DNA Relatedness among *Bacillus thuringiensis* Serovars

L. K. NAKAMURA*

Microbial Properties Research, National Center for Agricultural Utilization Research,
Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois 61604

The genetic relationships of *Bacillus cereus* and of the *Bacillus thuringiensis* serovars were assessed from measurements of DNA reassociation. A study of 8 to 10 strains each of 13 of the most commonly encountered serovars revealed that the levels of intragroup DNA relatedness for most serovars ranged from 90 to 100%. In contrast, *B. thuringiensis* serovars canadensis and kenyae consisted of two DNA relatedness groups, each of which exhibited levels of intragroup relatedness of 80% or higher and levels of intergroup relatedness of 60 to 70%. Analyses of DNA relatedness performed with all of the serovars revealed that the taxa were segregated into 11 phena differentiated from each other at about the 65% level; within each phenon the level of relatedness was 80% or higher. Three phena contained strains belonging to more than one serovar; *B. thuringiensis* serovars alesti and dendrolimus clustered in phenon 1, serovars aizawai, kurstaki, galleriae, and morrisoni clustered in phenon 7, and serovar darmstadensis and some strains of serovar kenyae clustered in phenon 11. The levels of DNA relatedness between *B. cereus* and *B. thuringiensis* strains ranged between 65 and 70%. My results suggest that many of the *B. thuringiensis* serovars are genetically distinct but closely related.

Bacillus thuringiensis was first isolated by Ishiwata (17) in 1901 from diseased silkworms, the larvae of the moth *Bombyx mori* (L.). A subsequent isolation was made by Berliner (4) in 1911 from diseased larvae of the Mediterranean flour moth, *Anagasta kuehniella* (Zeller). Discovery of the biocidal activity on the larvae of many lepidopterids suggested that *B. thuringiensis* could be used as a biological control agent for protection of crops and forests from insects.

A striking morphological and taxonomically distinguishing characteristic of *B. thuringiensis* is the formation of parasporal crystals. These parasporal bodies consist of crystallized protein which is responsible for the insecticidal activity of the organism (1, 16). The protein is solubilized at the alkaline pHs that occur in the intestines of susceptible insect larvae and is then cleaved by proteases into peptides which produce the toxic effects (29). Crystal formation is not a stable characteristic (6, 30). Synthesis of the paracrystalline protoxin is controlled by large plasmids (11, 12, 30), the maintenance of which is sensitive to frequency of transfer (6) and incubation conditions, such as temperature (30). Interestingly, to some researchers, strains of *B. thuringiensis* are virtually indistinguishable phenotypically from *Bacillus cereus* except for crystal formation (5, 13, 26), but other workers have observed distinct differences between the two taxa (8, 15).

de Barjac and Bonnefoi (7) noted that *B. thuringiensis* strains could be divided into 24 serovars on the basis of the immunological reactions of the flagellum antigen. Numerical analyses of phenotypic characteristics failed to differentiate the serovars from each other and *B. cereus* from *B. thuringiensis* (3). Opinions regarding the classification of the parasporal body-producing organisms have ranged from the proposal that these bacteria should be separated into several species (8, 15) to the belief that these pathogens are so similar to *B. cereus* they do not merit even the varietal status suggested by Smith et al. (26). In *Bergey's Manual of Systematic Bacteriology* (5), *B. cereus* and *B. thuringiensis*

are treated as separate species. DNA relatedness (19, 25, 28) and 16S rRNA sequencing (2) studies performed with small numbers of strains showed that *B. thuringiensis* and *B. cereus* were closely related genetically. The uncertainties concerning the taxonomic positions of *B. cereus*, *B. thuringiensis*, and the various *B. thuringiensis* serovars prompted the present study, in which the levels of DNA relatedness among large numbers of these taxa were examined.

MATERIALS AND METHODS

Bacterial strains. The *Bacillus* strains used in this study are listed in Table 1 and are maintained in the Agricultural Research Service Culture Collection at the National Center for Agricultural Utilization Research. Each Northern Regional Research Laboratory (NRRL) designation includes the prefix B-, which designates strains that were obtained directly from individuals or strains that were isolated at the National Center for Agricultural Utilization Research, or the prefix HD-, which identifies strains deposited in the Agricultural Research Service Culture Collection by H. T. Dulmage. Working stocks were cultured at 30°C on nutrient agar amended with 5 mg of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ per liter until sporulation occurred and were then stored at 4°C.

DNA isolation, G+C contents, and DNA reassociation. The organisms were grown in TGY broth (14) with agitation at 28°C and were harvested by centrifugation at 5°C in the mid- to late logarithmic growth phase when microscopic examination showed absence of sporulation.

DNA was extracted and purified by a modification of the method of Marmur (23). The modification involved the utilization of CsCl gradient centrifugation (22) to produce highly purified DNA samples. The purity and quality of each DNA preparation were such that the A_{260}/A_{280} and A_{260}/A_{230} ratios were consistently 1.8 to 1.9 and 2.0 to 2.3, respectively. The quality of the DNA preparations was confirmed from melting curves that showed hyperchromicities ranging from 38 to 40% (24).

The procedure used for estimating DNA reassociation values spectrophotometrically has been described previously (24). Reassociation was carried out in the presence of 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate) and 20% dimethyl sulfoxide; under these conditions

* Mailing address: National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, IL 61604. Phone: (309) 685-4011. Fax: (309) 681-6686.

TABLE 1. *B. cereus* and *B. thuringiensis* strains used in this study

Strain	Received as:	Source	Serovar and/or strain history
NRRL HD-11	HD-11	de Barjac	Serovar aizawai
NRRL HD-115	HD-115	Dubois (145)	Serovar aizawai; from A. Heimpel (2-IH-A)
NRRL HD-133	HD-133	Burges HDB-20	Serovar aizawai; isolated from <i>Plodia interpunctella</i> (Hubner)
NRRL HD-40	HD-40	CCEB 558 ^a	Serovar alesti; J. Weiser, " <i>Bacillus dendrolimus</i> "
NRRL HD-100	HD-100	Dubois 95	Serovar alesti; from A. Heimpel
NRRL HD-145	HD-145	Krieg III/36	Serovar alesti
NRRL HD-224	HD-224	de Barjac	Serovar canadensis
NRRL HD-552	HD-552	de Barjac 50-1	Serovar canadensis; from O. Morris (Zeller)
NRRL HD-554	HD-554	de Barjac 50-4	Serovar canadensis; from M. Jacquemard
NRRL HD-592	HD-592	Aizawai 74-E-74-7	Serovar canadensis; isolated from a silkworm farm in Japan
NRRL HD-768	HD-768	DeLucca 56	Serovar canadensis; isolated from grain dust
NRRL HD-826	HD-826	DeLucca 56	Serovar canadensis; isolated from grain dust; second deposition by DeLucca
NRRL HD-146	HD-146	Krieg 10/103	Serovar darmstadiensis
NRRL HD-147	HD-147	Krieg 10/102	Serovar darmstadiensis
NRRL HD-499	HD-499	DeLucca E-311	Serovar darmstadiensis; isolated from soil
NRRL HD-7	HD-7	de Barjac	Serovar dendrolimus
NRRL HD-34	HD-34	CCEB 306	Serovar dendrolimus; " <i>Bacillus dendrolimus</i> "; isolated by E. Talalaev from <i>Dendrolimus sibericus</i> (Tshetverikov)
NRRL HD-106	HD-106	Dubois 124 (119)	Serovar dendrolimus; from A. Heimpel
NRRL HD-9	HD-9	de Barjac	Serovar entomocidus
NRRL HD-110	HD-110	Dubois 143	Serovar entomocidus; from A. Heimpel (2-1328)
NRRL HD-198	HD-198	Hunter	Serovar entomocidus; isolated from <i>Cadra figulilla</i> (Gregson)
NRRL HD-29	HD-29	CCEB 565	Serovar galleriae; " <i>Bacillus finitimus</i> "; from O. Schvetsova
NRRL HD-129	HD-129	Burges HBD-15	Serovar galleriae; isolated from <i>Galleria mellonella</i> (Linnaeus)
NRRL HD-173	HD-173	de Barjac	Serovar galleriae; mucoid variant
NRRL HD-563	HD-563	Aronson R-153-78	Serovar israelensis; from Pasteur Institute
NRRL HD-567	HD-567	de Barjac	Serovar israelensis
NRRL HD-653	HD-653	Singer 2013-1	Serovar israelensis; isolated in Romania
NRRL HD-5	HD-5	de Barjac	Serovar kenya
NRRL HD-63	HD-63	Tanada 92-1-1	Serovar kenya; from E. Steinhaus
NRRL HD-123	HD-123	Burges HBD-6	Serovar kenya; isolated from <i>Ephestia cautella</i> (Walker)
NRRL HD-136	HD-136	Burges HBD-23	Serovar kenya; isolated <i>Corcyra cephalonica</i> (Stainton)
NRRL HD-268	HD-268	Burges PIL-94	Serovar kenya; isolated from <i>Trichophaga tapetzella</i> (Linnaeus) by G. Ayerst (19.6.61-5)
NRRL HD-277	HD-277	Burges PIL-138	Serovar kenya; isolated from <i>Ephestia cautella</i> (Walker) by H. Hyde
NRRL HD-291	HD-291	Martinez	Serovar kenya; agar plate contaminant
NRRL HD-549	HD-549	de Barjac 400-9	Serovar kenya; from S. Amonkar
NRRL HD-1	HD-1	Dulmage	Serovar kurstaki; isolated from diseased <i>Pectinophora gossypiella</i> (Saunders)
NRRL HD-88	HD-88	Correa	Serovar kurstaki; isolated from <i>Trichoplusia ni</i> (Hubner)
NRRL HD-187	HD-187	de Barjac	Serovar kurstaki; mucoid variant
NRRL HD-12	HD-12	de Barjac	Serovar morrisoni
NRRL HD-116	HD-116	Dubois 157 (156)	Serovar morrisoni; from A. Heimpel (2-G-2)
NRRL HD-325	HD-325	Gingrich AA-248B	Serovar morrisoni
NRRL HD-771	HD-771	Aizawai AO-31	Serovar sotto
NRRL HD-772	HD-772	Aizawai AO-73	Serovar sotto
NRRL HD-773	HD-773	Aizawai AO-87	Serovar sotto
NRRL HD-2	HD-2	de Barjac	Serovar thuringiensis
NRRL HD-20	HD-20	CCEB 056	Serovar thuringiensis; " <i>Bacillus finitimus</i> "; isolated by E. Steinhaus from <i>Ephestia kuehniella</i> (Zeller)
NRRL HD-362	HD-362	Gingrich AA-285C	Serovar thuringiensis
NRRL B-569		Raper 8	Laboratory contaminant
NRRL B-1877	NRS-768	Smith	<i>Bacillus cereus</i> ; from Knaysi
NRRL B-3711 ^T	ATCC 14579 ^{Tb}	Gordon	<i>Bacillus cereus</i> type strain; from ATCC

^a CCEB, Culture Collection of Entomogeneous Bacteria, Institute of Biology, Prague, Czech Republic.

^b ATCC, American Type Culture Collection, Rockville, Md. T = type strain.

the melting temperatures for *B. thuringiensis* DNAs having G+C contents of approximately 36 mol% were about 75°C. Reassociation rates were monitored at a relatively highly stringent temperature, 65°C (10°C below the melting temperature). The method of De Ley et al. (9) was used to calculate reassociation values.

Numerical analyses. DNA relatedness clustering was based on the unweighted pair group arithmetic average algorithm (27); the computations were accomplished by using the PC-SAS version 6.04 (SAS Institute, Inc., Cary,

N.C.) of the SAS/STAT cluster procedure. A dendrogram was generated with SAS/GRAPH by using the SAS macro GRAFTREE written and kindly provided by Dan Jacobs, University of Maryland. The SAS analyses were carried out with a DTK PC-486 computer.

RESULTS

An examination of the levels of intragroup DNA relatedness of 7 to 10 strains each of 13 *B. thuringiensis* serovars

TABLE 2. Levels of DNA relatedness between *B. cereus* and *B. thuringiensis* strains

Strain	Serovar	% Reassociation with DNA from strain ^a :		
		NRRL B-569	NRRL B-1877	NRRL B-3711
<i>B. thuringiensis</i>				
NRRL HD-5	kenyae	64.5	69.0	61.2
NRRL HD-7	dendrolimus	65.6	63.1	67.9
NRRL HD-11	aizawai	59.9	60.0	60.0
NRRL HD-20	thuringiensis	62.4	61.8	67.0
NRRL HD-29	galleriae	67.9	66.3	65.9
NRRL HD-40	alesti	64.9	67.0	63.0
NRRL HD-88	kurstaki	64.9	65.4	69.7
NRRL HD-110	entomocidus	67.9	63.4	67.5
NRRL HD-224	canadensis	68.7	67.7	69.1
NRRL HD-325	morrisoni	64.3	63.2	68.2
NRRL HD-499	darmstadiensis	59.7	65.3	60.9
NRRL HD-653	israelensis	65.7	62.6	63.2
NRRL HD-771	sotto	69.0	66.5	67.1
<i>B. cereus</i>				
NRRL B-569		(100)		
NRRL B-1877		99.0	(100)	
NRRL B-3711 ^T		100.0	98.0	(100)

^a Reassociation values are averages of two determinations; the maximum difference observed was 5%.

revealed that 11 serovars (serovars aizawai, alesti, darmstadiensis, dendrolimus, entomocidus, galleriae, israelensis, kurstaki, morrisoni, sotto, and thuringiensis) were very homogeneous; i.e., the levels of intragroup relatedness ranged from 80 to 100% (data not shown). Table 2 shows that the levels of DNA relatedness between representative strains of the *B. thuringiensis* serovars and *B. cereus* strains were generally greater than 60% but less than 70%. Two serovars (canadensis and kenyae) were composed of two groups (designated group 1 and group 2); each of these groups exhibited intragroup DNA relatedness values of 80 to 100%, but intergroup relatedness values of 60 to 70% (Tables 3 and 4).

The levels of genetic relatedness among several groups and serovars were estimated by measuring the levels of DNA relatedness among up to three reference strains from each of the serovars or DNA relatedness groups. Interestingly, levels of DNA relatedness of 80 to 90, 85 to 100, and 89 to 100% were obtained for strains of the alesti-dendrolimus, aizawai-galleriae-kurstaki-morrisoni, and darmstadiensis-kenyae (DNA relatedness group 2) complexes, respectively. For the remaining serovars, the levels of intragroup relatedness ranged from 80 to 100%, and the levels of

intergroup relatedness ranged from 56 to 70%. The dendrogram in Fig. 1 shows the clustering of the organisms into 11 phena, 3 of which (phenon 1, 7, and 11) contain members of two or more serovars at levels of relatedness of 85 to 100%. Phenon 1 includes members of serovars alesti and dendrolimus. Strains belonging to serovars aizawai, galleriae, kurstaki, and morrisoni cluster to form phenon 7, and strains belonging to serovar darmstadiensis and DNA relatedness group 2 of serovar kenyae form phenon 11. Strains belonging to serovars entomocidus, israelensis, sotto, thuringiensis, canadensis (groups 1 and 2), and kenyae (group 1) segregate into distinct phenon, each of which is delimited at a similarity level of 85 to 100%. The levels of intergroup similarity for the various phenon, including the phenon represented by the type strain of *B. cereus*, NRRL B-3711, range from 61 to 71%.

DISCUSSION

Phenotypic characterization, limited DNA relatedness studies, and 16S rRNA sequencing data have indicated that *B. cereus* and *B. thuringiensis* are closely related (2, 3, 5, 15, 20, 25, 26). Unfortunately, previous DNA relatedness studies were not extensive or stringent enough to determine the degree of genetic relatedness of the two taxa.

The levels of DNA relatedness of 8 to 10 strains of 13 of the most frequently encountered *B. thuringiensis* serovars were determined to obtain estimates of the levels of genetic relatedness among serovars and the levels of genetic relatedness to *B. cereus*. The results of this study revealed that *B. cereus* and *B. thuringiensis* are genetically related at DNA relatedness levels greater than 60% but less than 70%. If these organisms were members of the same species, the DNA relatedness values would be greater than 70% (18). 16S rRNA sequencing data (2) that revealed high levels of relatedness between *B. cereus* and *B. thuringiensis* and the levels of DNA relatedness found in this study emphasize that these two species are in the anomalous situation of being closely related yet separate taxonomic entities; this type of close relationship also applies to several of the *B. thuringiensis* serovars. Thus, the data appear to refute the species status of *B. thuringiensis*. Except for *B. thuringiensis* serovars canadensis and kenyae, the levels of intragroup DNA relatedness for the serovars ranged from 85 to 100%, indicating the apparent genetic homogeneity of the serovars. Serovars canadensis and kenyae were each composed of two groups that were related to each other at levels of 60 to 70%. Analyses to determine levels of DNA relatedness among representative strains of all of the serovars revealed that several serovars should be rearranged into new groups on the basis of the high levels of genetic relatedness observed. For example, a level of relatedness of 90% or higher clus-

TABLE 3. Levels of DNA relatedness between strains belonging to *B. thuringiensis* serovar canadensis

Strain	Group	% Reassociation with DNA from strain ^a :						
		NRRL HD-224	NRRL HD-552	NRRL HD-554	NRRL HD-565	NRRL HD-592	NRRL HD-768	NRRL HD-826
NRRL HD-224	1	100						
NRRL HD-552	1	100	100					
NRRL HD-554	2	63	65	100				
NRRL HD-565	2	69	60	100	100			
NRRL HD-592	2	69	60	100	95	100		
NRRL HD-768	2	65	69	80	90	91	100	
NRRL HD-826	2	60	69	90	89	99	99	100

^a Reassociation values are averages of two determinations; the maximum difference observed between determinations was 5%.

TABLE 4. Levels of DNA relatedness between *B. thuringiensis* serovar *kenyae* strains

Strain	Group	% Reassociation with DNA from strain ^a :							
		NRRL HD-5	NRRL HD-123	NRRL HD-268	NRRL HD-277	NRRL HD-63	NRRL HD-136	NRRL HD-291	NRRL HD-549
NRRL HD-5	1	100							
NRRL HD-123	1	100	100						
NRRL HD-268	1	100	99	100					
NRRL HD-277	1	83	94	100	100				
NRRL HD-63	2	69	60	69	65	100			
NRRL HD-136	2	70	66	65	70	100	100		
NRRL HD-291	2	68	70	60	66	98	97	100	
NRRL HD-549	2	70	65	69	69	86	80	85	100

^a Reassociation values are averages of two determinations; the maximum difference observed was 5%.

tered serovars *aizawai*, *galleriae*, *kurstaki*, and *morrisoni* into a common group. Similar high levels of DNA relatedness were observed between the strains belonging to serovars *alesti* and *dendrolimus* and between strains belonging to serovar *darmstadiensis* and serovar *kenyae* group 1. Levels of DNA relatedness in the 60% range have also been observed for insect pathogenic and nonpathogenic *Bacillus sphaericus* strains (21), but this observation has not been used as basis for partitioning the species.

Some bacterial taxonomists (10, 20, 31) use levels of DNA relatedness ranging from 60 to 70% and distinct phenotypic characteristics as criteria to delineate subspecies. My data suggest that the parasporal crystal-producing *B. cereus*-like organisms may represent several subspecies of *B. cereus*

rather than serological variants of a taxon now known as *B. thuringiensis*. However, any proposal for the creation of subspecies and for reclassification of this group of *Bacillus* species should not be implemented until a large number of strains of all known serovars have been examined and distinctive characteristics have been discovered.

The present data provide information concerning the levels of genetic relatedness between *B. cereus* and *B. thuringiensis* and between various serovars of *B. thuringiensis*. Such information may assist workers in determining whether genetic divergence of these organisms affects the nature of the insecticidal activity produced, in recognizing public health applications where distinguishing potential food-poisoning strains of *B. cereus* is important, and in resolving questions involving patenting actions.

ACKNOWLEDGMENTS

I thank James Swezey and Helen Gasdorf for their able technical assistance.

REFERENCES

1. Angus, T. A. 1954. A bacterial toxin paralyzing silkworm larvae. *Nature* (London) 173:545.
2. Ash, C., J. A. E. Farrow, M. Dorsch, E. Stackebrandt, and M. D. Collins. 1991. Comparative analysis of *Bacillus anthracis*, *Bacillus cereus*, and related species on the basis of reverse transcriptase sequencing of 16S rRNA. *Int. J. Syst. Bacteriol.* 41:343-346.
3. Baumann, L., K. Okamoto, B. M. Unterman, M. J. Lynch, and P. Baumann. 1984. Phenotypic characterization of *Bacillus thuringiensis* and *Bacillus cereus*. *J. Invertebr. Pathol.* 44:329-341.
4. Berliner, E. 1911. Über die Schlaffsucht der Mehlmotenraupe. *Z. Gesamte Getreidew.* 3:63-70.
5. Claus, D., and R. C. W. Berkeley. 1986. Genus *Bacillus* Cohn 1872, p. 1105-1207. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. Williams and Wilkins, Baltimore.
6. Dean, D. H. 1984. Biochemical genetics of the bacterial insect-control agent *Bacillus thuringiensis*. Basic principals and prospects for genetic engineering. *Biotechnol. Genet. Eng. Rev.* 2:341-363.
7. de Barjac, H., and A. Bonnefoi. 1962. Essai de classification biochimique et serologique de 24 souches de *Bacillus* du type *thuringiensis*. *Entomophaga* 7:5-31.
8. de Barjac, H., and A. Bonnefoi. 1968. A classification of strains of *Bacillus thuringiensis* Berliner with key to their differentiation. *J. Invertebr. Pathol.* 11:335-347.
9. De Ley, J., H. Cattoir, and A. Raynaerts. 1970. The quantitative measurement of DNA hybridization from renaturation rates. *Eur. J. Biochem.* 12:133-142.
10. Dzink, J. L., M. T. Sheenan, and S. S. Socransky. 1990. Proposal

FIG. 1. Dendrogram showing the levels of DNA relatedness among *B. thuringiensis* serovars, based on unweighted average-linkage clustering. sv, serovar. All strain designations are NRRL designations.

- of three subspecies of *Fusobacterium nucleatum* Knorr 1922: *Fusobacterium nucleatum* subsp. *nucleatum* subsp. nov., comb. nov.; *Fusobacterium nucleatum* subsp. *polymorphum* subsp. nov., nom. rev., comb. nov.; and *Fusobacterium nucleatum* subsp. *vincentii* subsp. nov., nom. rev., comb. nov. Int. J. Syst. Bacteriol. **40**:74–78.
11. **Gonzales, J. M., Jr., and B. C. Carlton.** 1984. A large transmissible plasmid is required for crystal production in *Bacillus thuringiensis* variety *israelensis*. Plasmid **11**:28–38.
 12. **Gonzales, J. M., Jr., H. T. Dulmage, and B. C. Carlton.** 1981. Correlation between specific plasmids and delta-endotoxin production in *Bacillus thuringiensis*. Plasmid **5**:351–365.
 13. **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.
 14. **Haynes, W. C., L. J. Wickerham, and C. W. Hesseltine.** 1955. Maintenance of cultures of industrially important microorganisms. Appl. Microbiol. **3**:361–368.
 15. **Heimpfel, A. M., and T. A. Angus.** 1958. The taxonomy of insect pathogens related to *Bacillus cereus* Frankland and Frankland. Can. J. Microbiol. **4**:531–541.
 16. **Hofte, H., and H. R. Whiteley.** 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiol. Rev. **53**:242–255.
 17. **Ishiwata, S.** 1901. On a kind of severe flacherie (*sotto* disease) (I.). Dainihon Sanshi Kaiho **9**:1–5.
 18. **Johnson, J. L.** 1986. Nucleic acids in bacterial classification, p. 972–975. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. Williams and Wilkins, Baltimore.
 19. **Kaneko, T., R. Nozaki, and K. Aizawa.** 1978. Deoxyribonucleic acid relatedness between *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*. Microbiol. Immunol. **22**:639–641.
 20. **Kloos, W. E., and J. F. Wolfshohl.** 1991. *Staphylococcus cohnii* subspecies: *Staphylococcus cohnii* subsp. *cohnii* subsp. nov. and *Staphylococcus cohnii* subsp. *urealyticum* subsp. nov. Int. J. Syst. Bacteriol. **41**:284–289.
 21. **Krych, V. K., J. L. Johnson, and A. A. Yousten.** 1980. Deoxyribonucleic acid homologies among strains of *Bacillus sphaericus*. Int. J. Syst. Bacteriol. **30**:476–484.
 22. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 23. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208–218.
 24. **Nakamura, L. K., and J. Swezey.** 1983. Taxonomy of *Bacillus circulans* Jordan 1890: base composition and reassociation of deoxyribonucleic acid. Int. J. Syst. Bacteriol. **33**:46–52.
 25. **Seki, T., C. Chung, H. Mikami, and Y. Oshima.** 1978. Deoxyribonucleic acid homology and taxonomy of the genus *Bacillus*. Int. J. Syst. Bacteriol. **28**:182–189.
 26. **Smith, N. R., R. E. Gordon, and F. E. Clark.** 1946. Aerobic sporeforming bacteria. Miscellaneous Publication 559. U. S. Department of Agriculture, Washington, D.C.
 27. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy. W. H. Freeman and Co., San Francisco.
 28. **Sommerville, H. J., and M. L. Jones.** 1972. DNA competition studies within the *Bacillus cereus* group of bacilli. J. Gen. Microbiol. **73**:257–265.
 29. **Stahly, D. P., R. E. Andrews, and A. A. Yousten.** 1992. The genus *Bacillus*—insect pathogens, p. 1670–1745. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), *The prokaryotes*, 2nd ed. Springer-Verlag, New York.
 30. **Ward, E. S., and D. J. Ellar.** 1983. Assignment of the delta-endotoxin gene of *Bacillus thuringiensis* var. *israelensis* to a specific plasmid by curing analysis. FEBS Lett. **180**:45–49.
 31. **Willems, A., M. Goor, S. Thielemans, M. Gillis, K. Kersters, and J. De Ley.** 1992. Transfer of several phytopathogenic *Pseudomonas* species to *Acidovorax* as *Acidovorax avenae* subsp. *avenae* subsp. nov., comb. nov., *Acidovorax avenae* subsp. *citrulli*, *Acidovorax avenae* subsp. *cattleyae*, and *Acidovorax konjaci*. Int. J. Syst. Bacteriol. **42**:107–119.