BACILLUS THURINGIENSIS & BACILLUS CEREUS

BACILLUS GENETIC STOCK CENTER CATALOG OF STRAINS SEVENTH EDITION VOLUME 2

Bacillus Genetic Stock Center Catalog of Strains, Seventh Edition, Part 2: *Bacillus thuringiensis and Bacillus cereus*

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Disclaimer: The information in this catalog is believed to be correct. Due to the dynamic nature of the scientific process and to normal human limitations in dealing with such a large amount of data, however, some undetected errors may persist. Users bear the responsibility of verifying all important data before making a significant investment of time or other physical or financial resources.

Cover: Scanning electron micrograph of an uncharacterized *B. thuringiensis* strain isolated on the Ohio State University campus. Spores and spherical crystals, covered with a mesh, are visible. Micrograph was digitized and converted to shadowed images with Macromedia Freehand® 8.0.

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OBTAINING MATERIALS FROM THE *BGSC*

What is the *Bacillus* Genetic Stock Center?

The primary mission of the *Bacillus* Genetic Stock Center (BGSC) is to maintain genetically characterized strains, cloning vectors, and bacteriophage for the genus *Bacillus* and related organisms and to distribute these materials without prejudice to qualified scientists and educators throughout the world. Since 1978, the National Science Foundation has funded the activities of the BGSC. The Department of Biochemistry in the College of Biological Sciences at the Ohio State University provides facilities and administrative support. The Director of the BGSC, Dr. Daniel R. Zeigler, is assisted by a technician and a data entry specialist.

What kinds of cultures are available from the BGSC?

This catalog lists only the *Bacillus thuringiensis* and *Bacillus cereus* cultures available from the BGSC, as well as a selection of *Escherichia coli* plasmids and clones that might be of interest to scientists working with these species. The BGSC maintains and distributes a wide range of other strains, however. Included in our collection as of July 2012 (and described in other existing and planned catalogs) are:

- The nomenclatural type strains for 34 species;
- 1291 mutant or plasmid bearing strains derived from *Bacillus subtilis* 168, including a collection of 115 genetically characterized sporulation mutants;
- 158 strains of round spore formers, comprised of 136 strains of *B. sphaericus,* 17 of *B. fusiformis,* and five of *Rommeliibacillus pycnus*;
- 239 genetically characterized wild-type, mutant, and plasmid-bearing strains of *B. megaterium*;
- 96 lytic or lysogenic *Bacillus* bacteriophages;
- 42 wild-type and mutant strains from the thermophilic genus *Geobacillus*
- 41 wild-type, mutant, and lysogenic strains of *Bacillus licheniformis*;
- 55 other wild-type, mutant, and plasmid-bearing *B. subtilis* isolates, including 13 from *B. subtilis* subsp. *spizizenii* and 42 from other *B. subtilis* backgrounds;
- 102 wild-type strains from the *Bacillus cereus* group, also including *B. mycoides* and *B. weihenstephanensis*;
- 18 wild-type isolates from the genus *Brevibacillus,* including *B. brevis, B. borstelensis, B. centrosporus,* and *B. laterosporus*;
- 18 wild-type and mutant strains from *B. amyloliquefaciens*;
- 30 wild-type isolates from the genus *Paenibacillus,* including *P. alvei, P. dendritiformus, P. macerans, P. polymyxa, P. popilliae, P. thiaminolyticus,* and *P. vorticalis*;
- 42 isolates from 22 other related species, including *Aneurinibacillus aneurinilyticus, A. migulanus, B. atrophaeus, B. badius, B. carboniphilus, B. circulans, B. clausii, B. coagulans, B. firmus, B. lentus, B. mojavensis, 'B. natto,' B. oleronius, B. pumilus, B. shackletonii, Marinibacillus marinus, Sporosarcina ureae*, and *Virgibacillus marismortui*
- 240 *Escherichia coli* strains bearing shuttle plasmids or cloned *Bacillus* DNA;
- Warehoused *Bacillus* strain collections of Joshua Lederberg, Eugene Nester, Bernard Reilly, Patiricia Vary, Allan Yousten, Stanley Zahler, and the late Ernst W. Freese.

Please note that the BGSC has never carried *B. anthracis* or products derived from it.

Please inquire about any of these strains that might be of interest to you.

What you can do to help the BGSC

Our NSF grant partially subsidizes many services we offer. User fees are vitally important if we are to close the funding gap and continue operations. We greatly appreciate your understanding! Additionally, we would be grateful for the following kinds of help:

- *Strain contributions:* Although we have obtained a few cultures from other strain repositories, the vast majority of our holdings were contributed by individual researchers. Please take a moment to look over our collection and consider: are there strains, vectors, phage, or clones that you have developed or acquired that we do not have? Would these materials be of some potential use to others in the research community? If so, please take the time to deposit the material in the BGSC. There is no charge whatsoever to you. Generally, all we would require would be a culture (or lysate) with appropriate reprints or other helpful information. Please contact us (see below) if you have any questions.
- *Financial Contributions:* The BGSC requires on corporate strain sales and contributions to purchase equipment and undertake special projects not covered by the NSF grant. The Ohio State University Development Fund has a separate account for the BGSC. Contributions are tax deductible to the full extent of the law. Please contact us if you wish to contribute.

How to order cultures

There are several ways to place orders with or request information from the BGSC:

- E-mail: zeigler.1@osu.edu
- Internet: www.bgsc.org
- Phone: (+1) 614-292-5550
- FAX: (+1) 614-292-3206
- Mail: Daniel R. Zeigler, Ph.D.
  Department of Biochemistry
  The Ohio State University
  484 West Twelfth Avenue
  Columbus, OH 43210
  USA

All users will be invoiced for strain, plasmid, or phage requests. Payment must be in US dollars via check, bank transfer, or procurement card (Visa, MasterCard, and American Express accepted). Orders can be placed via any of the five methods above with an institutional purchase order. Credit card orders should be made via phone or fax.

Pricing information

- *Academic, Government, and Non-Profit Users*—Not-for-profit users are requested to pay a $195 yearly subscription fee. This subscription entitles the user to receive up to 20 strains over a twelve-month period. Alternatively, individual strains may be purchased for $35 each. Users without research funds may request a fee-waiver for a particular order.
- *For-profit Corporate Users* — Users may purchase cultures as needed for a $135 per item charge. This charge includes shipment by UPS at no additional cost for domestic users. UPS shipping is included at no cost on international orders for two or more strains, while orders for single strains will include a surcharge for shipping. Alternatively, users may pay a $1950 fee, entitling them to up to 50 cultures within the next twelve calendar months at no further cost. Express delivery service is provided at no extra charge (maximum of five express deliveries per year on international shipments).

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Please read this notice before ordering materials from this catalog!

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BACILLUS THURINGIENSIS STRAINS BY BGSC CODE

Serotype 1—Serovar. *thuringiensis*

BGSC No.	Original Code	Reference	Description
4A1	NRRL-B4039	Bulla LA, donor; Berliner E (1915) Z f Angew Entomol 2:29	Wild type isolate
4A2	HD24 (CCEB206)	Dulmage HT, donor; Lysenko O, source	Wild type isolate
4A3	HD2	Dulmage HT, donor; Heimpel AM, source	"Bt *berliner*" Isolated in Canada from *Ephestia kühniella*; antisera standard
4A4	HD120 (HDB-2)	Dulmage HT, donor; Burges HD, source	Isolated in England from *Ephestia elutella*
4A5	HD14 (CCEB457)	Dulmage HT, donor; Lysenko O, source	Isolated in Czechoslovakia
4A6	1715	(unpublished)	Wild type isolate
4A7	Bt1	Sebesta K, Horska K (1970) Biochim Biophys Acta 209:357	Cry^- Exo^+ Spo^+
4A8	Bt131	Landen R. et al. (1981) J Gen Microbiol 123:49	*strA2*
4A9	Bt1627	Heierson A, et al. (1983) Mol Gen Genet 192:118	*asp-1 purA1*

Serotype 2—Serovar. *finitimus*

BGSC No.	Original Code	Reference	Description
4B1	HD19 (CCEB460)	Dulmage HT, donor; Lysenko O, source	Wild type isolate
4B2	HD3	Dulmage HT, donor; Heimpel AM, source	Isolated in the US from *Malacosoma distria*; antisera standard

Serotype 3a, 3c—Serovar. *alesti*

BGSC No.	Original Code	Reference	Description
4C1	HD16 (CCEB463)	Dulmage HT, donor; Lysenko O, source	Isolated in Czechoslovakia from *Bombyx mori*
4C2	HD72	Dulmage HT, donor; Vago C, source	Isolated in France
4C3	HD4 (*B. alesti* 143)	Dulmage HT, donor; Toumanoff C, Vago C (1951) C R Acad Sci 233:1504	Isolated in France from *Bombyx mori*; antisera standard

Serotype 3a, 3b, 3c—Serovar. *kurstaki*

BGSC No.	Original Code	Reference	Description
4D1	HD1	Dulmage HT (1970) J Invertebr Path 15:232	Isolated in US
4D2	2	Fettig P (source)	Wild type isolate
4D3	3	Fettig P (source)	Wild type isolate
4D4	HD73 (AP77BX17)	Kurstak E (unpublished; see J Invertebr Pathol 15:139)	Isolated in France from *Ephestia kühniella*
4D5	HD164	Dulmage HT (unpublished)	Wild type isolate
4D6	HD1	Yousten AA, donor; Dulmage HT (1970) J Invertebr Path 15:232	Wild type isolate
4D7	HD(CRY-1)	Yousten AA (unpublished)	Cry^-
4D8	HD1(CRY-6)	Yousten AA (unpublished)	Cry^-
4D9	HD1(CRY-7)	Yousten AA (unpublished)	Cry^-
4D10	HD1(CRY-8)	Yousten AA (unpublished)	Cry^-; Oligosporogenous
4D11	CRY(-)B	Stahley DP, et al. (1978) Bioch Biophys Res Comm 84:581	Cry^-; no reaction with known Bt flagellar antisera
4D12	HD263 (PIL-89)	Dulmage HT, donor; Burges HD, source	Isolated in England from *Ephestia cautella*

4D14	HD231	Dulmage HT	Wild type isolate
4D15	HD232	Dulmage HT	Wild type isolate
4D16	HD243	Dulmage HT	Wild type isolate
4D17	HD88	Dulmage HT (1971) J Invertebr Path 18:353	Isolated in US from *Trichoplusia ni*
4D18	HD89	Dulmage HT, donor; Correa, source	Isolated in US from *Trichoplusia ni*
4D19	HD270 (PIL-96)	Dulmage HT, donor; Burges HD, source	Isolated in England from *Carpophilus hemipterus*
4D20	HD1 (4432)	Fischer H-M, et al. (1984) Arch Microbiol 139:213	Wild type isolate
4D21	4432(pC194)	Fischer H-M, et al. (1984) Arch Microbiol 139:213	(pC194) Cm
4D22	HD-73-20	González J. *et al.* (1982) Proc Natl Acad Sci 79:6951	Crystal minus derivative of HD-73; cured of 75, 11, and 10 kb plasmids

Serotype 4a, 4b—Serovar. *sotto/dendrolimus*

BGSC No.	Original Code	Reference	Description
4E1	HD106 (2-124)	Dubois N, donor; Heimpel, source	Isolated in US
4E2	HD7	Talalev EV (1956) Mikrobiologija 25:99	Isolated in France from *Dendrolimus sibericus*; biotype *dendrolimus* standard
4E3	sotto	see J Bacteriol (1983) 154:419	Wild type isolate
4E4	Sotto G (HD6) (4-1)	Angus T (unpublished) (obtained through deBarjac)	Isolated in Canada
4E5	4E3 Cry-	Dean DH, unpublished	Crystal minus derivative of 4E3

Serotype 4a, 4c—Serovar. *kenyae*

BGSC No.	Original Code	Reference	Description
4F1	HD136 (HDB-23)	Dulmage HT, donor; Burges HD, source	Isolated in England from *Corcyra cephalonica*
4F2	HD278 (PIL-139)	Burges HD, source	Isolated in Kenya from *Cadra cautella*
4F3	HD293	Dulmage HT, donor; Allen J, source	Isolated in US from *Cadra cautella*
4F4	HD560(7304)	Tsai & Sha (unpublished)	Wild type isolate

Serotype 5a, 5b—Serovar. *galleriae*

BGSC No.	Original Code	Reference	Description
4G1	HD8	Isakova NP (1958) Dokl Akad Sci Naul Selsk 23:26	Isolated in the USSR from *Gallaeria mellonella;* antisera standard
4G2	HD210	Dulmage HT, donor; Heimpel AM, source	Isolated in US
4G3	HD161	Dulmage HT, donor; de Barjac H, source	Wild type isolate
4G4	HD305 (HDB-34)	Dulmage HT, donor; Burges HD, source	Isolated in England from *Plodia interpunctella*
4G5	HD29	Dulmage HT, donor; Lysenko O, source	Isolated in Czechoslovakia from *Dendrolimus sibericus*
4G6	HD168	Dulmage HT, donor	Reisolation of HD8

Serotype 5a, 5c—Serovar. *canadensis*

BGSC No.	Original Code	Reference	Description
4H1	HD30 (Dch-T) (CCEB566)	Dulmage HT, donor; Schvetsova O, source	Isolated in Czechoslovakia from *Notodonta aniera*
4H2	HD224	de Barjac H, Bonnefoi A (1972) J Invertebr Path 20:212	Isolated in Canada; antisera standard

SELECTED CLONING VECTORS AND HOSTS

Bacillus thuringiensis plasmid-cured hosts

BGSC No.	Original Code	Reference	Description
4D11	CRY(-)B	Stahley DP, *et al*. (1978) Bioch Biophys Res Comm 84:581	Reported to be plasmid-cured strain of *Bacillus thuringiensis* subsp. *kurstaki,* but reacts with no known Bt flagellar antisera
4D22	HD-73-20	González J. *et al.* (1982) Proc Natl Acad Sci 79:6951	Crystal minus derivative of *Bacillus thuringiensis* subsp. *kurstaki* HD-73; cured of 75, 11, and 10 kb plasmids
4E5	4E3 Cry-	Dean DH, unpublished	Crystal minus derivative of *Bacillus thuringiensis* subsp. *sotto* 4E3
4Q7	4Q2-81	Clark BD (1987) Ph.D. Thesis (Ohio St. Univ)	*Bacillus thuringiensis* subsp. *israelensis* plasmid-cured strain
4Q8	4Q2-81 str	Dean DH (unpublished)	*Bacillus thuringiensis* subsp. *israelensis* plasmid-cured strain with chromosomal streptomycin resistance mutation

Other *Bacillus* hosts

BGSC No.	Original Code	Reference	Description
1A748	1012M15	(unpublished)	*B. subtilis* host; restriction minus, sporulation plus; allows blue-white screening on X-gal with pHPS9; genotype glgB*::lacZ*Δ*M15* Km *leu met* r(-)m(+)
1A751	MW10	Wolf M. et al. (1995) Microbiology 141:281-290	*B. subtilis* host; deficient in major secreted proteases; genotype *eglS*Δ*102 bglT/bglS*Δ*EV npr apr his*
7A16	QMB1551	Quarter Master R&D Center, US Army (unpublished)	*B. megaterium* host; wild type isolate
13A4	WHO2297	Davisdon E, donor; Abbott Labs, source	*B. sphaericus* host; wild type isolate; isolated in Ceylon from *Culex pipiens quinquefasciatus*

Gram-positive plasmids

BGSC No.	Original Code	Reference	Description
1E6	BD366	Gryczan TJ, *et al*. (1978) J Bacteriol 134:318	*B. subtilis* host bearing pUB110; confers resistance to kanamycin or neomycin and phleomycin; host genotype *thr-5 trpC2*
1E9	DSM402 (pBC16)	Kreft J, *et al*. (1978) Mol Gen Genet 162:59	*B. subtilis* host bearing pBC16; confers resistance to tetracycline; host genotype *trpC2*
1E10	DSM402 (pBC16-1)	Kreft J, *et al*. (1978) Mol Gen Genet 162:59	*B. subtilis* host bearing pBC16-1; confers resistance to tetracycline; host genotype *trpC2*
1E17	168(pC194)	Erlich SD, source	*B. subtilis* host bearing pC194; confers chloramphenicol resistance; host genotype *trpC2*
1E18	pE194	Weisblum B *et al*. (1979) J Bacteriol 137:635	*B. subtilis* host bearing pE194; confers erythromycin resistance; host genotype *thr-5 trpC2*
1E60	1012M15 (pGVD1)	Eijsink, unpublished (see *Molecular Biological Methods for Bacillus*, Harwood & Cutting, eds., p 83)	*B. subtilis* host bearing pGVD1; confers chloramphenicol resistance; 2571 bp plasmid with copy number 150-200 in *B. subtilis*; contains multiple cloning site; host genotype *glgB::lacZ*Δ*M15 leu met*
7E2	PV311	Vary P (unpublished)	*B. megaterium* host bearing pUB110; confers resistance to kanamycin or neomycin and phleomycin

Bacillus-E. coli shuttle vectors

BGSC No.	Original Code	Reference	Description
ECE10	MM294(pBS42)	Band L, Henner DJ (1984) DNA 3:17	*E. coli* host bearing pBS42 ; confers chloramphenicol resistance to *Bacillus* strains
ECE15	JM83(pMK3)	Plasmid--Gene 29:21; transformed into *E. coli* at BGSC	*E. coli* host bearing pMK3; confers ampicillin resistance to *E. coli* strains and kanamycin resistance to *E. coli* or *Bacillus* strains; fusion of fragments from pUC8 and pUB110
ECE32	JM103(pHP13)	Haima P, *et al*. (1987) Mol Gent 209:342	*E. coli* JM103 bearing pHP13; confers chloramphenicol resistance to *E. coli* or *Bacillus* strains, erythromycin resistance to *Bacillus*; 4850 bp shuttle plasmid; hgh copy number in *E. coli*, low copy number in *B. subtilis*
ECE50	C600/pAMB22	Zukowski M, Miller L (1986) Gene 46:247	*E. coli* host bearing pAMB22; confers chloramphenicol and tetracycline resistance to *E. coli* or *Bacillus* strains; *xylE* fusion vector
ECE51	MC1000 (pHPS9)	Haima P, *et al*. (1990) Gene 86:63-69	*E. coli* MC1000 bearing pHPS9; confers chloramphenicol resistance to *E. coli* or *Bacillus* strains, erythromycin resistance to *Bacillus*; 5650 bp shuttle shuttle vector providing α-complementation in suitable *E. coli* or *B. subtilis* hosts (eg. 1A748)

Antibiotic resistance cassettes

BGSC No.	Original Code	Reference	Description
ECE90	pDG641	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG641; confers ampicillin resistance to *E. coli;* plasmid bears erythromycin resistance cassette for *Bacillus*, flanked by multiple restriction sites
ECE91	pDG646	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG646; confers ampicillin resistance to *E. coli;* plasmid bears erythromycin resistance cassette for *Bacillus*, flanked by multiple restriction sites
ECE92	pDG647	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG647; confers ampicillin resistance to *E. coli;* plasmid bears erythromycin resistance cassette for *Bacillus*, flanked by multiple restriction sites
ECE93	PDG780	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG780; confers ampicillin resistance to *E. coli;* plasmid bears kanamycin-neomycin resistance cassette for *Bacillus*, flanked by multiple restriction sites
ECE94	PDG783	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG783; confers ampicillin resistance to *E. coli;* plasmid bears kanamycin-neomycin resistance cassette for *Bacillus*, flanked by multiple restriction sites
ECE96	PDG782	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG782; confers ampicillin resistance to *E. coli;* plasmid bears kanamycin-neomycin resistance cassette for *Bacillus*, flanked by multiple restriction sites
ECE97	PDG792	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG792; confers ampicillin resistance to *E. coli;* plasmid bears kanamycin-neomycin resistance cassette for *Bacillus*, flanked by multiple restriction sites
ECE98	pDG1515	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG1515; confers ampicillin resistance to *E. coli;* plasmid bears tetracycline resistance cassette for *Bacillus*, flanked by multiple restriction sites
ECE99	pDG1513	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG1513; confers ampicillin resistance to *E. coli;* plasmid bears tetracycline resistance cassette for *Bacillus*, flanked by multiple restriction sites
ECE100	pDG1514	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG1514; confers ampicillin resistance to *E. coli;* plasmid bears tetracycline resistance cassette for *Bacillus*, flanked by multiple restriction sites
ECE101	pDG1726	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG1726; confers ampicillin resistance to *E. coli;* plasmid bears tetracycline resistance cassette for *Bacillus*, flanked by multiple restriction sites
ECE102	pDG1727	Géurot-Fleury AM, *et al*. 1995. Gene 167:335	*E. coli* host bearing pDG1727; confers ampicillin resistance to *E. coli;* plasmid bears tetracycline resistance cassette for *Bacillus*, flanked by multiple restriction sites

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PLASMID PROFILES OF *B. THURINGIENSIS* AND *B. CEREUS*

Adapted from: Jensen, G. B., *et al.* 1995. J. Bacteriol **177:**2914-2917.

Most strains of *Bacillus cereus* and *Bacillus thuringiensis* contain an array of plasmids, from the small to the very large (see González *et al.* 1982. Proc. Natl. Acad. Sci. **79:**6951; Carlson and Kolstø. 1993. J. Bacteriol. **175:**1053; Carlson *et al.* 1994. Appl. Environ. Microbiol. **60:**1719). A.-B. Kolstø cites unpublished work in which megaplasmids have been detected in over 50 strains of *B. cereus* and *B. thuringiensis* (Mol. Microbiol. **24:**241-248). Standard alkaline lysis or boiling protocols are efficient at isolating the smaller plasmids but have very low or negligent yields of the larger ones. The following protocol gives reproducible, complete plasmid profiles for *B. thuringiensis* or *B. cereus*.

1. Grow the *B. cereus* or *B. thuringiensis* strain overnight at 30°C in 2 ml LB. The final OD_{600} will be 11-15.
2. Transfer the culture to a microcentrifuge tube and pellet the cells with a brief spin.
3. Suspend the cells in 100 ˜l E buffer (15% w/v sucrose, 40 mM Tris-HCl, 2 mM EDTA, pH 7.9) by pipeting them up and down.
4. Add 200 µl lysing solution (3% SDS, 50 mM Tris-HCl, pH 12.5).
5. Heat lysate at 60°C for 30 min.
6. Add 5 U proteinase K; invert the tube 20 times.
7. Incubate at 37°C for 90 min.
8. Add 1 ml phenol-chloroform-isoamyl alcohol; invert 40 times.
9. Centrifuge at high speed for 15 min.
10. Analyze the aqueous supernatant by electrophoresis on a horizontal 0.5% agarose gel.

PURIFICATION OF *B. THURINGIENSIS* CRYSTALS

Purification of Crystals on NaBr Gradients

Chang, C., S.-M. Dai, R. Frutos, B. A. Federici, and S. S. Gill. 1992. Properties of a 72-kilodalton mosquitocidal protein from *Bacillus thuringiensis* subsp. morrisoni PG-14 expressed in *Bacillus thuringiensis* subsp. kurstaki by using the shuttle vector pHT3101. Appl. Environ. Microbiol. **58:**507-512.

1. Grow B. thuringiensis strain on nutrient agar plate about 5 days at 30°C or until cell autolysis is observed.
2. Harvest the lysates by centrifugation at 12,000 × g.
3. Wash three times with 10 mM EDTA-1 M NaCl-0.1 mM PMSF, each time collecting the top two layers of the three-layered pellet. Note: the bottom layer is composed of spores.
4. Resuspend in water and sonicate 5 min on ice.
5. Centrifuge at 20,000 rpm for 1 hr in SW28 rotor by using a discontinuous NaBr gradient of 38.5% (4 ml), 41.9% (6 ml), 45.3% (6 ml), 48.9% (6 ml), 52.7% (6 ml), 56.3% (3 ml).
6. Partially purified inclusions recentrifuged in another discontinuous NaBr gradient of 38.5% (5 ml), 40% (7 ml), 42% (7 ml), 44% (6 ml), and 47% (1.5 ml) under same conditions.
7. Collect purified inclusions and wash three times in cold, deionized water to remove all of the NaBr.
8. Resuspend in 5-10 ml water with Complete™ tablet and store at 4°Cuntil needed.

Purification of Crystals on Sucrose Gradients

Debro, L., P. C. Fitz-James, and A. Aronson. 1986. Two different parasporal inclusions are produced by *Bacillus thuringiensis subsp. finitimus. J. Bacteriol. 58:507-512.*

1. Grow B. thuringiensis in G-Tris medium with shaking at 30°C for 36-48 hr.
2. Recover spores and crystals by centrifugation and wash once with 1 M NaCl and 2-3 times in ddH_2O.
3. If desired, boil 2 min before the final water wash to limit protease activity.
4. Layer concentrated suspension of spores and crystals in ddH_2O on a step sucrose gradient consisting of 5 ml 60%, 3 ml of 40%, 5 ml of 30%, and 5 ml of 10% sucrose (wt/vol) in water. Centrifuge 4,080 × g for 20-30 min in Sorvall HB4 swinging bucket rotor. Free inclusions band; spores pellet.
5. Repeat step gradient as deemed necessary.
6. Wash recovered crystals in water and use immediately, or store at 4°C for up to 48 hr, or desiccate for longer storage periods.

Purification of Crystals on Renografin Gradients

Aronson, A. I., E. S. Han, W. McGaughey, D. Johnson. 1991. The solubility of inclusion proteins from *Bacillus thuringiensis* is dependent upon protoxin composition and is a factor in toxicity to insects. Appl. Environ. Microbiol. **57:**981-986.

1. Grow B. thuringiensis strain on G-Tris agar plate 36-40 hr at 30°C or 4 days at 27°C.
2. Scrape spores from the surface of chilled Petri plates into 1 M KCl-5 mM EDTA.
3. Wash once with deionized water containing 5 mM PMSF and twice with water (10 ml each).
4. Suspend pellets in water plus 0.2% Triton X-100 and 1% Renografin.
5. Layer portions over step gradients consisting of 6 ml of 50% Renografin and 4 ml of 40% Renografin in water.
6. Centrifuge in Sorvall HB4 rotor at 8,000 rpm for 50 min.
7. Remove inclusion band and if necessary purify through a second step gradient.
8. Dilute [3] fivefold with water, pellet at 8,000 rpm for 20 min in Sorvall SS-1 rotor.
9. Wash twice with deionized water, then dry in Speed-Vac.

Purification of Crystals in a Separatory Funnels

Delafield, F. P., H. J. Somerville, and S. C. Rittenberg. 1968. Immunological homology between crystal and spore protein of *Bacillus thuringiensis.* J. Bacteriol. **96:**713-720.

1. Grow *B. thuringiensis* on medium (per liter ddH_2O: 8.0 g nutrient broth, 20.0 g agar, 0.08 g $CaCl_2$, 0.05 g $MnCl_24{\cdot}H_2O$, 0.005 g $ZnSO_4 \cdot 7H_2O$, and 0.005 g $CuSO_4 \cdot 5H_2O$) for 72 hr @ 30°C.
2. Scrape cultures from surface of petri dishes and suspend in 1 M NaCl-0.02 M potassium phosphate buffer (pH 7.0) containing 0.01% Triton X-100.
3. Filter suspension through cheesecloth to remove small pieces of agar and pellet spores and crystals through centrifugation.
4. Wash sediment repeatedly until A_{260} of supernatant is negligible.
5. Wash particles once in 0.2 M NaCl-0.004 M phosphate buffer (pH 7.0)-0.01% Triton X-100 and once in 0.01% Triton X-100, then suspend in water.
6. Remove residual cells by extracting five times with 1.5 liters of Phase Mixture I of Sacks and Alderton.
7. Centrifuge and wash three times in 0.02 M phosphate buffer (pH 7.0)-0.01% Triton X-100.
8. Add suspension, in 182 ml of buffer, to cylindrical separatory funnel containing 105 g of a 20% (w/v) aqueous solution of sodium dextran sulfate 500, 13.2 g of solid PEG 6000, 3.3 ml of 3 M phosphate buffer (pH 7.0), and 7.5 g NaCl. After shaking to dissolve the solids, the volume is adjusted to 600 ml by adding a well-shaken solution of the same composition. Shake vigorously and place at 5°C for 30 min.
9. The mixture separates into two phases, an upper PEG-rich phase and a lower dextran-rich phase. Spores partition to the upper phase, crystals to the lower.
10. Draw off upper phase; centrifuge to remove spores. Add back to funnel and repeat extraction. Repeat a total of ten times.
11. Collect crystals from lower phase by centrifugation and wash five times in cold distilled water. Store at 5°C as suspensions in water.

DETECTION OF *CRY* AND *CYT* GENES BY PCR

The polymerase chain reaction (PCR) offers a powerful tool for detecting, characterizing, and isolating novel *cry* genes in *Bacillus thuringiensis* and other bacteria. The alternating blocks of conserved and variable nucleotides among *cry* genes make it possible to select primers to amplify entire gene subfamilies on the one hand or specific gene types on the other. Table 1 lists primer pairs from published studies, together with the spectrum of genes each amplifies and the sizes of the PCR products. Table 2 lists the DNA sequence for each primer. Please: these lists are planning tools only. Errors or omissions may have crept their way in, so it is essential that a researcher consult the primary references and the appropriate GenBank sequence files to confirm the exact DNA sequence required *before* synthesizing primers!

The primers may be used in several different ways. The primer pairs given should amplify the genes listed. *Triplex PCR* employs two general primers that amplify a family of genes, together with a third, more specific primer. The products of a triplex PCR reaction include both the family fragment and the specific fragment, if the specific gene type exists. *Exclusion PCR* makes use of a pair of family primers, plus a set of specific primers located internal to the family primers. If no other members of the gene family are found in the strain tested, then the specific primers will out-compete the family primers and the family fragment will be excluded from the PCR products. If at least one novel gene is present, however, the family primers alone will be able to amplify it, and the family fragment will appear in the products. Consult the references given after table 2 for detailed explanations and protocols for these strategies.

Table 1. PCR primer pairs and the *cry* or *cyt* genes they amplify

Direct	Reverse	Amplifies	Products (bp)
gral-cry1(d)	gral-cry1(r)	*cry1*	543-594
CJI-1	CJI-2	*cry1*	272-290
Un1(d)	Un1(r)	*cry1*	274-277
I(+)	I(-)	*cry1*	1500-1600
IA's	I(-)	*cry1A*	1720
Lep1A	Lep1B	*cry1A*	490
Lep2A	Lep2B	*cry1A*	908-986
CJ1	CJ2	*cry1Aa,d*	246
SB-1	U8-15c	*cry1Aa*	1500
IAa	I(-)	*cry1Aa*	1023
CJ4	CJ5	*cry1Ab,c*	216
IAb	I(-)	*cry1Ab*	940
SB-2	U3-18c	*cry1Ab*	858
IAc	I(-)	*cry1Ac*	1452
RB-19	U8-15c	*cry1Ac*	653
CJ6	CJ7	*cry1Ac*	180
IAd	I(-)	*cry1Ad*	1057
CJ3	CJ2	*cry1Ad*	171
IAe	I(-)	*cry1Ae*	1169
IB	I(-)	*cry1B*	1063
CJ8	CJ9	*cry1B*	367
IC	I(-)	*cry1C*	1160
CJ10	CJ11	*cry1C*	130
ID	I(-)	*cry1D*	1126
CJ12	CJ13	*cry1D*	290
IE	I(-)	*cry1E*	1155
CJ14	CJ15	*cry1E*	147
IF	I(-)	*cry1F*	1302
CJ16	CJ17	*cry1F*	177
V(+)	V(-)	*cry1I*	587
13091/1	05091/2	*cry1IA*	1124
II(+)	II(-)	*cry2*	1556
Un2(d)	Un2(r)	*cry2*	689-701
IIA	II(-)	*cry2A*	694
Un2(d)	EE-2Aa(r)	*cry2Aa*	498
Un2(d)	EE-2Ab(r)	*cry2Ab*	546
Un2(d)	EE-2Ac(r)	*cry2Ac*	725
IIB	II(-)	*cry2B*	694
CJIII20	CJIII21	*cry3, 7, 8*	652-733
III(+)	III(-)	*cry3*	858
Un3(d)	Un3(r)	*cry3*	589-604
Col2A	Col2B	*cry3A, 3B*	1060
Un3(d)	EE-3Aa(r)	*cry3A*	951
Col1A	Col1B	*cry3A*	649
CJIIIcte 22	CJIIIA23	*cry3A*	285
Un3(d)	EE-3Ba(r)	*cry3B*	1103
CJIIIcte 22	CJIIIB24	*cry3Ba*	437
CJIIIcte 22	CJIIIC25	*cry3Bb*	535
Un3(d)	EE-3C(r)	*cry3C*	461
CJIIIcte 22	CJIIID27	*cry3C*	312
Dip1A	Dip1B	*cry4*	797
Un4(d)	Un4(r)	*cry4*	439
EE-4A(d)	Un4(r)	*cry4A*	1529
Dip2A	Dip2B	*cry4A*	1290
EE-4B(d)	Un4(r)	*cry4B*	1951
gral-nem(d)	gral-nem(r)	*cry5,12, 14, 21*	474-489
VI(+)	VI(-)	*cry6*	587
7/8(+)	7/8(-)	*cry7, 8*	1704
Un7,8(d)	Un7,8(r)	*cry7, 8*	420-423
CJIIIcte 22	CJIIICg26	*cry7*	211
EE-7Aa(d)	Un7,8(r)	*cry7A*	916
gral-cry8(d)	gral-cry8(r)	*cry8*	373-376
EE-8A(d)	Un7,8(r)	*cry8A*	679
spe-cry8A(d)	spe-cry8A(r)	*cry8A*	338
CJIIIE28	CJIIIE29	*cry8A*	394
EE-8B(d)	Un7,8(r)	*cry8B*	775
spe-cry8B(d)	spe-cry8B(r)	*cry8B*	510
spe-cry8C(d)	spe-cry8C(r)	*cry8C*	963
EE-8C(d)	Un7,8(r)	*cry8C*	511
IG	I(-)	*cry9*	1300
CJ18	CJ19	*cry9A*	177
spe-cry9A(d)	spe-cry9A(r)	*cry9A*	571
spe-cry9B(d)	spe-cry9B(r)	*cry9B*	402
spe-cry9C(d)	spe-cry9C(r)	*cry9C*	306
gral-cry11(d)	gral-cry11(r)	*cry11*	305
EE-11A(d)	EE-11A(r)	*cry11A*	445
spe-cry13(d)	spe-cry13(r)	*cry13*	313
$DA5_c$	$CR3_c$	*cry16*	1415
OX7as	CR8	*cry17*	1400
gral-cyt(d)	gral-cyt(r)	*cyt1*	522-525

NOMENCLATURE FOR CRY AND CYT PROTEINS

The Cry and Cyt proteins of *B. thuringiensis* and related bacteria are named by a logical set of rules developed by the Cry Nomenclature Committee, a standing committee of the *Bacillus* Genetic Stock Center. Each protein name consists of the mnemonic "Cry" or "Cyt" with four characters appended--an Arabic numeral, an uppercase letter, a lowercase letter, and another Arabic numeral, e.g. Cry1Aa1. All proteins sharing the first numeral in their names share at least 45% amino acid identity with other members of the group. Proteins sharing both the first numeral and the uppercase letter share at least 75% identity. Proteins sharing the same first numeral, uppercase, and lowercase letters share at least 95% identity. A phylogram showing the relative amino acid identity of the aligned Cry and Cyt sequences appears on page . The table beginning below lists all sequences that have received official names as of April 1999. Only sequences deposited in public databases are eligible to receive names. In addition, a protein must satisfy at least one of the two following criteria: (1) It must display significant homology to known Cry or Cyt proteins or (2) must be accumulated in a crystal and have a demonstrated toxic effect to a target organism. A much more thorough explanation of the nomenclature system can be found in Crickmore *et al.* 1998. Microbiol. Mol. Biol. Rev. 62:807-813. An up-to-date listing of genes can be found on Neil Crickmore's web site at the following URL: http://www.biols.susx.ac.uk/Home/Neil_Crickmore/Bt/. If you have a predicted sequence encoding a protein you wish named, please contact Dan Zeigler at zeigler.1@osu.edu.

NAME	ORIGINAL	ACCESSION NUMBER(S)	CODING REGION	REF.
Cry1Aa1	CryIA(a)	M11250	527 ... 4054	100
Cry1Aa2	CryIA(a)	M10917, E00881	153 ... >2955	106
Cry1Aa3	CryIA(a)	D00348, E01529, E01601	73 ... 3600	107
Cry1Aa4	CryIA(a)	X13535	1 ... 3528	65
Cry1Aa5	CryIA(a)	D17518, E01217	81 ... 3608	121
Cry1Aa6	CryIA(a)	U43605	1 ... >1860	66
Cry1Aa7	CryIA(a)	AF081790		78
Cry1Aa8	CryIA(a)	I26149	148 ... 3675	63
Cry1Ab1	CryIA(b)	M13898	142 ... 3606	127
Cry1Ab2	CryIA(b)	M12661	155 ... 3622	119
Cry1Ab3	CryIA(b)	M15271, A03793, A09398	156 ... 3620	33
Cry1Ab4	CryIA(b)	D00117, E01218	163 ... 3627	52
Cry1Ab5	CryIA(b)	X04698, I24776	141 ... 3605	43
Cry1Ab6	CryIA(b)	M37263	73 ... 3537	40
Cry1Ab7	CryIA(b)	X13233, X16315	1 ... 3465	39
Cry1Ab8	CryIA(b)	M16463, E01173, E01279, E01308, E01600	157 ... 3621	74
Cry1Ab9	CryIA(b)	X54939	73 ... 3537	13
Cry1Ab10	CryIA(b)	A29125	Peptide sequence	30
Cry1Ab11	CryIA(b)	I12419		28
Cry1Ab12	CryIA(b)	AF059670	41 ... 3505	111
Cry1Ac1	CryIA(c)	M11068	388 ... 3921	4
Cry1Ac2	CryIA(c)	M35524	239 ... 3769	125
Cry1Ac3	CryIA(c)	X54159	339 ... >2192	18
Cry1Ac4	CryIA(c)	M73249	1 ... 3534	93
Cry1Ac5	CryIA(c)	M73248	1 ... 3531	92
Cry1Ac6	CryIA(c)	U43606	1 ... >1821	66
Cry1Ac7	CryIA(c)	U87793	976 ... 4509	41
Cry1Ac8	CryIA(c)	U87397	153 ... 3686	76
Cry1Ac9	CryIA(c)	U89872	388 ... 3921	35
Cry1Ac10	CryIA(c)	AJ002514	388 ... 3921	116
Cry1Ac11	CryIA(c)	AJ130970	156 ... 3689	64
Cry1Ac12	CryIA(c)	I12418	81 ... >2990	28
Cry1Ad1	CryIA(d)	M73250, I76414, I76775	1 ... 3537	88
Cry1Ad2	CryIA(d)	A27531	1 ... 3537	1
Cry1Ae1	CryIA(e)	M65252	81 ... 3623	61
Cry1Af1	icp	U82003	172 ... >2905	50
Cry1Ag1		AF081248		78
Cry1Ba1	CryIB	X06711	1 ... 3684	10
Cry1Ba2	CryIB	X95704	186 ... 3869	113
Cry1Bb1	ET5	L32020, I38760, I70138	67 ... 3753	26
Cry1Bc1	CryIB(c)	Z46442	141 ... 3839	7

PESTICIDAL ACTIVITY OF CRY AND CYT PROTEINS

Cry proteins exhibit toxicity to insects and other invertebrates. Typically, a given Cry protein has a fairly narrow range of target organisms against which it is effective. The mode of action of Cry proteins is complex. It is thought to involve solubilization and proteolytic processing in the target organism's gut, binding to receptor molecules in specific gut cells, and insertion into the cell membrane. At some point in this process certain structural rearrangements must occur and oligomers of the protein must form. Eventually, the inserted protein functions as an ion channel, disrupting the electrophysiology of the gut cells. For a Cry protein to function effectively in a given target organism, then, an entire series of events must occur at a rate and frequency above a certain threshold. It can be difficult to predict which organisms might be susceptible to a newly discovered Cry protein. Although numerous exceptions exist, a useful first approximation would be that proteins sharing a primary rank (the Cry1 proteins, for example) are toxic to the same orders of insects or other invertebrates. Proteins sharing the same secondary rank (the Cry1A proteins, for example) are generally toxic to the same families. Finally, proteins sharing the same tertiary rank (such as the Cry1Aa proteins) typically are toxic to the same species. The following table, derived from the Toxin Specificity Database (http://www.glfc.forestry.ca/Bacillus/Web98.adb), primary journal articles, and patent applications is intended to illustrate the activity spectrum for selected toxins. Consult the Toxin Specificity Database for a much more complete, searchable list.

NAME	SOURCE STRAIN	KNOWN TOXICITY
Cry1Aa1	*B.t. kurstaki* HD-1; *B.t. aizawai* HD-68	*Heliothis virescens, Mamestra brassicae, Pseudoplusia includens* (Lepidoptera: Noctuidae); *Manduca sexta* (Lepidoptera: Sphingidae); *Pieris brassicae* (Lepidoptera: Pieridae); *Bombyx mori* (Lepidoptera: Bombycidae); (Lepidoptera: Lymantriidae); *Sciropophaga incertulas, Chilo suppressalis, Ostrinia nubilalis* (Lepidoptera: Pyralidae); *Choristoneura fumiferana* (Lepidoptera: Tortricidae); *Hyphantria cunea* (Lepidoptera: Arctiidae); *Plutella xylostella* (Lepidoptera: Plutellidae)
Cry1Ab2	*B.t. kurstaki* HD-1	*Lymantria dispar* (Lepidoptera: Lymantriidae); *Heliothis virescens, Trichoplusia ni* (Lepidoptera: Noctuidae); *Manduca sexta* (Lepidoptera: Sphingidae)
Cry1Ac1	*B.t. kurstaki* HD-73, *B.t. kurstaki* HD-244	*Bombyx mori* (Lepidoptera: Bombycidae); *Agrotis segetum, Helicoverpa zea, Heliothis virescens, Mamestra brassicae, Trichoplusia ni, Spodoptera exigua* (Lepidoptera: Noctuidae); *Ephestia kuehniella, Sciropophaga incertulas, Chilo suppressalis, Ostrinia nubilalis* (Lepidoptera: Pyralidae); *Manduca sexta* (Lepidoptera: Sphingidae); *Lymantria dispar* (Lepidoptera: Lymantriidae); *Pieris brassicae* (Lepidoptera: Pieridae)
Cry1Ad1	*B.t. aizawai* PS81I	*Trichoplusia ni, Spodoptera exigua* (Lepidoptera: Noctuidae); *Choristoneura fumiferana* (Lepidoptera: Tortricidae); *Plutella xylostella* (Lepidoptera: Plutellidae)
Cry1Ae1	*B.t. alesti*	*Heliothis virescens, Trichoplusia ni* (Lepidoptera: Noctuidae)
Cry1Af1	*B. thuringiensis* NT0423	Reported dual activity against Diptera and Lepidoptera
Cry1Ba1	*B. thuringiensis* HD-290-I; *B. thuringiensis* HD2	*Chrysomela scripta* (Coleoptera: Chrysomelidae); *Manduca sexta* (Lepidoptera: Sphingidae); *Artogeia rapae* (Lepidoptera: Pieridae)
Cry1Bb1	*B. thuringiensis* EG5847	*Spodoptera frugiperda, Pseudoplusia includens, Trichoplusia ni* (Lepidoptera: Noctuidae); *Plutella xylostella* (Lepidoptera: Plutellidae); *Lymantria dispar* (Lepidoptera: Lymantriidae); *Ostrinia nubilalis* (Lepidoptera: Pyralidae);
Cry1Be1	*B. thuringiensis* 158C2	Strain of origin active against lepidopterans
Cry1Ca1	*B.t. entomocidus* 60.5, *B.t. aizawai* HD-229	*Sciropophaga incertulas, Chilo suppressalis* Lepidoptera: Pyralidae); *Heliothis virescens, Spodoptera exigua, Spodoptera frugiperda, Trichoplusia ni* (Lepidoptera: Noctuidae); *Pieris brassicae* (Lepidoptera: Pieridae)
Cry1Cb1	*B.t. galleriae* HD-29	*Spodoptera exigua, Trichoplusia ni* (Lepidoptera: Noctuidae)
Cry1Da1	*B.t. aizawai* HD-68	*Plutella xylostella* (Lepidoptera: Plutellidae); *Choristoneura fumiferana* (Lepidoptera: Tortricidae); *Bombyx mori* (Lepidoptera: Bombycidae); *Lymantria dispar, Orgyia leucostigma* (Lepidoptera: Lymantriidae); *Manduca sexta* (Lepidoptera: Sphingidae); *Malacosoma disstria* (Lepidoptera: Lasiocampidae); *Lambdina fiscellaria fiscellaria* (Lepidoptera: Geometridae); *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

THE REVIEW LITERATURE FOR *B. THURINGIENSIS*

Bacillus thuringiensis is the subject of intensive research. Nearly 4000 primary research articles covering some aspect of Bt or Cry protein biology will be published in *this decade alone.* Fortunately, many of these topics have been reviewed in recent years. The following list of reviews is by no means complete. Perhaps it will suffice, however, to provide researchers new to the field with an entry into the fascinating *Bacillus thuringiensis* research literature. The list is organized by the kinds of questions raised in each review.

- **Where can I find a thorough, general review of *Bacillus thuringiensis* and Cry proteins?**

  Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D. R. Zeigler, D. H. Dean. 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. **62**:775-806.

  Kumar, P. A., R. P. Sharma, V. S. Malik. 1996. The insecticidal proteins of *Bacillus thuringiensis*. Adv. Appl. Microbiol. **42:**1-43.

  Aronson, I. 1993. Insecticidal toxins. pp. 953-963 in: *Bacillus subtilis* and Other Gram-Positive Bacteria. Biochemistry, Physiology, and Molecular Genetics. (Sonenshein, A. L., J. A. Hoch, and R. Losick, eds.) American Society for Microbiology, Washington, D. C.

- **How are Cry and Cyt proteins named?**

  N. Crickmore, D. R. Zeigler, J. Feitelson, Schnepf, E., J. Van Rie, D. Lereclus, J. Baum, D. H. Dean. 1998. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. **62:**807-813.

- **What are the phylogenetic relationships found among the Cry proteins?**

  Bravo, A. 1997. Phylogenetic relationships of *Bacillus thuringiensis* delta-endotoxin family proteins and their functional domains. J. Bacteriol. **179:**2793-801.

- **How is the expression of *cry* genes regulated in *Bacillus thuringiensis*?**

  Agaisse, H., D. Lereclus. 1995. How does *Bacillus thuringiensis* produce so much insecticidal crystal protein? J. Bacteriol. **177:**6027-6032.

- **What role might transposable elements play in *cry* gene biology?**

  Mahillon, J., R. Rezsohazy, B. Hallet, J. Delcour. 1994. IS*231* and other *Bacillus thuringiensis* transposable elements: a review. Genetica **93:**13-26.

- **How might Cry proteins contribute to the fitness of *Bacillus thuringiensis*?**

  Aronson, A. I. 1993. The two faces of *Bacillus thuringiensis*: insecticidal proteins and post-exponential survival. Mol. Microbiol. **7:**489-496.

B. CEREUS & B. THURINGIENSIS—THE SPECIES QUESTION

The species status of members of the *B. cereus* group has been a persistent question among bacterial taxonomists. The data summarized below suggest strongly that these organisms are as closely related genetically as are members of other recognized bacterial species. Further, no physiological or molecular character has been discovered that correlates with the presence of parasporal crystals, the classical definition of *B. thuringiensis*, other than the presence of the *cry* genes themselves. A model in which *B. cereus*-like organisms exchange genetic material, especially plasmid borne genes, could easily account for the occurrence of crystal-producing strains in nature. It is perhaps inconvenient that highly beneficial organisms and pathogenic ones co-exist under the same taxonomic identifier. Yet the safe use of Bt products for over three decades argues strongly that many *B. cereus*-like organisms can indeed be harnessed for applications in industry and agriculture. If so, it may be more productive to work towards eliminating a few hazardous genes than to regard an entire species as somehow unsuitable for use.

Numbers Analyzed[a]				Method	Conclusions	Ref
Bc	Bt	Bm	Ba			
44	15	13	23	30 morphological and physiological characters	"We are bound by our data" that there is no "basis for separation" into more than one species	6
35	137	-	-	99 phenotypic traits	"Strains of Bt were indistinguishable from *B. cereus*, except for their ability to produce parasporal crystals."	2
39	12	16	-	329 physiological tests	Strains clustered in one main group, distinct from the other *Bacillus* species tested	7
149	55	25	37	API test strips	"The results suggest that *B. mycoides* and Bt should be considered as varieties of *B. cereus*."	10
17	35	4	-	118 morphological and physiological tests	"The numerical phenetic data underline the close relationship between *B. cereus* and *B. thuringiensis*."	12
33	9	-	-	Fatty acid analysis	All strains tested clustered together, distinct from the other *Bacillus* species tested	8
24	12	-	-	*NotI* profiles, multilocus enzyme electrophoresis	"On the basis of these results…we conclude that strains typed as *B. cereus* and Bt belong to the same species."	4
1	2	1	78	Amplification fragment length polymorphism	"AFLP similarities are consistent with…close relationships" among these strains.	9
4	3	-	-	Physical mapping of chromosomes	Some *B. cereus* genomes are more similar to Bt genomes than to those of other *B. cereus* strains.	5
3	3	-	3	Phospholipid analysis	The three "species" tested clustered into a "B. cereus group" readily distinguishable from B. subtilis	3
2	6	1	2	DNA reassociation	"The available DNA reassociation data indicate a single species."	11
2	1	1	1	16S rRNA sequencing	"These 'species' form a genealogically tight group" comparable to "other gram-positive species."	1

[a]Species abbreviations: Bc, *B. cereus;* Bt, *B. thuringiensis;* Bm, *B. mycoides;* Ba, *B. anthracis*

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