 ^T	NRRL NRS-1029b	NRRL NRS-948	HP926
5	NRRL NRS-1206 ^T	100	100	0	0	9	9	1	NT ^b
6	NRRL NRS-1207	96	100	NT	0	12	9	0	8
7	NRRL NRS-1208	97	100	0	11	4	10	0	NT
8	NRRL NRS-863 ^T	3	NT	100	86	0	NT	NT	NT
9	NRRL NRS-1210	5	9	79	100	9	10	5	NT
10	NRRL NRS-910	0	NT	87	94	0	NT	NT	NT
11	NRRL NRS-818 ^T	4	2	0	0	100	100	92	73
12	NRRL NRS-1029b	9	11	0	0	85	100	100	75
13	HP926	5	7	20	8	100	99	99	100
14	NRRL NRS-948	3	0	NT	0	93	100	100	77
15	NRRL NRS-1373	6	11	NT	11	85	74	74	72
16	CCM 1613	3	0	NT	1	99	100	81	71
17	NCFB 1133	4	21	NT	18	100	100	100	97
18	NCFB 1158	10	NT	NT	0	93	100	90	73
19	NCFB 1165	19	16	NT	11	100	100	79	79
20	S038	26	0	NT	15	100	100	98	73
21	S042	13	0	NT	5	100	100	85	89
22	S043	5	9	NT	3	100	100	76	89
23	S044	17	4	NT	1	100	100	90	100
24	S047	5	9	NT	6	100	100	93	89
25	E15	19	0	NT	0	92	100	76	81
26	E16	3	0	NT	0	89	100	100	75

^a Reassociation values are the averages of two determinations. The maximum variation observed was 9%.^b NT, not tested.

phenotypically from each other and from *B. brevis*, *B. agri*, *B. centrosporus*, *B. migulanus*, *B. choshinensis*, and *B. parabrevis*. Differences in as few as 2 and as many as 11 characteristics distinguish groups 5 through 7 from the previously described species.

The results of previous studies (6, 7, 11) and this study strongly suggested that groups 5 through 7 are genetically and phenotypically distinct taxa and merit recognition as separate species. We propose the name *Bacillus reuszeri* sp. nov. for group 5 (strains NRRL NRS-1206^T, NRRL NRS-1207, and NRRL NRS-1208). The name *Bacillus formosus* sp. nov., nom. rev. is proposed for group 6 (strains NRRL NRS-863^T, NRRL

NRS-910, and NRRL NRS-1210), and the name *Bacillus borstelensis* sp. nov., nom. rev. is proposed for group 7 (strains NRRL NRS-818^T, NRRL NRS-1029b, HP926, NRRL NRS-948, NRRL NRS-1373, CCM 1613, NCFB 1133, NCFB 1158, NCFB 1165, S038, S042, S043, S044, S047, E15, and E16). The revived names are used for the same taxa to which they were originally applied, in accordance with Rules 27, 28a, 33a, and 33c of the *International Code of Nomenclature of Bacteria* (4).

Takagi et al. (11) and Shida et al. (9) pointed out that the presence of immunologically and genetically related S-layer proteins is a taxonomic marker which suggests that the species *B. brevis*, *B. agri*, *B. centrosporus*, *B. choshinensis*, and *B.*

TABLE 4. Cellular fatty acid compositions of *B. brevis* sensu stricto, *B. agri*, *B. centrosporus*, *B. choshinensis*, *B. parabrevis*, and the ungrouped *B. brevis*-like organisms

Strain		% of total cellular fatty acids:									
Laboratory no.	Designation	Saturated acids									Unsaturated acids
		C ₁₄	C ₁₅	C ₁₆	iso-C ₁₄	iso-C ₁₅	iso-C ₁₆	iso-C ₁₇	anteiso-C ₁₅	anteiso-C ₁₇	
Group 5 strains											
5	NRRL NRS-1206 ^T	1.0	0	3.3	1.3	8.9	3.3	1.1	64.5	5.1	1.8
6	NRRL NRS-1207	0.6	0	2.0	1.8	7.8	3.7	0.9	65.7	4.7	3.0
Group 6 strains											
8	NRRL NRS-863 ^T	0	0.2	1.9	0.2	24.4	1.8	8.5	43.6	4.4	14.4
10	NRRL NRS-910	0	0	2.1	0.5	14.7	1.8	5.5	48.0	6.6	9.7
Group 7 strains											
11	NRRL NRS-818 ^T	0.4	0.2	0.8	0.8	41.9	1.7	4.3	32.0	2.7	11.9
14	NRRL NRS-948	0.3	0.3	0.8	0.8	39.1	1.4	4.9	34.6	3.2	9.1
Type strains											
27	<i>B. brevis</i> JCM 2503 ^T	0.4	0.7	2.7	0.6	17.6	3.5	5.3	54.4	9.6	4.2
28	<i>B. agri</i> NRRL NRS-1219 ^T	0.6	0.4	2.5	0.6	41.0	2.6	4.4	40.4	4.2	1.9
29	<i>B. centrosporus</i> NRRL NRS-664 ^T	0.7	0.3	1.8	1.3	22.5	2.6	1.9	62.4	3.8	0.9
31	<i>B. choshinensis</i> HPD52 ^T	1.4	0.8	3.5	1.3	11.7	2.4	0.9	71.7	4.1	1.2
33	<i>B. parabrevis</i> IFO 12334 ^T	0.6	0.6	1.7	0.9	32.3	1.5	2.9	53.5	3.9	1.2

FIG. 1. Detection of S-layer proteins of strains formerly assigned to *B. brevis*. (A) Sodium dodecyl sulfate-7.5% polyacrylamide Coomassie blue-stained gel of whole-cell proteins. (B) Western blot analysis. Proteins on a sodium dodecyl sulfate-7.5% polyacrylamide gel were transferred electrophoretically to nitrocellulose sheets. Immunoreactive protein bands containing antiserum against the S-layer protein (protein HWP) of *B. choshinensis* HPD31 were detected with an immunostaining HRP kit (Konica Co., Tokyo, Japan). The lane numbers correspond to the strain numbers in Table 1. Lane M contained marker proteins.

parabrevis are closely related taxonomically. This group of organisms may be distinguished by the *B. brevis* group type of S layer (11). A different immunologically and genetically distinct S layer is found in the cell walls of *Bacillus aneurino-*

lyticus and *B. migulanus* (9). These species may informally be designated the *B. aneurinolyticus* group. The data in this study revealed the presence of S-layer proteins in the three new species that were immunologically and genetically related to

FIG. 2. Detection of homologous regions of the S-layer protein genes of strains formerly assigned to *B. brevis* by Southern blot analysis. The probe used for hybridization was the 1.0-kb *HpaI*-*Bgl*III fragment containing the HWP gene of *B. choshinensis* HPD31 (1, 11). Chromosomal DNAs were completely digested with *Eco*RI. Hybridization was detected with the enhanced chemiluminescence gene detection system (Amersham International plc) by using the manufacturer's protocol. The lane numbers correspond to the strain numbers in Table 1.

TABLE 5. Distinctive phenotypic characteristics of *B. reuszeri*, *B. formosus*, and *B. borstelensis*

Characteristic	<i>B. reuszeri</i> (group 5) (n = 3) ^a	<i>B. formosus</i> (group 6) (n = 3)	<i>B. borstelensis</i> (group 7) (n = 16)
Nitrate reduction	0 ^b	100	100
Hydrolysis of:			
Casein	0	100	100
Gelatin	0	100	100
DNA	0	100	100
Decomposition of tyrosine	100	0	100
Growth at:			
pH 5.5 or 5.6	100	0	0
15°C	100	100	0
50°C	0	0	100
Growth in the presence of:			
2% NaCl	100	0	0
3% NaCl	100	0	0
0.001% lysozyme	0	100	0
Utilization of:			
Citrate	0	100	0
Propionate	0	0	0
Acetate	0	100	0
Fumarate	0	100	0
L-Malate	0	100	0
Succinate	0	100	0
L-Glutamate	0	100	0
L-Aspartate	0	66	0
Gluconate	0	66	0
α-Ketoglutarate	0	100	0
Acid production from:			
D-Glucose	100	100	13
Maltose	100	100	13
Glycerol	100	100	0
D-Mannitol	100	0	0
D-Cellobiose	100	0	0
D-Ribose	100	0	13
Raffinose	100	0	0

^a n, number of strains examined.^b Values are the percentages of strains that exhibit positive reactions. In addition, the G+C contents of *B. reuszeri*, *B. formosus*, and *B. borstelensis* are 46 to 47, 47, and 50 to 52 mol%, respectively.

the S-layer proteins found in members of the *B. brevis* group. This observation allowed us to place the three new species in the *B. brevis* group.

Description of *Bacillus reuszeri* sp. nov. *Bacillus reuszeri* (reus.ze'ri. M. L. adj. *reuszeri*, of Reuszer, referring to H. W. Reuszer, the person who isolated the organism). Cells are rods that are 0.5 to 0.9 by 2.0 to 5.0 µm. Gram positive. Motile with peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia. Colonies are flat, smooth, circular, and entire. Soluble pigment is not produced on nutrient agar.

Strictly aerobic.

Catalase positive and oxidase negative.

Acetylmethylcarbinol is not produced (as determined by the Voges-Proskauer test). The pH in Voges-Proskauer broth is higher than 7.0.

Hydrogen sulfide, indole, and lecithinase (as determined by the egg yolk test) are not produced.

Nitrate is not reduced to nitrite.

Casein, gelatin, starch, DNA, and urea are not hydrolyzed.

Tyrosine is decomposed.

Citrate, propionate, acetate, fumarate, L-malate, DL-lactate, succinate, L-glutamate, L-aspartate, alginate, gluconate, α-ketoglutarate, malonate, and tartrate are not utilized.

Growth occurs at pHs 5.5 and 5.6 and in the presence of 2%

NaCl. Growth is inhibited by 3% NaCl, by 0.001% lysozyme, and at 50°C.

The optimum growth temperature is 30°C, the maximum growth temperature is 45°C, and the minimum growth temperature is 10°C (lowest temperature tested).

Acid but no gas is produced from D-glucose, D-fructose, maltose, glycerol, D-mannitol, D-cellobiose, D-ribose, and raffinose. Neither acid nor gas is produced from L-arabinose, D-galactose, lactose, sucrose, D-xylose, trehalose, salicin, D-sorbitol, L-sorbose, D-mannose, melibiose, L-rhamnose, inositol, erythritol, and adonitol.

Specific S-layer proteins are present.

The major fatty acid is anteiso-C_{15:0} acid; the major quinone is menaquinone 7.

The G+C content ranges from 46.4 to 46.7 mol%; the G+C content of the type strain is 46.5 mol%. The type strain is NRRL NRS-1206, which was isolated from soil. The type strain has been deposited in the Japan Collection of Microorganisms, Saitama, Japan, as JCM 9170 and in the Institute for Fermentation, Osaka, Japan, as IFO 15719.

Description of *Bacillus formosus* sp. nov., nom. rev. *Bacillus formosus* (for.mo'sus. M. L. adj. *formosus*, beautiful). Cells are rods that are 0.5 to 0.9 by 2.0 to 5.0 µm. Gram positive. Motile with peritrichous flagella. Ellipsoidal spores are formed in swollen sporangia. Colonies are flat, smooth, circular, and entire. Soluble pigment is not produced on nutrient agar.

Strictly aerobic.

Catalase positive and oxidase negative.

Acetylmethylcarbinol is not produced (as determined by the Voges-Proskauer test). The pH in Voges-Proskauer broth is higher than 7.0.

Hydrogen sulfide, indole, and lecithinase (as determined by the egg yolk test) are not produced.

Nitrate is reduced to nitrite.

Casein, gelatin, and DNA are hydrolyzed. Starch and urea are not hydrolyzed.

Tyrosine is not decomposed.

Citrate, acetate, fumarate, L-malate, succinate, L-glutamate, and α-ketoglutarate are utilized. L-Aspartate and gluconate are utilized by some strains. Propionate, DL-lactate, alginate, and tartrate are not utilized.

Growth occurs at pHs 5.5 and 5.6 and in the presence of 0.001% lysozyme. Growth is inhibited by 2% NaCl and at 50°C.

The optimum growth temperature is 30°C, the maximum growth temperature is 45°C, and the minimum growth temperature is 10°C (lowest temperature tested).

Acid but no gas is produced from D-glucose, D-fructose, maltose, and glycerol. Neither acid nor gas is produced from L-arabinose, D-galactose, lactose, sucrose, D-xylose, trehalose, D-mannitol, D-cellobiose, D-ribose, salicin, D-sorbitol, D-mannose, melibiose, L-rhamnose, raffinose, inositol, erythritol, and adonitol.

Specific S-layer proteins are present.

The major cellular fatty acids are anteiso-C_{15:0} and iso-C_{15:0} acids; the major quinone is menaquinone 7.

The G+C content ranges from 46.8 to 47.2 mol%; the G+C content of the type strain is 47.2 mol%. The type strain is NRRL NRS-863, which was isolated from soil. The type strain has been deposited in the Japan Collection of Microorganisms, Saitama, Japan, as JCM 9169 and in the Institute for Fermentation, Osaka, Japan, as IFO 15716.

Description of *Bacillus borstelensis* sp. nov., nom. rev. *Bacillus borstelensis* (bor.stel.en'sis. M. L. adj. *borstelensis*, referring to Borstel, the German city where the organism was isolated). Cells are rods that are 0.5 to 0.9 by 2.0 to 5.0 µm. Gram positive. Motile with peritrichous flagella. Ellipsoidal spores

TABLE 6. Differentiation of the species belonging to the *B. brevis* group and *B. migulanus*

Characteristic	<i>B. reuszeri</i> ^a	<i>B. formosus</i> ^a	<i>B. borstelensis</i> ^a	<i>B. brevis</i> ^{b,c}	<i>B. agri</i> ^d	<i>B. centrosporus</i> ^d	<i>B. migulanus</i> ^e	<i>B. choshinensis</i> ^{b,d}	<i>B. parabrevis</i> ^b
Nitrate reduction	— ^f	+	+	v	—	v	+	—	+
Oxidase	—	—	—	v	—	—	v	v	v
Hydrolysis of:									
Casein	—	+	+	+	+	—	—	—	+
Gelatin	—	+	+	+	NT	NT	—	—	+
DNA	—	+	+	+	NT	NT	+	—	+
Decomposition of tyrosine	+	—	+	NT	+	+	+	—	NT
Growth at:									
pH 5.5 or 5.6	+	—	—	—	+	—	+	—	v
15°C	+	+	—	v	+	+	—	+	v
50°C	—	—	+	—	—	—	+	—	v
Growth in the presence of:									
2% NaCl	+	—	—	v	NT	NT	+	—	v
3% NaCl	+	—	—	NT	—	—	NT	—	NT
0.001% lysozyme	—	+	—	NT	—	—	+	—	NT
Egg yolk reaction	—	—	—	NT	—	—	+	—	NT
Utilization of citrate	—	+	—	v	—	v	—	—	v
Acid produced from:									
D-Glucose	+	+	v	v	+	+	—	+	+
D-Fructose	+	+	+	v	+	—	v	v	—
Maltose	+	+	v	v	+	+	—	v	v
Sucrose	—	—	—	v	+	+	—	v	v
Trehalose	—	—	—	v	+	+	—	v	+
D-Mannitol	+	—	—	+	+	+	—	v	+
Major cellular fatty acid(s)	anteiso-C _{15:0}	iso-C _{15:0} , anteiso-C _{15:0}	iso-C _{15:0} , anteiso-C _{15:0}	iso-C _{15:0} , anteiso-C _{15:0}	iso-C _{15:0} , anteiso-C _{15:0}	iso-C _{15:0} , anteiso-C _{15:0}	iso-C _{15:0} , C _{16:0} , iso-C _{16:0}	iso-C _{15:0} , anteiso-C _{15:0}	iso-C _{15:0} , anteiso-C _{15:0}
G+C content (mol%)	46–47	47	50–52	46–48	52–54	49–50	42–43	48–51	51–53

^a Data obtained in this study.^b Data from reference 11.^c Data from reference 6.^d Data from reference 7.^e Data from reference 9.^f —, negative reaction; +, positive reaction; v, variable reaction; NT, not tested.

are formed in swollen sporangia. Colonies are flat, smooth, circular, and entire. NRRL NRS-948 produces a soluble brown-red pigment(s) on nutrient agar.

Strictly aerobic.

Catalase positive and oxidase negative.

Acetylmethylcarbinol is not produced (as determined by the Voges-Proskauer test). pH values higher than 7.0 are measured in Voges-Proskauer broth.

Hydrogen sulfide, indole, and lecithinase (as determined by the egg yolk test) are not produced.

Nitrate is reduced to nitrite.

Casein, gelatin, and DNA are hydrolyzed. Starch and urea are not hydrolyzed.

Tyrosine is decomposed.

Citrate, propionate, acetate, fumarate, L-malate, DL-lactate, succinate, L-glutamate, L-aspartate, alginate, gluconate, α-ke-toglutarate, malonate, and tartrate are not utilized.

Growth occurs at pH 5.5 and 5.6. Growth is inhibited by 2% NaCl, by 0.001% lysozyme, and at 55°C.

The optimum growth temperature is 30°C, the maximum growth temperature is 50°C, and the minimum growth temperature is 20°C (lowest temperature tested).

Acid but no gas is produced from D-fructose. Variable reactions are observed with D-glucose, maltose, and D-ribose. Neither acid nor gas is produced from L-arabinose, D-galactose, lactose, sucrose, D-xylose, trehalose, glycerol, D-mannitol, L-sorbose, D-mannose, melibiose, L-rhamnose, raffinose, inositol, erythritol, and adonitol.

Specific S-layer proteins are present.

The major cellular fatty acids are iso-C_{15:0} and anteiso-C_{15:0} acids; the major quinone is menaquinone 7.

The G+C content ranges from 50.4 to 51.8 mol%; the G+C content of the type strain is 51.3 mol%. The type strain is NRRL NRS-818, which was isolated from soil in Borstel, Germany. The type strain has been deposited in the Japan Collection of Microorganisms, Saitama, Japan, as JCM 9022 and in the Institute for Fermentation, Osaka, Japan, as IFO 15714.

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