Taxonomy of *Bacillus circulans* Jordan 1890: Base Composition and Reassociation of Deoxyribonucleic Acid

L. K. NAKAMURA* AND J. SWEZEY

Northern Regional Research Center, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois 61604

The guanine-plus-cytosine (G+C) contents of 123 strains previously identified as *Bacillus circulans* Jordan 1890 were determined. Of the strains examined, only 18 had G+C contents of 37 to 41 mol%, a range that included the G+C content (38.1 mol%) of the type strain. A major portion of the strain (93 strains) had G+C contents in the range from 46 to 55 mol%. For seven strains the G+C contents ranged from 42 to 45 mol%, and for another small group of five strains the G+C contents ranged from 56 to 61 mol%. Deoxyribonucleic acid reassociation studies revealed that 10 of the 18 strains with G+C contents of 37 to 41 mol% were closely related genetically to each other and to the type strain. This genetically related group was phenotypically quite homogeneous. Our results strongly suggested that most of the strains were misclassified as *B. circulans*. Consequently, the phenotypic heterogeneity of the species *B. circulans* Jordan 1890 was not due to inherent variability of genetically related strains, but to variability introduced by inclusion of genetically unrelated organisms.

In 1890, Jordan described a new species and named it *Bacillus circulans* because the interior of its colonies, when viewed under low magnification, flowed in a circular pattern (11). Later, in 1916, Ford isolated a strain that he considered to be the same as the strain of Jordan because its colonies also displayed interior circular movement (4). However, an examination of the descriptions revealed that whereas the rod-shaped organisms described by Jordan measured 1.0 by 2.5 μm and had nonswelling sporangia, those described by Ford measured 0.5 by 2.5 to 4 μm and produced swollen sporangia. Because the strain of Jordan has been lost, strain 26 of Ford, the only historical strain available, has been designated the type strain.

From the beginning, *B. circulans* has been a poorly defined species. Gibson and Topping (T. Gibson and L. E. Topping, Soc. Agric. Bacteriol. Proc. Abstr., p. 43–44, 1938) referred to this species as a complex “exhibiting variation in several directions.” Ensuing studies of the taxon by other researchers reinforced this view (1, 9, 10, 17, 21, 24). Such studies have disclosed a morphologically, nutritionally, physiologically, immunologically, and chemically heterogeneous group. Currently, the situation remains unchanged. In *Bergey's Manual of Determinative Bacteriology*, 8th ed., the *B. circulans* group is considered to be an assemblage in which “variant features occur in many combinations without any obvious association in discrete groups” (5).

Very limited analyses of guanine-plus-cytosine (G+C) contents have disclosed widely varying values for *B. circulans* strain (5). These limited observations suggest that genetic diversity exists among the strains identified as *B. circulans*, thus accounting for the heterogeneity of the species. Therefore, we undertook studies to assess the extent of G+C variation among a large number of existing bacterial strains identified as *B. circulans* and to determine by deoxyribonucleic acid (DNA) reassociation experiments which of the strains, if any, were genetically related to the type strain. The fundamental characteristics of the species were established by examining the group of organisms determined to be related genetically to the type strain.

MATERIALS AND METHODS

Bacterial strains. The *B. circulans* strains used in this study are listed in Table 1. The Northern Regional Research Laboratory (NRRL) designations include the prefixes B-, NRS-, and BD-. The prefix B- indicates the strains obtained directly from individuals or the strains isolated at the Northern Regional Research Center; the prefix NRS- designates strains from the *Bacillus* collection of N. R. Smith, which has been deposited in toto in the Agricultural Research Service Culture Collection (NRRL) by R. E. Gordon; and the prefix BD- designates the strains from the collection of B. Delaporte, a part of which has also been deposited in the Agricultural Research Service Culture Collection. In this paper all references to strains are to serial numbers (Table 1).

Characterization. Unless specified otherwise, the strains were characterized by the method of Gordon et

TABLE 1. List of *B. circulans* strains used in this study

Serial no.	Laboratory no.	Received as strain:	Source ^a	Strain history ^b
1	NRRL B-142	PCI 221	1	Streptomycin assay
2	NRRL B-745	8 · iv	2	Deposited as " <i>Vibrio perimastix</i> " ^c
3	NRRL B-1359	Q-19	3	Circulin production
4	NRRL B-1373	82	2	Deposited as " <i>Vibrio perimastix</i> "
5	NRRL B-1488		2	Deposited as " <i>Vibrio perimastix</i> "
6	NRRL B-4031	G3-7	4	Isolated for phosphomannase activity
7	NRRL B-4255	1	5	Isolated from honeybee broodcomb in Finland
8	NRRL NRS-290	NRS-290	6	K. F. Kellerman, " <i>Bacillus amylolyticus</i> "
9, 10	NRRL NRS-294, -295	NRS-294, -295	6	N. R. Smith, isolated from soil
11	NRRL NRS-313	NRS-313	6	ATCC 4516 from W. W. Ford 7 from Kral collection, " <i>Bacillus globigii</i> "
12	NRRL NRS-358	NRS-358	6	AMNH from W. W. Ford
13	NRRL NRS-359	NRS-359	6	N. R. Smith
14	NRRL NRS-375	NRS-375	6	N. R. Smith, isolated from soil
15-24	NRRL NRS-381 to -390	NRS-381 to -390	6	N. R. Smith, isolated from soil
25-28	NRRL NRS-396 to -399	NRS-396 to -399	6	N. R. Smith, isolated from soil
29-31	NRRL NRS-625, -626, -639	NRS-625, -626, -639	6	J. R. Porter from G. Bredemann, " <i>Bacillus nitidus</i> "
32	NRRL NRS-666	NRS-666	6	University of Washington, " <i>Bacillus latus</i> "
33-36	NRRL NRS-676 to -679	NRS-676 to -679	6	N. R. Smith, migratory colonies 1 through 4, isolated from soil
37, 38	NRRL NRS-715, -716	NRS-715, -716	6	A. Russ-Munzer W1, WII
39	NRRL NRS-726	NRS-726	6	ATCC 4513 from W. W. Ford 26, type strain
40	NRRL NRS-727	NRS-727	6	ATCC 61 from AMC 732 (" <i>Bacillus fusiformis</i> ") from AMNH from W. W. Ford 10
41	NRRL NRS-728	NRS-728	6	ATCC 4515 from W. W. Ford 24, " <i>B. fusiformis</i> "
42	NRRL NRS-729	NRS-729	6	ATCC 4516 from W. W. Ford from Kral collection, " <i>Bacillus globigii</i> "
43	NRRL NRS-746	NRS-746	6	ATCC 4530 from W. W. Ford 28, " <i>Bacillus terminalis</i> "
44	NRRL NRS-760	NRS-760	6	S. F. Snieszko, " <i>Bacillus krzemieniewski</i> ," isolated from soil
45	NRRL NRS-765	NRS-765	6	R. P. Myers, isolated from flour
46	NRRL NRS-826	NRS-826	6	J. R. Porter from G. Bredemann from G. Stapp, " <i>Bacillus carotarum</i> "
47	NRRL NRS-831	NRS-831	6	J. R. Porter from NCTC 2610 from W. W. Ford 26
48	NRRL NRS-832	NRS-832	6	J. R. Porter from H. G. Thornton, " <i>Bacillus cloisteroides</i> "
49	NRRL NRS-838a	NRS-838a	6	J. R. Porter from G. Bredemann, " <i>Bacillus cohaerens</i> "
50	NRRL NRS-839a	NRS-839a	6	J. R. Porter from C. Stapp, " <i>B. cohaerens</i> "
51	NRRL NRS-841	NRS-841	6	J. R. Porter from G. Bredemann, " <i>B. cohaerens</i> "
52	NRRL NRS-924	NRS-924	6	J. R. Porter from M. Schiebllich, " <i>Bacillus pabuli</i> "
53	NRRL NRS-977	NRS-977	6	J. R. Porter from G. Bredemann, " <i>Bacillus suprarresistans</i> "
54	NRRL NRS-1000	NRS-1000	6	J. R. Porter from G. Bredemann, " <i>Bacillus validus</i> "
55	NRRL NRS-1029a	NRS-1029a	6	J. Eisenberg, " <i>Bacillus schuykilliensis</i> "
56	NRRL NRS-1061	NRS-1061	6	NCA
57	NRRL NRS-1108	NRS-1108	6	ATCC 9500 from W. H. Fuller, " <i>Bacillus aporrhoeus</i> "

TABLE 1—Continued

Serial no.	Laboratory no.	Received as strain:	Source ^a	Strain history ^b
58	NRRL NRS-1136	NRS-1136	6	ATCC 9966 from FDA strain PCI 221
59	NRRL NRS-1145	NRS-1145	6	A. L. Pollard, unknown strain
60-70	NRRL NRS-1167 to -1177	NRS-1167 to -1177	6	H. W. Reuszer, Army 297B, 353, 629, 707, 857, 1292C, 1304, 1324, 1329, 1335, and 1376
71	NRRL NRS-1218	NRS-1218	6	C. McLeod
72	NRRL NRS-1250	NRS-1250	6	P. H. H. Gray, " <i>Bacillus effluens</i> "
73	NRRL NRS-1251	NRS-1251	6	P. H. H. Gray, " <i>Bacillus kellermani</i> "
74	NRRL NRS-1254	NRS-1254	6	P. H. H. Gray, " <i>Bacillus torquens</i> "
75, 76	NRRL NRS-1275, -1278	NRS-1275, -1278	6	M. Shaw 4204, N-3
77	NRRL NRS-1280	NRS-1280	6	S. D. Henriksen
78, 79	NRRL NRS-1281, -1282	NRS-1281, -1282	6	S. D. Henriksen from A. W. Turner and C. E. Eales, strain A, strain B
80	NRRL NRS-1337	NRS-1337	6	M. O'Brien
81-97	NRRL NRS-1340 to -1356	NRS-1340 to -1356	6	F. E. Clark 1, 4, 13, 15, 19, 20, 2, 3, 5, 6, 8, 12, 22, 23, 10, 11, and 14, isolated from soil
98-102	NRRL NRS-1431, -1433 to -1436	NRS-1431, -1433 to -1436	6	M. B. Allen, thermophilic strains 15-3, 15-2, 7-2, 1, and 2
103-107	NRRL BD-525 to -529	525 to 529	7	Isolated from coarse Swedish sand
108, 109	NRRL BD-530, 531	530, 531	7	Isolated from Canadian soil
110	NRRL BD-532	532	7	Isolated from Indian soil
111-116	NRRL BD-533 to -538	533 to 538	7	Isolated from Canadian soil
117, 118	NRRL BD-539, -540	539, 540	7	Isolated from Trinidadian soil
119	NRRL BD-541	541	7	Isolated from tadpole intestine
120-123	NRRL BD-542, -543, -661, -662	542, 543, 661, 662	7	Isolated from Canadian soil

^a 1, Food and Drug Administration, Washington, D.C.; 2, P. H. H. Gray, McGill University, Montreal, Canada; 3, the Upjohn Co., Kalamazoo, Mich.; 4, M. C. Cadmus, Northern Regional Research Center, Peoria, Ill.; 5, depositor not known; 6, the N. R. Smith *Bacillus* collection maintained by R. E. Gordon, Rutgers University, New Brunswick, N.J.; 7, B. Delaporte, Laboratoire Cytologie Vegetale, Paris, France.

^b FDA, Food and Drug Administration, Washington, D.C.; ATCC, American Type Culture Collection, Rockville, Md.; AMNH, American Museum of Natural History, New York, N.Y.; AMC, Walter Reed Army Medical Center, Washington, D.C.; NCTC, National Collection of Type Cultures, London, England; NCA, National Canners Association, San Francisco, Calif.

^c Species names not on the Approved Lists of Bacterial Names (21) or validated since 1 January 1980 are in quotation marks.

al. (6). The carbohydrate fermentation tests were expanded to include the following as substrates: arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, melibiose, rhamnose, ribose, salicin, sorbitol, sucrose, trehalose, and xylose. In addition to citrate, the utilization of acetate, fumarate, malate, and succinate was assessed. Hydrolysis of Tween 80 was determined by the method of Breuil and Gounot (2). The decomposition of arginine, lysine, and ornithine was determined in Moeller decarboxylase broth (16). The production of H₂S was detected by stab culturing in triple sugar iron agar (7). The method of Steel (22) was used for the oxidase test.

DNA isolation, G+C ratios, and DNA reassociation. For DNA extraction, cells were grown for 24 h in tryptone glucose yeast extract broth (8) at 28°C on a rotary shaker at 200 rpm. Sporulation was not observed in any of the cultures after 24 h of growth.

DNA was extracted and purified by a modification of the method of Marmur (14). The modification involved the use of hydroxyapatite (Bio-Gel HTP; Bio-Rad Laboratories, Richmond, Calif.) chromatography (13) to produce highly purified DNA preparations. The

purity and quality of each DNA preparation were determined by monitoring the ratio of absorbance at 260 nm to absorbance at 280 nm (1.8 to 1.9) and the ratio of absorbance at 260 nm to absorbance at 230 nm (2.0 to 2.3), using the method of Marmur (14), and by determining the melting curves, which include hyperchromicity values from 38 to 40% (12).

The G+C content of the DNA was estimated by the buoyant density method (18). Buoyant density of DNA was measured by CsCl density gradient centrifugation in a Beckman model E ultracentrifuge. *Micrococcus luteus* (synonym, "*Micrococcus lysodeikticus*") DNA was purchased from the Sigma Chemical Co., St. Louis, Mo., and was used as an internal standard.

In preparation for renaturation studies, native double-stranded DNA was sheared by two passages through a French pressure cell at 10,000 lb/in². After filtration through a membrane filter (pore size, 0.45 µm; Millipore Corp., Bedford, Mass.), the sheared DNA solution was dialyzed exhaustively against a 0.001 M ethylenediaminetetraacetate solution containing 5 ml of 1× SSC (0.15 M NaCl plus 0.015 M trisodium citrate, pH 7.0) per liter. After dialysis, the

DNA solution was dried by lyophilization. The dried material was dissolved in 2 ml of double-distilled water.

The kinetics of DNA renaturation was measured in reaction mixtures that contained 0.25 ml of $10\times$ SSC, 0.2 ml of dimethyl sulfoxide, approximately 70 μ g of DNA, and double-distilled water to bring the volume to 1 ml. In reaction mixtures containing two different DNAs, the quantities (about 35.0 μ g) of the nucleic acids were adjusted to differ by no more than 0.15 μ g. The reaction mixtures were placed in 0.4-ml quartz cuvettes that were sealed with Teflon-lined stoppers. Denaturation and renaturation were effected and monitored with a Gilford UV-VIS thermal analyzer system. A denaturation temperature of 90°C was reached rapidly and was held for 10 min after the hyperchromic shift at 260 nm. Subsequently, the temperature was lowered rapidly to 65°C; the optical density at this point was taken to represent 0% renaturation (although some reassociation undoubtedly occurred). The absorbance change at 260 nm was monitored for 15 min, during which the rate of renaturation was apparently linear even though second-order kinetics were displayed over a longer period of time. All determinations were repeated three times. Reassociation values (percentages) were calculated by using the equation of De Ley et al. (3).

RESULTS

Analyses of the DNAs of 123 strains identified as *B. circulans* revealed G+C contents ranging from 37 to 60 mol% (Table 2). Of the strains examined, 18 clustered into a group having G+C contents of 37 to 41 mol%. The type strain (strain 39) belonged to this group. A major portion of the strains (93 strains) had G+C contents ranging from 46 to 55 mol%. In seven strains, the G+C contents ranged from 42 to 45 mol%. Another small group (five strains) had G+C contents of 56 to 61 mol%.

DNA reassociation studies were done to determine the genetic relationship of the strains with G+C contents ranging from 37 to 41 mol% (Table 3). High relatedness values (90 to 100%) were measured among the type strain and strains 11, 12, 40 through 43, 45, and 47. Strain 57 showed moderate DNA relatedness (50 to 60%) to these nine strains. These 10 strains were designated group I. Except for the moderate value of 60% when strains 19 and 21 were compared, the levels of DNA relatedness among the group II strains (strains 19, 21, 26, 46, 63, 82, and 94) were low (Table 3).

Table 4 shows a comparison of the phenotypic characteristics of group I and II strains. Our data indicated that group I consisted of a phenotypically homogeneous cluster of organisms. (Because of the homogeneity, the data for group I are not presented on a per strain basis.) Variability occurred only in the ability to grow in 7% NaCl, Tween 80 hydrolysis, and the utilization of succinate. Strains 40, 45, and 57 failed to grow in 7% NaCl. Strain 57 also utilized succinate and

TABLE 2. Grouping of *B. circulans* strains according to G+C content

G+C range (mol%)	No. of strains	Laboratory no.
37.1-38	3	NRRL NRS-313, -729, -831
38.1-39	10	NRRL NRS-358, -726, -727, -728, -746, -765, -826, -1108, -1341, -1353
39.1-40	3	NRRL NRS-397, -398, -1170
40.1-41	2	NRRL NRS-385, -387
41.1-42	2	NRRL BD-535, -661
42.1-43	2	NRRL NRS-1433; NRRL BD-528
43.1-44	2	NRRL BD-539, -543
44.1-45	1	NRRL BD-530
45.1-46	5	NRRL NRS-1351, -1434, -1435; NRRL BD-531, -540
46.1-47	6	NRRL B-745; NRRL NRS-389, -396, -399, -1431, -1436
47.1-48	11	NRRL B-1373, -1488; NRRL NRS-375, -677, -1175, -1346, -1347, -1350, -1352, -1356; NRRL BD-525
48.1-49	14	NRRL B-1359; NRRL NRS-1173, -1176, -1251, -1280, -1344, -1345, -1349; NRRL BD-526, -527, -529, -533, -537, -541
49.1-50	10	NRRL NRS-715, -716, -924, -1061, -1174, -1177, -1348; NRRL BD-533, -536, -538
50.1-51	15	NRRL B-142, -4031; NRRL NRS-383, -386, -678, -679, -977, -1167, -1281, -1337, -1342, -1343, -1354; NRRL BD-532, -662
51.1-52	10	NRRL NRS-295, -381, -382, -384, -666, -676, -1145, -1168, 1171, -1282
52.1-53	6	NRRL NRS-290, -359, -832, -1136, -1254, -1340
53.1-54	9	NRRL NRS-388, -390, -625, -626, -839a, -841, -1000, -1169, -1355
54.1-55	7	NRRL B-4255; NRRL NRS-639, -838a, -1029a, -1218, -1275; NRRL BD-542
55.1-56	1	NRRL NRS-1250
57.1-58	1	NRRL NRS-1278
58.1-59	1	NRRL NRS-1172
59.1-60	1	NRRL NRS-294
60.1-61	1	NRRL NRS-760

was not able to hydrolyze Tween 80. In contrast to group I strains, the group II strains exhibited variable reactions for the following characteristics: growth in lysozyme and 5% NaCl; acid production in litmus milk; nitrate reduction;

TABLE 3. DNA relatedness among *B. circulans* strains with G+C contents ranging from 37 to 41 mol%

Strain	% Reassociation to DNAs from the following strains: ^a															
	12 ^b	19	21	26	39 ^c	40	41	42	43	45	46	47	57	63	82	94
11 ^b	100	6	10	9	100	95	100	98	97	100	36	90	58	11	35	6
12		5	27	18	98	99	100	100	100	97	29	85	51	12	33	10
19			63	21	10	15	8	9	17	23	7	5	26	9	30	4
21				26	4	12	11	7	10	8	16	4	9	6	20	8
26					24	14	25	10	25	18	16	6	21	10	8	14
39 ^c						100	100	99	99	99	31	90	63	25	35	3
40							98	100	100	98	36	87	56	28	32	5
41								97	96	100	27	86	67	31	30	11
42									96	100	26	90	51	21	26	8
43										100	32	91	47	19	8	13
45											32	88	48	18	10	10
46												26	35	20	19	14
47													38	15	30	7
57														23	33	16
63															23	33
82																10

^a Values are the averages of three determinations.^b Serial number (see Table 1).^c Type strain.

oxidase activity; Tween 80 hydrolysis; urease activity; utilization of acetate, citrate, fumarate, and succinate; and fermentation of arabinose, cellobiose, lactose, mannitol, melibiose, rhamnose, ribose, salicin, sorbitol, and xylose.

Morphologically, the cells of group I strains generally were 0.5 to 1 by 3 to 5 μ m. Except for strain 94, whose cells measured 1 to 2 by 5 to 10 μ m, the cells of group II strains had dimensions similar to those of the cells of group I strains. All group I strains were gram variable; group II included gram-variable and gram-positive strains. Except for strain 94, all group I and II strains produced swollen sporangia.

DISCUSSION

According to Table 2, only 18 of the 123 strains identified as *B. circulans* had G+C contents in the range from 37 to 41 mol%, which included the G+C content (38.1 mol%) of the type strain. It has been generally accepted that organisms with similar G+C values may or may not be related. In the present study, high DNA relatedness values (90 to 100%) showed that 9 of these 18 strains were closely related genetically. Since the nine strains included the type strain, these organisms must represent the species *B. circulans* as it was described by Ford (4). The extent of DNA relatedness (50 to 60%) suggested that a tenth strain, strain 57, was a subspecies. In general, the eight remaining strains were not related genetically.

Table 4 shows that the 10 genetically related strains (group I) displayed a minimum amount of phenotypic variation. Three strains failed to

grow in 7% NaCl, and, in addition, one of these three did not hydrolyze Tween 80 but did utilize succinate. These three divergent characteristics were shown by strain 57, an organism tentatively classified as a subspecies. Unfortunately, the small number of closely related strains was not suitable for obtaining a good estimate of the extent of phenotypic variability of the species. However, our data suggested that *B. circulans* could be characterized fundamentally as follows: gram-variable rod-shaped cells that measure 0.5 to 1 by 3 to 5 μ m; are motile, produce swollen sporangia, grow anaerobically, are catalase positive, usually grow in 7% NaCl, do not reduce nitrate to nitrite, are oxidase negative, usually hydrolyze Tween 80, hydrolyze starch, do not utilize citrate, do not produce acetyl-methylcarbinol, and ferment the common pentoses, hexoses, hexitols, and disaccharides. In contrast to group I strains, the seven genetically unrelated group II strains exhibited variable reactions for a wide range of characteristics (Table 4). Except for its resistance to lysozyme and its failure to hydrolyze casein, strain 94 generally showed the characteristics of *Bacillus megaterium*. On the basis of their characteristics, however, the other group II strains could not be readily associated with any of the other firmly established *Bacillus* species. These unidentified strains are being studied to determine their taxonomic positions.

In 106 of the 123 strains, the G+C contents ranged from 45 to 60 mol%. Sueoka (23) suggested that the probability that DNA molecules have identical nucleotide sequences of gene length is low when the differences in G+C values

TABLE 4. Characteristics of *B. circulans* with G+C contents ranging from 37 to 41 mol%^a

Characteristics ^b	Group I ^c	Strain (serial no.)						
		19	21	26	46	63	82	94
Morphology								
Width (μm)	0.5-1	0.5-1	0.5-1	0.5-1	0.5-1	0.5-1	0.5-1	1-2
Length (μm)	3-7	3-5	3-5	3-5	3-5	3-5	4-8	5-10
Gram stain	v	v	v	+	v	+	v	+
Swollen sporangium	+	+	+	+	+	+	+	-
Anaerobic growth	+	+	+	+	+	+	+	-
Growth in lysozyme	-	-	-	+	-	-	-	+
Growth in:								
5% NaCl	+	-	-	+	+	-	-	+
7% NaCl	+	-	-	-	-	-	-	-
Amino acid-decomposing enzyme: argine dihydrolase	-	-	-	-	-	-	-	+
Litmus milk	A	nc	nc	A	A	nc	A	A
Nitrate reduction to nitrite	-	+	+	+	+	-	-	+
Oxidase	-	+	+	+	-	+	+	+
Tween 80 hydrolysis	+	-	+	+	-	+	-	-
Urease	-	-	-	-	-	+	-	+
pH in VP broth	4.9	5.0	5.0	4.9	4.9	4.9	5.0	4.9
Utilization of:								
Acetate	+	+	+	+	-	+	+	+
Citrate	-	-	+	-	-	-	-	+
Fumarate	+	+	+	-	-	+	-	+
Malate	+	+	+	-	+	+	-	+
Succinate	-	-	+	-	-	+	-	+
Acid from:								
Arabinose	+	-	-	+	+	+	+	-
Cellobiose	+	-	-	+	+	+	+	+
Lactose	+	+	-	+	+	+	+	-
Mannitol	+	-	+	+	+	+	+	-
Melibiose	+	+	-	+	+	+	+	-
Rhamnose	+	+	-	+	+	-	+	-
Ribose	+	+	+	+	+	-	+	+
Salicin	+	+	+	+	+	-	+	-
Sorbitol	+	-	-	-	+	+	+	-
Xylose	+	-	-	+	+	+	+	-

^a Symbols: +, positive; -, negative; v, variable; A, acid produced; nc, no change.^b All strains were motile; grew at pH 5.7; produced catalase; hydrolyzed starch; and produced acid from fructose, galactose, glucose, maltose, mannose, sucrose, and trehalose. No strain grew in 10% NaCl; produced lysine decarboxylase, ornithine decarboxylase, egg yolk lecithinase, H₂S, or indole; hydrolyzed casein; or gave a positive Voges-Proskauer (VP) test reaction.^c Type strain included in this group.

exceed 10 mol%. Furthermore, accumulated data have indicated that within a genus, strains with G+C contents varying by 2 mol% or more usually are members of separate species (15, 19). Therefore, the significant differences in G+C content among many of the organisms which we

studied indicated that this group of 106 strains consisted of members of several different species. Perhaps some of these will prove to be related, because a number of the strains may share a common spore antigen (24) and have similar G+C contents (Table 2). Whether these

strains are strains of new species or existing species will be determined in continuing studies.

DNA relatedness studies and G+C analyses showed that the majority of strains which we examined were erroneously classified as *B. circulans*. Consequently, the phenotypic heterogeneity of this species is not due to inherent variability of genetically related strains, but is due to inclusion of genetically unrelated organisms.

ACKNOWLEDGMENT

We thank Judith Scheppeler for her able technical assistance.

LITERATURE CITED

- Bradley, D. E., and J. G. Franklin. 1958. Electron microscope survey of the surface configuration of spores of the genus *Bacillus*. *J. Bacteriol.* **76**:618-630.
- Breuil, C., and A. M. Gounot. 1972. Recherches préliminaires sur les bactéries lipolytiques psychrophiles des sols et des eaux. *Can. J. Microbiol.* **18**:1445-1451.
- De Ley, J., H. Cattoir, and A. Reynaerts. 1970. The quantitative measurement of DNA hybridization from renaturation rates. *Eur. J. Biochem.* **12**:133-142.
- Ford, W. W. 1916. Studies on aerobic spore-bearing non-pathogenic bacteria. II. Miscellaneous cultures. *J. Bacteriol.* **1**:518-526.
- Gibson, T., and R. E. Gordon. 1974. Genus I. *Bacillus* Cohn 1872, p. 529-550. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Gordon, R. E., W. C. Haynes, and C. H. Pang. 1973. The genus *Bacillus*. *Agricultural handbook no. 427*. U.S. Department of Agriculture, Washington, D.C.
- Hajna, A. A. 1945. Triple-sugar iron agar medium for the identification of the intestinal group of bacteria. *J. Bacteriol.* **49**:516-517.
- Haynes, W. C., L. J. Wickerham, and C. W. Hesseltine. 1955. Maintenance of cultures of industrially important microorganisms. *Appl. Microbiol.* **3**:361-368.
- Hill, I. R., and T. R. G. Gray. 1967. Application of the fluorescent-antibody technique to an ecological study of bacteria in soil. *J. Bacteriol.* **93**:1888-1896.
- Jayne-Williams, D. J., and G. C. Cheeseman. 1960. The differentiation of bacterial species by paper chromatography. IX. The genus *Bacillus*: a preliminary investigation. *J. Appl. Bacteriol.* **23**:250-268.
- Jordan, E. O. 1890. A report on certain species of bacteria observed in sewage, p. 821-844. In Sedgewick, A report of the biological work of the Lawrence Experiment Station, including an account of methods employed and results obtained in the microscopical and bacteriological investigation of sewage and water. Report on water supply and sewerage, part 2. Massachusetts State Board of Health, Boston.
- Mandel, M., and J. Marmur. 1968. Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine of DNA. *Methods Enzymol.* **12B**:195-206.
- Markov, G. G., and I. G. Ivanov. 1974. Hydroxyapatite column chromatography procedure for isolation of purified DNA. *Anal. Biochem.* **59**:559-563.
- Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.* **3**:208-218.
- Marmur, J., S. Falkow, and M. Mandel. 1963. New approaches to bacterial taxonomy. *Annu. Rev. Microbiol.* **17**:329-372.
- Moeller, V. 1955. Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. *Acta Pathol. Microbiol. Scand.* **36**:158-172.
- Proom, H., and B. C. J. G. Knight. 1955. The minimal nutritional requirements of some species of the genus *Bacillus*. *J. Gen. Microbiol.* **13**:474-480.
- Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *J. Mol. Biol.* **4**:430-443.
- Seidler, R. J., M. D. Knittel, and C. Brown. 1975. Potential pathogens in the environment: cultural reactions and nucleic acid studies on *Klebsiella pneumoniae* from clinical and environmental sources. *Appl. Microbiol.* **29**:819-825.
- Skerman, V. B. D., V. McGowan, and P. H. A. Sneath (ed.). 1980. Approved lists of bacterial names. *Int. J. Syst. Bacteriol.* **30**:225-420.
- Smith, N. R., R. E. Gordon, and F. E. Clark. 1952. Aerobic sporeforming bacteria. *Agriculture monograph no. 16*. U.S. Department of Agriculture, Washington, D.C.
- Steel, K. J. 1961. The oxidase reaction as a taxonomic tool. *J. Gen. Microbiol.* **25**:297-306.
- Sueoka, N. 1961. Variation and heterogeneity of base composition of deoxyribonucleic acids: a compilation of new and old data. *J. Mol. Biol.* **3**:31-40.
- Wolf, J., and M. S. U. Chowdhury. 1971. The *Bacillus circulans* complex: biochemical and immunological studies, p. 227-245. In A. N. Barker, G. W. Gould, and J. Wolf (ed.), *Spore research 1971*. Academic Press, Inc., New York.