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Taxonomic Relationship of Black-Pigmented *Bacillus subtilis* Strains and a Proposal for *Bacillus atrophaeus* sp. nov.

L. K. NAKAMURA

Northern Regional Research Center, Peoria, Illinois 61604

The taxonomic position of *Bacillus subtilis* strains that produce soluble black pigment is unclear. To assess the genetic relatedness between the pigmented and nonpigmented strains, deoxyribonucleic acid (DNA) reassociation was measured spectrophotometrically. Among the 40 pigmented strains examined, two distinct DNA relatedness groups were found. A total of 25 strains (group 1) showed 24 to 34% DNA relatedness and 15 strains (group 2) showed 70 to 100% relatedness to *Bacillus subtilis* type strain NRRL NRS-744. The intragroup DNA relatedness values for each group ranged from 85 to 100%; the intergroup relatedness values ranged from 20 to 35%. A multilocus enzyme electrophoresis analysis revealed a low level of similarity between group 1 and group 2 or the nonpigmented group. The group 2 strains and the nonpigmented strains clustered in a common group, indicating the close genetic relationship of these organisms. My results strongly suggest that group 2 is a pigmented variant of *B. subtilis*, but group 1 is a new species, for which the name *Bacillus atrophaeus* is proposed. The type strain of the new species is strain NRRL NRS-213.

Smith et al. (13) observed and studied two black-pigmented varieties of *Bacillus subtilis*. One variety, designated "*Bacillus subtilis* var. *aterrimus*," developed a soluble black pigment in media containing glucose or other utilizable carbohydrates; the other, called "*Bacillus subtilis* var. *niger*," formed a soluble dark pigment in tyrosine-containing media. Early workers presumed that pigmentation in "*B. subtilis* var. *niger*" resulted from tyrosinase activity. Because it was repeatedly observable in the crude agar media available to Smith et al. (13), black-pigment development was considered to be a stable characteristic and, therefore, a dependable and distinctive basis for varietal designation.

Some black-pigmented *B. subtilis* strains have important uses or characteristics. For example, "*B. subtilis* var. *niger*" strains produce 1-deoxynojirimycin, a substance with antibiotic as well as glucosidase-inhibiting activities (10). Selected "*B. subtilis* var. *niger*" strains are also used as standards for autoclave sterility testing (*Catalogue of Bacteria, Phages, and rRNA Vectors*, 16th ed., American Type Culture Collection, Rockville, Md.).

Except for pigment production, the colored strains are generally phenotypically indistinguishable from nonpigmented *B. subtilis* strains. However, in an extensive numerical phenetic survey carried out by Priest et al. (8), *B. subtilis* and "*B. subtilis* var. *niger*" did segregate into distinct but adjacent clusters. Furthermore, studies based on a small number of strains have indicated that strains classified as "*B. subtilis* var. *aterriums*" are genetically unrelated to strains classified as "*B. subtilis* var. *niger*" (2). In this study I augmented the sparse previously existing taxonomic data with guanine-plus-cytosine (G+C) and deoxyribonucleic acid (DNA) relatedness measurements and with enzyme electrophoresis pattern analyses of 40 black-pigmented and 12 nonpigmented strains identified as *B. subtilis*.

MATERIALS AND METHODS

Bacterial strains. Table 1 lists the pigmented and nonpigmented *B. subtilis* strains used in this study. Also used in this study were *Bacillus alvei* Cheshire and Cheyne 1885 NRRL B-383^T (T = type strain), *Bacillus badius* Batchelor 1919 NRRL NRS-663^T, *Bacillus brevis* Migula 1900 NRRL NRS-604^T, *Bacillus coagulans* Hammer 1915 NRRL NRS-609^T, *Bacillus firmus* Bredemann and Werner 1933 NRRL B-14307^T, *Bacillus licheniformis* (Weigmann) Chester 1901 NRRL NRS-1264^T, *Bacillus polymyxa* (Prazmowski) Mace 1889 NRRL NRS-1105^T, and *Bacillus pumilus* Meyer and Gottheil 1901 NRRL NRS-272^T. The Northern Regional Research Laboratory (NRRL) strain designations include the prefixes B- and NRS-; the prefix B- indicates strains that were obtained directly from a source or strains that were isolated at the Northern Regional Research Center, and the prefix NRS- indicates strains that were obtained from N. R. Smith. Working cultures were grown at 30°C in soil extract agar (5) until sporulation occurred, and they were stored at 4°C.

DNA investigations. The cells were grown in TGY broth (6) with agitation and were harvested by centrifugation at 5°C in the mid- or late logarithmic growth phase. All cultures were checked microscopically for the absence of sporulation before harvesting. Previous publications have described the procedure used for preparing highly purified DNA samples by hydroxyapatite chromatography and the method used for measuring the extent of DNA reassociation by determining DNA renaturation rates spectrophotometrically with a model 250 ultraviolet spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) equipped with a model 2527 thermoprogramer (7). The equation of De Ley et al. (3) was used to calculate DNA relatedness values.

The G+C contents of DNA samples were determined by measuring buoyant densities by CsCl density centrifugation in a Beckman model E ultracentrifuge (9). *Micrococcus luteus* (synonym, "*Micrococcus lysodeikticus*") DNA with a buoyant density of 1.724 g/cm^3, which was purchased from Sigma Chemical Co., St. Louis, Mo., was used as an internal standard.

Characterization. The physiological, morphological, and biochemical characteristics were determined as described previously (5, 7).

Enzyme electrophoresis. Cells were grown at 30°C for 24 h in 3 liters of TGY broth with agitation, harvested by centrifugation at 30,000 × *g* for 10 min, and suspended in 10 ml of pH 6.8 buffer containing 10 mM tris (hydroxymethyl) aminomethane (Tris), 1 mM ethylenediaminetetraacetate, and 0.5 mM $NaH(PO_4)_2$. The cells were disrupted by passage through a chilled French pressure cell at 10,000 lb/in^2. After centrifugation at 30,000 × *g* for 15 min at 4°C, portions of the

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

NRRL no.	Received as strain(s):	Source[a]	Strain history[b]
B-357	NRS-242	1	From soil
B-360	NRS-230	1	C. Thom, from corn
B-361	NRS-259	1	I. C. Hall
B-362	NRS-624	1	J. R. Porter from F. W. Fabian
B-363	NRS-212	1	From Colorado soil
B-364	NRS-220	1	AMNH from Kral Collection
B-365	NRS-356	1	"*B. subtilis* var. *niger*"[c]
B-447	20	2	"*Bacillus mesentericus*"
B-554	12-H	3	
B-627		4	"*B. subtilis* var. *niger*"
B-765	ATCC 6633	5	N. R. Smith 231 from K. F. Kellerman
B-971	398	6	
B-4418	GL 100	7	"*B. subtilis* subsp. *niger*"
NRS-162	NRS-162	1	D. M. Webley FFT, from grass compost
NRS-163	NRS-163	1	D. M. Webley HFT, from grass compost
NRS-193	NRS-193	1	W. Bohrer, C-1889, from okra
NRS-211, NRS-213^T, NRS-214 to NRS-216, NRS-218, NRS-219, NRS-221 to NRS-224, NRS-226 to NRS-228	NRS-211, NRS-213^T, NRS-214 to NRS-216, NRS-218, NRS-219, NRS-221 to NRS-224, NRS-226 to NRS-228	1	N. R. Smith, "*B. subtilis* var. *niger*," from Colorado soil
NRS-229	NRS-229		N. R. Smith, "*B. subtilis* var. *niger*," from Utah soil
NRS-253	NRS-253		N. R. Smith, "*B. subtilis* var. *niger*," from air
NRS-261, NRS-262	NRS-261, NRS-262	1	I. C. Hall 620, I. C. Hall 621A, "*B. subtilis* var. *aterrimus*"
NRS-263	NRS-263	1	I. C. Hall 798B from W. W. Ford, "*Bacillus aterrimus*"
NRS-264	NRS-264	1	I. C. Hall 799, "*Bacillus niger*," from W. W. Ford from Kral collection
NRS-265	NRS-265	1	I. C. Hall 1509, (ATCC 6455)[d]
NRS-274	NRS-274	1	USDA, from air
NRS-275	NRS-275	1	USDA, from Maryland soil, (ATCC 6461)
NRS-276	NRS-276	1	USDA, from Maryland soil
NRS-330	NRS-330	1	NCTC 2590, "*Bacillus aterrimus*," from W. W. Ford 5A
NRS-561	NRS-561	1	NIH 4
NRS-651	NRS-651	1	NCA, "*Bacillus niger*," from R. S. Breed from NCTC 2592, from W. W. Ford 6
NRS-652	NRS-652	1	NCA, "*Bacillus aterrimus*," from R. S. Breed from NCTC 2590 from W. W. Ford 5A
NRS-653	NRS-653	1	NCA, "*Bacillus aterrimus*," from R. S. Breed from NCTC 2591 from W. W. Ford 5B
NRS-655	NRS-655	1	NCA, "*Bacillus lactis niger*," from R. S. Breed from C. Gorini 2
NRS-704	NRS-704	1	M. L. Rakieten C3, "*B. subtilis* (*niger*)"
NRS-730	NRS-730	1	ATCC 7003, "*Bacillus graveolens*," from F. S. Orcutt
NRS-740	NRS-740	1	ATCC 4295, "*Bacillus nigrificans*," from F. W. Fabian from pickle brine
NRS-744^T	NRS-744^T	1	ATCC 6051^T from H. J. Conn from NCTC 3610^T
NRS-748	NRS-748	1	USDA, from decomposed wheat

[a] 1, N. R. Smith, U. S. Department of Agriculture Research Center, Beltsville, Md.; 2, C. E. Georgi, University of Nebraska, Lincoln; 3, J. Naghski, Eastern Regional Research Laboratory, Chestnut Hill, Pa.; 4, L. J. Wickerham, Northern Regional Research Laboratory, Peoria, Ill.; 5, American Type Culture Collection, Rockville, Md.; 6, P. Stansly, American Cyanamid Co., Pearl River, N.Y.; 7, R. Gillis, Amsco Co., Erie Pa.

[b] AMNH, American Museum of Natural History, Washington, D.C.; ATCC, American Type Culture Collection, Rockville, Md.; USDA United States Department of Agriculture, Washington, D.C.; NCTC, National Collection of Type Cultures, London, England; NIH, National Institutes of Health, Washington, D.C.; NCA, National Canners Association, San Francisco, Calif.

[c] Names in quotation marks are not on the Approved Lists of Bacterial Names (12) and have not been validly published since January 1980.

[d] Designations in parentheses are equivalent strain designations.

supernatant were transferred to capped, 1-ml plastic centrifuge tubes and stored at −20°C. Fresh cell lysates were prepared at weekly intervals.

Enzymes were separated by vertical electrophoresis through polyacrylamide slab gels (0.75 mm by 15.5 cm by 16 cm). A stacking gel (0.75 mm by 4.5 cm by 16 cm) was also used. The separating gel (12%) contained 11.68% acrylamide, 0.32% *N,N'*-methylene bisacrylamide, 0.05% ammonium persulfate, and 0.05% *N,N,N',N'*-tetramethylethylenediamine. The stacking gel (4%) contained 3.9% acrylamide, 0.1% *N,N'*-methylene bisacrylamide, 0.05% ammonium persulfate, and 0.1% *N,N,N',N'*-tetramethylethylenediamine. The separating gel buffer was 0.375 M Tris hydrochloride (pH 8.8), and the stacking gel buffer was 0.125 M Tris hydrochloride (pH 6.8). The running buffer (pH 8.3) was a mixture of 0.123 M Tris and 0.959 M glycine. Electro-

TABLE 2. DNA relatedness of pigmented *B. subtilis* strains

Strain (NRRL no.)	% Reassociation with DNA from strain[a]:			Soluble pigment color on[b]:	
	NRRL NRS-213[T]	NRRL NRS-261	NRRL NRS-744[T]	TGY agar	Glycerol-glutamate agar
Group 1					
B-363	97	29	35	Brownish black	Brown
B-364	95	28	27	Brownish black	Brown
B-365	93	27	25	Brownish black	Brown
B-627	90	33	35	Brownish black	Brown
B-4418	98	34	27	Brownish black	Brown
NRS-211	88	27	25	Brownish black	Brown
NRS-213[T]	(100)[c]	24	30	Brownish black	Brown
NRS-214	100	26	25	Brownish black	Brown
NRS-215	100	29	23	Brownish black	Brown
NRS-216	94	26	30	Brownish black	Brown
NRS-218	94	33	29	Brownish black	Brown
NRS-219	91	27	30	Brownish black	Brown
NRS-221	99	24	25	Brownish black	Brown
NRS-222	97	26	22	Brownish black	Brown
NRS-223	91	29	30	Brownish black	Brown
NRS-224	98	26	36	Brownish black	Brown
NRS-226	100	34	25	Brownish black	Brown
NRS-227	96	32	32	Brownish black	Brown
NRS-228	96	28	32	Brownish black	Brown
NRS-229	91	30	25	Brownish black	Brown
NRS-253	96	26	33	Brownish black	Brown
NRS-265	94	31	27	Brownish black	Brown
NRS-651	88	30	30	Brownish black	Brown
NRS-704	96	24	30	Brownish black	Brown
NRS-748	92	29	25	Brownish black	Brown
Group 2					
B-360	20	100	96	Brown	Bluish black
B-361	29	100	93	Brown	Bluish black
B-362	25	100	96	Brown	Bluish black
NRS-261	25	(100)	96	Brown	Bluish black
NRS-262	30	84	92	Brown	Bluish black
NRS-263	24	93	99	Brown	Bluish black
NRS-264	21	95	100	Brown	Bluish black
NRS-274	23	98	100	Brown	Bluish black
NRS-275	25	90	85	Brown	Bluish black
NRS-276	26	93	92	Brown	Bluish black
NRS-330	35	99	98	Brown	Bluish black
NRS-652	27	100	98	Brown	Bluish black
NRS-653	27	90	97	Brown	Bluish black
NRS-655	29	99	70	Brown	Bluish black
NRS-740	30	90	95	Brown	Bluish black
Group 3					
B-357	25	100	100	None	None
B-447	23	100	98	None	None
B-554	30	100	93	None	None
B-765	30	75	89	None	None
B-971	28	80	70	None	None
NRS-161	25	91	92	None	None
NRS-162	30	96	95	None	None
NRS-163	28	100	91	None	None
NRS-193	36	94	88	None	None
NRS-561	33	93	100	None	None
NRS-730	22	95	98	None	None
NRS-744[T]	30	96	(100)	None	None

[a] Reassociation values are averages of two determinations; the maximum difference found between determinations was 7%.

[b] Brownish black pigmentation of group 1 strains was observed after 2 to 6 days in TGY agar, and brown pigmentation of group 2 strains was observed after 14 days. Brown pigmentation of group 1 strains in glycerol-glutamate agar was observed after 6 to 13 days, and bluish black pigmentation of group 2 strains was observed after 1 to 2 days.

[c] Values in parentheses indicate that, by definition, the reassociation value was 100%.

phoresis was carried out at 5°C at a constant amperage of 13 mA per slab to stack the samples and 18 mA per slab to effect enzyme separation. Sample proteins were diluted to a concentration of 600 μg/ml in 0.125 M Tris hydrochloride (pH 6.8) containing 10% glycerol and 0.00125% bromophenol blue; 50-μl portions of the diluted samples were analyzed electrophoretically.

The 12 enzymes studied were alanine dehydrogenase (EC 1.4.1.1), alcohol dehydrogenase (EC 1.1.1.1), aspartate dehydrogenase (EC 1.4.3.×), fumarase (EC 4.2.1.2), glucose-

TABLE 3. Levels of DNA relatedness of group reference strains and selected *Bacillus* spp. type strains

Strain	G+C content (mol%)[a]	% Reassociation with DNA from group reference strain[b]:	
		NRRL NRS-213^T	NRRL NRS-261
B. firmus NRRL B-14307^T	41.5	27	23
B. pumilus NRRL NRS-272^T	42.0	17	24
B. badius NRRL NRS-663^T	43.8	26	30
B. polymyxa NRRL NRS-1105^T	44.5	37	23
B. alvei NRRL B-383^T	44.6	29	32
B. coagulans NRRL NRS-609^T	45.0	29	25
B. licheniformis NRRL NRS-1264^T	46.5	21	22
B. brevis NRRL NRS-604^T	47.5	28	30

[a] Data from reference 4.
[b] Reassociation values are averages of two determinations; the maximum difference found between determinations was 7%.

6-phosphate dehydrogenase (EC 1.1.1.49), glutamate dehydrogenase (EC 1.4.1.2), hexokinase (EC 2.7.1.1), indophenol oxidase (EC 1.15.1.1), leucine dehydrogenase (EC 1.4.3.2), lysine dehydrogenase (EC 1.4.3.×), malic dehydrogenase (EC 1.1.1.40), and phosphoglucose isomerase (EC 5.3.1.9). The enzymes were stained by using the method of Selander et al. (11).

The relative mobilities of alternative forms of each enzyme in the strains were compared directly on the electrophoresis gels. These allozymes (electromorphs) were assumed to be encoded by chromosomal genes and thus were equated with alleles at each locus. The electromorphs were numbered in order of increasing anodal mobility, and the combination of electromorphs at the 12 enzyme loci was determined for each strain. The absence of enzyme activity was scored as a null allele. Each distinctive combination of alleles was designated an electrophoretic type (ET).

Levels of similarity among strains were determined by using the simple matching coefficient, and clustering was based on the unweighted pair group arithmetic average algorithm (14). Computations were carried with an DTK AT computer by using the TAXAN program of David Swartz, University of Maryland, College Park.

RESULTS

B. subtilis strains that produced a soluble black pigment segregated into two groups on the basis of DNA relatedness (Table 2). Group 1 strains (which produced a brownish black pigment) showed 88 to 100% DNA relatedness to reference strain NRRL NRS-213^T and a range of relatedness to reference strains NRRL NRS-261 (which produced a bluish black pigment) and NRRL NRS-744^T (nonpigmented) of 22 to 35%. Strains in group 2 (which produced a bluish black pigment) had levels of DNA complementarity of 84 to 100 and 70 to 100% with reference strains NRRL NRS-261 and NRRL NRS-744^T, respectively. The levels of DNA relatedness of group 2 strains to strain NRRL NRS-213^T ranged from 20 to 35%. The nonpigmented group 3 strains showed 70 to 100% DNA relatedness to reference strain NRRL NRS-744^T and 75 to 100% DNA relatedness to strain NRRL NRS-261. The levels of DNA relatedness between group 3 strains and strain NRRL NRS-213^T ranged from 22 to 36%. The intragroup DNA relatedness values (data not shown) for all three groups ranged from 85 to 100%.

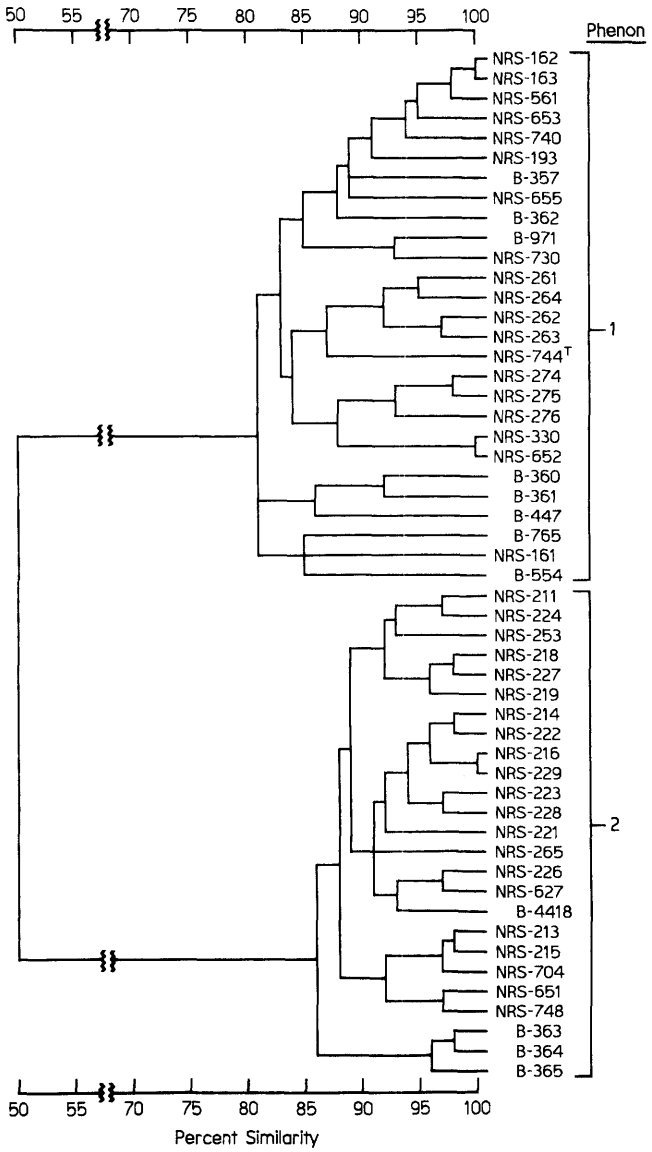

FIG. 1. Relationships of pigmented *B. subtilis* strains. The dendrogram was generated by unweighted average linkage clustering from a matrix of simple matching coefficients based on 12 enzyme loci.

The data in Table 3 show that reference strains NRRL NRS-213^T and NRRL NRS-261 yielded low DNA complementarity values (17 to 37%) with the following type strains: *B. alvei* NRRL B-383, *B. badius* NRRL NRS-663, *B. brevis* NRRL NRS-604, *B. coagulans* NRRL NRS-609, *B. firmus* NRRL B-14307, *B. licheniformis* NRRL NRS-1264, *B. polymyxa* NRRL NRS-1105, and *B. pumilus* NRRL NRS-272. These species had G+C contents ranging from 40.5 to 47.5 mol%, a range that includes the values (41 to 43 mol%) exhibited by the pigmented strains.

Analyses of the multilocus enzyme electrophoresis data revealed 49 ETs. The overall genetic diversity (11) of the 49 ETs was 0.39. The dendrogram in Fig. 1 shows the relationships of ETs based on the enzyme electrophoresis data. At a level of about 50% similarity, two distinct phena were identified. Phenon 1, with a genetic diversity of 0.3, contained 27 strains that represented 25 ETs (2 ETs contained

two strains each; the other ETs were one-member entities). Within this phenon, two enzymes were monomorphic, and 10 were polymorphic. The strains included in this phenon correspond exactly to the strains in DNA relatedness groups 2 and 3. Phenon 2, with a genetic diversity of 0.27, contained 25 strains that were equivalent to 24 ETs (1 ET contained two strains, and the other ETs contained one strain each). The strains in this phenon were identical to the strains found in DNA relatedness group 1. Of 12 enzymes, 4 were monomorphic and 8 were polymorphic.

Except for the color of the soluble pigment, all of the strains were indistinguishable by the standard characterization method (data not shown); i.e., they exhibited the traits typical of *B. subtilis* (5). The G+C contents of all of the strains ranged from 41 to 43 mol%. All group 1 strains produced a soluble brownish black pigment in 2 to 6 days in TGY agar (5); group 2 strains produced a brown pigment slowly in TGY agar. On the glycerol-glutamate medium of Arai and Mikami (1), group 2 strains synthesized a distinctly blue pigment in 1 to 2 days, the color of which intensified to a bluish black hue after 6 days. Group 1 strains produced only a brown pigment in glycerol-glutamate agar in 6 to 13 days.

DISCUSSION

The results of DNA relatedness studies indicate that the soluble pigment-forming strains of *B. subtilis* consist of two distinct genetically unrelated groups. Low DNA relatedness values show that the producers of the brownish black pigment are genetically unrelated to the bluish black strains and the nonpigmented strains. Moreover, the brownish black-pigmented organisms are also not closely related genetically to previously described species with G+C contents ranging from about 40 to 48 mol% (Table 3). Thus, the brownish black pigment producers, once classified as "*B. subtilis* var. *niger*," are members of a separate species. High DNA relatedness levels indicate that the bluish black pigment producers and nonpigmented *B. subtilis* strains are closely related genetically and thus are cospecific. Thus, the bluish black-pigmented strains are truly variants of *B. subtilis*.

The results of multilocus enzyme electrophoresis analyses supported the conclusions drawn from the DNA relatedness studies. Basically, the reduction of the genetic diversity value from 0.39 to about 0.30 upon segregation into the brownish black- and bluish black-pigmented groups suggested genetic heterogeneity of the whole group. If the group were genetically homogeneous, subgrouping should not have affected the genetic diversity value. Furthermore, organisms that form tight DNA relatedness groups are closely related on the basis of enzyme electrophoresis comparisons. While subgroups occur in phenon 1 at the 84 to 85% similarity level, the blue-pigmented strains are dispersed in a roughly even pattern among these subgroups. This suggests that mutations causing blue pigmentation occurred independently of mutations causing allelic enzyme variation.

Although conventional classification procedures barely differentiate one pigmented group from the other, DNA relatedness and multilocus enzyme electrophoresis analyses have established clearly that the *B. subtilis*-like organisms which produce a soluble brownish black pigment are members of a distinct, previously unnamed species. Since it is phenotypically virtually identical to *B. subtilis*, the pigmented taxon can be differentiated from *B. alvei*, *B. badius*, *B. brevis*, *B. coagulans*, *B. firmus*, *B. licheniformis*, *B. polymyxa*, and *B. pumilus* on the same bases as *B. subtilis* is. These brownish black pigment producers represent between 10 and 15% of the 300 organisms identified as *B. subtilis* in the Agricultural Research Service Culture Collection. Based on their demonstrated distinctiveness, rather common occurrence in nature, and usefulness, these organisms merit designation as members of a new species, for which I propose the name *Bacillus atrophaeus*. A description of the species is given below.

Bacillus atrophaeus sp. nov. *Bacillus atrophaeus* (a.tro.phae'.us L. adj. *ater*, black; Gr. adj. *phaeus*, brown; M.L. adj. *atrophaeus*, dark brown) vegetative cells are rods that are 0.5 to 1.0 μm wide by 2.0 to 4.0 μm long (as determined by phase microscopy) and occur singly and in short chains. Motile. Gram positive. Produces ellipsoidal spores centrally or paracentrally in unswollen sporangia.

Agar colonies are opaque, smooth, circular, entire, and 1.0 to 2.0 mm in diameter after 2 days at 28°C. A dark brown pigment is formed in 2 to 6 days in media containing an organic nitrogen source.

Catalase is produced. Oxidase is not produced. Aerobic. Acetylmethylcarbinol (Voges-Proskauer test) is produced. Hydrogen sulfide, indole, and dihydroxyacetone are not produced. The pH in Voges-Proskauer broth ranges from 5.3 to 5.7. Nitrate is reduced to nitrite. Starch and casein are hydrolyzed. Citrate but not propionate is utilized. Egg yolk lecithin, Tween 80, and urea are not decomposed. The pH in litmus milk is alkaline; casein is digested.

Arginine, lysine, ornithine, phenylalanine, and tyrosine are not decomposed.

The optimum growth temperature ranges from 28 to 30°C, the maximum growth temperature ranges from 50 to 55°C, and the minimum growth temperature ranges from 5 to 10°C. Grows at pH 5.6 or 5.7 and in the presence of 7% NaCl. Growth is usually inhibited by 0.001% lysozyme.

Acid but no gas is produced from L-arabinose, D-fructose, D-glucose, mannitol, salicin, sucrose, trehalose, and D-xylose. Acid production from cellobiose, D-galactose, maltose, D-mannose, D-ribose, L-rhamnose, and sorbitol is variable. Lactose and melibiose are not fermented.

The DNA buoyant density ranges from 1.6946 to 1.6966 g/cm^3, and the G+C contents determined from these values are 41 to 43 mol%.

The description above is virtually identical to that of *B. subtilis*. The new species is differentiated from *B. subtilis* on the basis of DNA relatedness and multilocus enzyme electrophoresis analyses, as well as pigment production.

Isolated mainly from soil.

The type strain is strain NRS-213, which has been deposited as NRRL NRS-213 in the Agricultural Research Service Culture Collection, Peoria, Ill.

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