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).

Important Notice

Please read this notice before ordering materials from this catalog!

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   (i) the composition, characterization, purity, stability, safety or utility of Materials,

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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

Serotype 6—Serovar. *entomocidus/subtoxicus*

BGSC No.	Original Code	Reference	Description
4I1	HD10	Heimpel AM, Angus TA (1958) Can J Microbiol 4:531	Isolated in Canada from *Plodia interpunctella*; biotype *subtoxicus*
4I2	HD198	Dulmage HT, donor; Hunter K, source	Isolated in US from *Cadra figulilela*
4I3	HD320	Dulmage HT, donor; Krywienczyk J, source	Isolated in US
4I4	HD9	Dulmage HT, donor; Heimpel AM, Angus TA (1958) Can J Microbiol 4:531	Isolated in Canada from *Aphonia gularis*; antisera standard
4I5	HD109	Dulmage HT, donor; Heimpel AM source	Isolated in Canada; biotype *subtoxicus*

Serotype 7—Serovar. *aizawai/pacificus*

BGSC No.	Original Code	Reference	Description
4J1	HD112 (DD-742)	Dulmage HT, donor; Hall IM, source; see J Invertebr Path 9:364	Isolated in Japan from *Heliothis assulta*
4J2	HD137 (HDB-24)	Dulmage HT, donor; Burges HD, source	Isolated in England from *Plodia interpunctella*
4J3	HD133 (HDB-20)	Dulmage HT, donor; Burges HD, source	Isolated in England from *Plodia interpunctella*
4J4	HD11	Dulmage HT, donor; Aizawa K, source	Isolated in Japan from *Heliothis assulta;* antisera standard
4J5	HD137 (HDB-24)	Dulmage HT, donor; Burges HD, source	Isolated in England from *Plodia interpunctella*

Serotype 8a, 8b—Serovar. *morrisoni*

BGSC No.	Original Code	Reference	Description
4K1	HD12	Dulmage HT, donor; Norris JR, source	Isolated in the US; antisera standard
4K3	HD518	Dulmage HT, donor; DeLucca, source	Isolated in the US
4AA1	tenebrionis	McPherson S A, et al. (1989) Bio/technology 6: 61	Biovar. tenebrionis
4AB1	san diego	Herrnstadt C, et al. (1987) Gene 57:37-46	Biovar. sandiego

Serotype 9—Serovar. *tolworthi*

BGSC No.	Original Code	Reference	Description
4L1	HD125 (HDB-8)	Dulmage HT, donor; Burges HD, source	Isolated in England from *Cadra cautella*
4L2	HD301 (HDB-28)	Dulmage HT, donor; Burges HD, source	Isolated in England from *Cadra cautella*
4L3	HD537	Dulmage HT, donor; deBarjac H, source	Wild type isolate

Serotype 10a, 10b—Serovar. *darmstadiensis*

BGSC No.	Original Code	Reference	Description
4M1	HD146 (103)	Krieg A, et al. (1968) J Invertebr Path 10:428	Isolated in England
4M2	HD199 (102)	Krieg A, et al. (1968) J Invertebr Path 10:428	Isolated in England
4M3	HD146 (103)	Krieg A, et al. (1968) J Invertebr Path 10:428	Isolated in England

Serotype 11a, 11b—Serovar. *toumanoffi*

BGSC No.	Original Code	Reference	Description
4N1	HD201 (B-30-2)	Toumanoff C (1956) Ann Inst Pasteur 90:660	Isolated in England from *Galleria mellonella;* antisera standard

Serotype 12—Serovar. *thompsoni*

BGSC No.	Original Code	Reference	Description
4O1	HD542	deBarjac H, source	Wild type isolate

Serotype 13—Serovar. *pakistani*

BGSC No.	Original Code	Reference	Description
4P1	HD395	Shaikh, source	Isolated in Pakistan from *Cydia pomonella*; antisera standard

Serotype 14—Serovar. *israelensis*

BGSC No.	Original Code	Reference	Description
4Q1	HD567 (ONR60A)	Goldberg LJ, Margalit J (1977) Mosquito News 37:55	Isolated in Israel from *Culicidae* larvae; antisera standard
4Q2	HD500	Goldberg LJ, Margalit J (1977) Mosquito News 37:55	Isolated in Israel from *Culicidae* larvae
4Q3	IPS 70	Goldberg LJ, Margalit J (1977) Mosquito News 37:55	Isolated in Israel from *Culicidae* larvae
4Q4	WHO2013-9	Nosec I (unpublished) (see: J Am Mosq Cont Assc 1:1)	Wild type isolate
4Q5	4Q2-72	Clark BD (1987) Ph.D. Thesis (Ohio St. Univ.)	Plasmid cured mutant; bears only 72 mDal plasmid
4Q6	4Q2-72 str azi	(unpublished)	Azi^r Str^r; bears only 72 mDal plasmid
4Q7	4Q2-81	Clark BD (1987) Ph.D. Thesis (Ohio St. Univ)	Plasmidless mutant
4Q8	4Q2-81 str	(unpublished)	Str^r; Plasmidless mutant

Serotype 15—Serovar. *dakota*

BGSC No.	Original Code	Reference	Description
4R1	Oats 43	DeLucca AJ, et al. (1979) J Invertebr Path 34:323	Wild type isolate

Serotype 16—Serovar. *indiana*

BGSC No.	Original Code	Reference	Description
4S2	HD521	Dulmage HT, donor; deLucca, source	Wild type isolate
4S3	HD516	Dulmage HT, donor; deBarjac H, source	Wild type isolate

Serovar. wuhanensis (no flagellar antigen)

BGSC No.	Original Code	Reference	Description
4T1	HD525 (140)	Hubei Institute Microbiol (1976) Acta Microbiol Sin 16:12	no flagellar antigen

Serotype 11a, 11c—Serovar. *kyushuensis*

BGSC No.	Original Code	Reference	Description
4U1	HD541 (74-F-6-18)	Ohba M, Aizawa K (1979) J Invertebr Path 33:387	Isolated in Japan from *B. mori* colony; antisera standard

Serotype 17—Serovar. *tohokuensis*

BGSC No.	Original Code	Reference	Description
4V1	78-FS-29-17	Ohba M, et al. (1981) J Invertebr Path 38:307	Isolated in Japan from *B. mori* colony; antisera standard

Serotype 18a, 18b—Serovar. *kumamtoensis*

BGSC No.	Original Code	Reference	Description
4W1	HD867 (3-71)	Ohba M, et al. (1981 J Invertebr Path 38:184	Isolated in Japan from *B. mori* colony; antisera standard

Serotype 21—Serovar. *colmeri*

BGSC No.	Original Code	Reference	Description
4X1	IS720	DeLucca AJ, et al. (1984) J Invertebr Path 43:437	Wild type isolate

Serotype 19—Serovar. *tochigiensis*

BGSC No.	Original Code	Reference	Description
4Y1	HD868 (117-72)	Ohba M, et al. (1981) J Invertebr Path 38:184	Isolated in Japan from soil; antisera standard

Serotype 8a, 8c—Serovar. *ostriniae*

BGSC No.	Original Code	Reference	Description
4Z1	HD501	Gaixin R, et al. (1975) Acta Microbiol Sin 19:117	Isolated in China

Serotype 27—Serovar. *mexicanensis*

BGSC No.	Original Code	Reference	Description
4AC1	GM54	Rodriguez-Padilla and Galan-Wang (1988)	Isolated in Mexico; antisera standard

Serotype 31—Serovar. *toguchini*

BGSC No.	Original Code	Reference	Description
4AD1	toguchini	Hodirev VP (1990) Izvestiia Akademii Nauk SSSR. SerIIA Biologicheskaia. 5:789-91	Isolated in USSR from soil; antisera standard

Serotype 29—Serovar. *amagiensis*

BGSC No.	Original Code	Reference	Description
4AE1	84 F 58.20	Ohba M, source; see Saitoh H, et al. (1996) Microbiol Res 151: 263-71	Isolated in Japan from *Bombyx mori* litter; antisera standard

Serotype 32—Serovar. *cameroun*

BGSC No.	Original Code	Reference	Description
4AF1	273B	Jacquemard, source	isolated from soil samples collected in Cameroon; produces cuboidal crystals with major proteins of 53 and 35 kDa; antisera standard

Serotype 26—Serovar. *silo*

BGSC No.	Original Code	Reference	Description
4AG1	SLM5.A	Lecadet M, source	Isolated from grain silo in France; antisera standard

Serotype 34—Servoar. *konkukian*

BGSC No.	Original Code	Reference	Description
4AH1	HL47	Lee HH (1994) J Invertebr Pathol 63:217	Isolated in South Korea; antisera standard; produces bipyrimidal crystals; toxic to *Bombyx mori* but not *Culex pipiens*

Serotype 28a, 28b—Serovar. *monterrey*

BGSC No.	Original Code	Reference	Description
4AJ1	GM33	Rodriquez-Padilla, source	Isolated in Mexico; antisera standard

Serotype 33—Serovar. *leesis*

BGSC No.	Original Code	Reference	Description
4AK1	HL51	Lee HH (1994) J Invertebr Pathol 63:217	Isolated in South Korea; antisera standard; produces cuboidal crystals; toxic to *Bombyx mori* but not *Culex pipiens*

Serotype 25—Serovar. *coreanensis*

BGSC No.	Original Code	Reference	Description
4AL1	HL1	Lee HH (1994) J Invertebr Pathol 63:217	Isolated in South Korea; antisera standard; produces irregular crystals; not toxic to *Bombyx mori* or *Culex pipiens*

Serotype 20a, 20b—Serovar *yunnanensis*

BGSC No.	Original Code	Reference	Description
4AM1	T20 001	Yu W, Fang Q, Ping X, You W (1979)	Isolated in China from *Prodenia litura*; antisera standard

Serotype 22—Serovar. *shanongiensis*

BGSC No.	Original Code	Reference	Description
4AN1	T22 001	Ying, Jie, Xichang (1986)	Isolated in China; antisera standard

Serotype 3a, 3d—Serovar. *sumiyoshiensis*

BGSC No.	Original Code	Reference	Description
4AO1	T03B001	Ohba M, Aizawa (1989)	Isolated in Japan; antisera standard

Serotype 3a, 3d, 3e—Serovar. *fukuokaensis*

BGSC No.	Original Code	Reference	Description
4AP1	T03C001	Ohba M, Aizawa (1989)	Isolated in Japan; antisera standard

Serotype 35—Serovar. *seoulensis*

BGSC No.	Original Code	Reference	Description
4AQ1	T35 001	Shim JC, unpublished	Isolated in South Korea from soil; antisera standard

Serotype 42—Serovar. *jinghongiensis*

BGSC No.	Original Code	Reference	Description
4AR1	T42 001	Rong SL, unpublished	Isolated in China from soil; antisera standard

Serotype 38—Serovar. *oswaldocruzi*

BGSC No.	Original Code	Reference	Description
4AS1	T38 001	Rabinovitch L, unpublished	Isolated in Brasil from black pepper powder; antisera standard

Serotype 23—Serovar. *japonensis*

BGSC No.	Original Code	Reference	Description
4AT1	T23 001	Ohba M, Aizaawa (1986)	Isolated in Japan; antisera standard

Serotype 44—Serovar. *higo*

BGSC No.	Original Code	Reference	Description
4AU1	T44 001	Ohba M, unpublished	Isolated in Japan from *Bombyx mori* litter; antisera standard

Serotype 36—Serovar. *malayensis*

BGSC No.	Original Code	Reference	Description
4AV1	T36 001	Ho TM, unpublished	Isolated in Malaysia; antisera standard

Serotype 37—Serovar. *andalousiensis*

BGSC No.	Original Code	Reference	Description
4AW1	T37 001	Santiago-Alvarez C, unpublished	Isolated in Spain; antisera standard

Serotype 24a, 24c—Serovar. *novosibirsk*

BGSC No.	Original Code	Reference	Description
4AX1	T24A 001	Burtseva L, Kalmikowa G (unpublished)	Isolated in the USSR from soil; antisera standard

Serotype 39—Serovar. *brasilensis*

BGSC No.	Original Code	Reference	Description
4AY1	T39 001	Rabionovitch L, unpublished	Isolated in Brazil from black pepper powder; antisera standard

Serotype 8b, 8d—Serovar. *nigeriensis*

BGSC No.	Original Code	Reference	Description
4AZ1	T08B 001	Weiser J, Prasertphon (1984)	Isolated in Czechoslovakia; antisera standard

Serotype 20a, 20c—Serovar. *pondicheriensis*

BGSC No.	Original Code	Reference	Description
4BA1	T20A 001	Rajagopalan PK	Isolated in India from soil; antisera standard

Serotype 41—Serovar. *sooncheon*

BGSC No.	Original Code	Reference	Description
4BB1	T41 001	Lee HH (unpublished)	Isolated in South Korea; antisera standard

Serotype 43—Serovar. *guiyangiensis*

BGSC No.	Original Code	Reference	Description
4BC1	T43 001	Rong SL, unpublished	Isolated in China from soil; antisera standard

Serotype 40—Serovar. *huazhongensis*

BGSC No.	Original Code	Reference	Description
4BD1	T40 001	Yu Z, unpublished	Isolated in China; antisera standard

Serotype 24a, 24b—Serovar. *neoleonensis*

BGSC No.	Original Code	Reference	Description
4BE1	T24 001	Rodriguez-Padilla, et al. (1988)	Isolated in Mexico; antisera standard

Serotype 10a, 10c—Serovar. *londrina*

BGSC No.	Original Code	Reference	Description
4BF1	T10A 001	Aramtes O, unpublished	Isolated in Germany; antisera standard

Serotype 45—Serovar. *roskildiensis*

BGSC No.	Original Code	Reference	Description
4BG1	T45 001	Hinrinschen & Hansen, unpublished	Isolated in Denmark from ivy leaves; antisera standard

Serotype 46—Serovar. *chanpaisis*

BGSC No.	Original Code	Reference	Description
4BH1	JC 51	IEBC, donor; Cangpaisan J, source	Isolated in Thailand from rice paddy

Serotype 47—Serovar. *wratislaviensis*

BGSC No.	Original Code	Reference	Description
4BJ1	PO 12	IEBC, donor; Lonc E, source	Isolated in Poland from soil

Serotype 48—Serovar. *balearica*

BGSC No.	Original Code	Reference	Description
4BK1	PM9	IEBC, donor; Caballero P, source	Isolated in Spain from soil

Serotype 49—Serovar. *muju*

BGSC No.	Original Code	Reference	Description
4BL1	A39	IEBC, donor; Campos Dias S, source	Isolated in Argentina

Serotype 50—Serovar. *navarrensis*

BGSC No.	Original Code	Reference	Description
4BM1	NA69	IEBC, donor; Caballero P, source	Isolated in Spain from soil

Serotype 51—Serovar. *xiaguangiensis*

BGSC No.	Original Code	Reference	Description
4BN1	3397	IEBC, donor; Jianping Yan, source	Isolated in China from beans

Serotype 52—Serovar. *kim*

BGSC No.	Original Code	Reference	Description
4BP1	HL 175	IEBC, donor; Kim S, source	Isolated in Korea from sesame field

Serotype 53—Serovar. *asturiensis*

BGSC No.	Original Code	Reference	Description
4BQ1	EA 34594	IEBC, donor; Santiago-Alvarez C, source	Isolated in Spain from soil

Serotype 54—Serovar. *poloniensis*

BGSC No.	Original Code	Reference	Description
4BR1	Pbt 23	IEBC, donor; Damgaard P, source	Isolated in Denmark from *Lymantria monacha* larvae

Serotype 55—Serovar. *palmanyolensis*

BGSC No.	Original Code	Reference	Description
4BS1	EA 40694	IEBC, donor; Santiago-Alvarez C, source	Isolated in Spain from soil

Serotype 56—Serovar. *rongseni*

BGSC No.	Original Code	Reference	Description
4BT1	Scg04-02	IEBC, donor; Li R, source	Isolated in China from red soil

Serotype 57—Serovar. *pirenaica*

BGSC No.	Original Code	Reference	Description
4BU1	NA210	IEBC, donor; Caballero P, source	Isolated in Spain

Serotype 58—Serovar. *argentinensis*

BGSC No.	Original Code	Reference	Description
4BV1	A20	IEBC, donor; Campos Dias S, source	Isolated in Argentina

Serotype 59—Serovar. *iberica*

BGSC No.	Original Code	Reference	Description
4BW1	L60	IEBC, donor; Caballero P, source	Isolated in Spain

Serotype 60—Serovar. *pingluonsis*

BGSC No.	Original Code	Reference	Description
4BX1	NXP15-04	IEBC, donor; Li R, source	Isolated in China from sandy soil

Serotype 61—Serovar. *sylvestriensis*

BGSC No.	Original Code	Reference	Description
4BY1	Pbt 53	IEBC, donor; Damgaard P, source	Isloated in Denmark from Scotch pine

Serotype 62—Serovar. *zhaodongensis*

BGSC No.	Original Code	Reference	Description
4BZ1	HZ39-04	IEBC, donor; Li R, source	Isolated in China from black soil

Serotype 18a, 18c—Serovar. *yosoo*

BGSC No.	Original Code	Reference	Description
4CA1	HL94	IEBC, donor; Lee HH, source	Isolated in South Korea

Serotype 64—Serovar. *azorensis*

BGSC No.	Original Code	Reference	Description
4CB1	EA11996	IEBC, donor; Santiago-Alvarez C, source	Isolated in Azores from soil

Serotype 65—Serovar. *pulsiensis*

BGSC No.	Original Code	Reference	Description
4CC1	NARC Bt17	IEBC, donor; Khalique A., source	Isolated in Pakistan from grain field

Serotype 66—Serovar. *graciosensis*

BGSC No.	Original Code	Reference	Description
4CD1	EA15196	IEBC, donor; Santiago-Alvarez C, source	Isolated in Azores from soil

Serotype 67—Serovar. *vazensis*

BGSC No.	Original Code	Reference	Description
4CE1	EA14696	IEBC, donor; Santiago-Alvarez C, source	Isolated in Azores from soil

Serotype 28a, 28c—Serovar. *jegathesan*

BGSC No.	Original Code	Reference	Description
4CF1	367	IEBC, donor; Lee HL, source	Isolated in Malaysia

B. THURINGIENSIS STRAINS BY ORIGINAL CODE

Original Code	BGSC
102	4M2
103	4M1
117-72	4Y1
140	4T1
143	4C3
1715	4A6
2	4D2
2-124	4E1
273B	4AF1
3	4D3
367	4CF1
3397	4BN1
3-71	4W1
4432	4D20
4432(pC194)	4D21
4Q2-72	4Q5
4Q2-72 str azi	4Q6
4Q2-81	4Q7
4Q2-81 str	4Q8
7304	4F4
74-F-6-18	4U1
78-FS-29-17	4V1
84 F 58.20	4AE1
A20	4BV1
A39	4BL1
AP77BX17	4D4
B-30-2	4N1
Bt1	4A7
Bt131	4A8
Bt1627	4A9
CCEB206	4A2
CCEB457	4A5
CCEB460	4B1
CCEB463	4C1
CCEB566	4H1
CRY(-)B	4D11
Dch-T	4H1
DD-742	4J1
EA 11996	4CB1
EA 14696	4CE1
EA 15196	4CD1
EA 34594	4BQ1
EA 40694	4BS1
GM33	4AJ1
GM54	4AC1
HD(CRY-1)	4D7
HD1	4D1
HD1	4D20
HD1	4D6
HD1(CRY-6)	4D8
HD1(CRY-7)	4D9
HD1(CRY-8)	4D10
HD2	4A3
HD3	4B2
HD4	4C3
HD6	4E4
HD7	4E2
HD8	4G1
HD9	4I4
HD10	4I1

Original Code	BGSC
HD11	4J4
HD12	4K1
HD14	4A5
HD16	4C1
HD19	4B1
HD24	4A2
HD29	4G5
HD30	4H1
HD72	4C2
HD73	4D4
HD88	4D17
HD89	4D18
HD106	4E1
HD109	4I5
HD112	4J1
HD120	4A4
HD125	4L1
HD133	4J3
HD136	4F1
HD137	4J2
HD137	4J5
HD146	4M1
HD146	4M3
HD161	4G3
HD164	4D5
HD168	4G6
HD198	4I2
HD199	4M2
HD201	4N1
HD201	4N1
HD210	4G2
HD224	4H2
HD231	4D14
HD232	4D15
HD243	4D16
HD263	4D12
HD270	4D19
HD278	4F2
HD293	4F3
HD301	4L2
HD305	4G4
HD320	4I3
HD395	4P1
HD500	4Q2
HD501	4Z1
HD516	4S3
HD518	4K3
HD521	4S2
HD525	4T1
HD537	4L3
HD541	4U1
HD541	4U1
HD542	4O1
HD560	4F4
HD567	4Q1
HD867	4W1
HD867	4W1
HD868	4Y1
HDB-2	4A4
HDB-8	4L1

Original Code	BGSC
HDB-20	4J3
HDB-23	4F1
HDB-24	4J2
HDB-24	4J5
HDB-28	4L2
HDB-34	4G4
HL1	4AL1
HL47	4AH1
HL51	4AK1
HL94	4CA1
HL175	4BP1
HZ39-04	4BZ1
IPS 70	4Q3
IS720	4X1
JC 51	4BH1
L60	4BW1
NA69	4BM1
NA210	4BU1
NARC Bt17	4CC1
NRRL-B4039	4A1
NXP15-04	4BX1
Oats 43	4R1
ONR60A	4Q1
Pbt 23	4BR1
Pbt 53	4BY1
PIL-89	4D12
PIL-96	4D19
PIL-139	4F2
PM9	4BK1
PO 12	4BJ1
san diego	4AB1
Scg04-02	4BT1
SLM5.A	4AG1
sotto	4E3
Sotto G	4E4
T03B001	4AO1
T03C001	4AP1
T08B 001	4AZ1
T10A 001	4BF1
T20 001	4AM1
T20A 001	4BA1
T22 001	4AN1
T23 001	4AT1
T24 001	4BE1
T24A 001	4AX1
T35 001	4AQ1
T36 001	4AV1
T37 001	4AW1
T38 001	4AS1
T39 001	4AY1
T40 001	4BD1
T41 001	4BB1
T42 001	4AR1
T43 001	4BC1
T44 001	4AU1
T45 001	4BG1
tenebrionis	4AA1
toguchini	4AD1
WHO2013-9	4Q4

B. THURINGIENSIS STRAINS BY SEROTYPE

Serotype	Serovar	BGSC No.
1	thuringiensis	4A1-4A9
2	finitimus	4B1-4B2
3a,3b,3c	kurstaki	4D1-4D21
3a,3c	alesti	4C1-4C3
3a,3d	sumiyoshiensis	4AO1
3a,3d,3e	fukuokaensis	4AP1
4a,4b	sotto/dendrolimus	4E1-4E4
4a,4c	kenyae	4F1-4F4
5a,5b	galleriae	4G1-4G6
5a,5c	canadensis	4H1-4H2
6	entomocidus/subtoxicus	4I1-4I5
7	aizawai/pacificus	4J1-4J5
8a,8b	morrisoni	4K1-4K3
8a,8c	ostriniae	4Z1
8b,8d	nigeriensis	4AZ1
9	tolworthi	4L1-4L3
10a,10b	darmstadiensis	4M1-4M3
10a,10c	londrina	4BF1
11a,11b	toumanoffi	4N1
11a,11c	kyushuensis	4U1
12	thompsoni	4O1
13	pakistani	4P1
14	israelensis	4Q1-4Q8
15	dakota	4R1
16	indiana	4S2-4S3
17	tohokuensis	4V1
18a,18b	kumamtoensis	4W1
18a,18c	yosoo	4CA1
19	tochigiensis	4Y1
20a,20b	yunnanensis	4AM1
20a,20c	pondicheriensis	4BA1
21	colmeri	4X1
22	shanongiensis	4AN1
23	japonensis	4AT1
24a,24b	neoleonensis	4BE1
24a,24c	novosibirsk	4AX1
25	coreanensis	4AL1
26	silo	4AG1
27	mexicanensis	4AC1
28a,28b	monterrey	4AJ1
28a,28c	jegathesan	4CF1
29	amagiensis	4AE1
31	toguchini	4AD1
32	cameroun	4AF1
33	leesis	4AK1
34	konkukian	4AH1
35	seoulensis	4AQ1
36	malayensis	4AV1
37	andalousiensis	4AW1
38	oswaldocruzi	4AS1
39	brasilensis	4AY1
40	huazhongensis	4BD1
41	sooncheon	4BB1
42	jinghongiensis	4AR1
43	guiyangiensis	4BC1
44	higo	4AU1
45	roskildiensis	4BG1
46	chanpaisis	4BH1
47	wratislaviensis	4BJ1
48	balearica	4BK1
49	muju	4BL1
50	navarrensis	4BM1
51	xiaguangiensis	4BN1
52	kim	4BP1
53	asturiensis	4BQ1
54	poloniensis	4BR1
55	palmanyolensis	4BS1
56	rongseni	4BT1
57	pirenaica	4BU1
58	argentinensis	4BV1
59	iberica	4BW1
60	pingluonsis	4BX1
61	sylvestriensis	4BY1
62	zhaodongensis	4BZ1
64	azorensis	4CB1
65	pulsiensis	4CC1
66	graciosensis	4CD1
67	vazensis	4CE1
none	wuhanensis	4T1

B. THURINGIENSIS STRAINS BY SUBSPECIES

Serovar	Serotype	BGSC No.
aizawai/pacificus	7	4J1-4J5
alesti	3a,3c	4C1-4C3
amagiensis	29	4AE1
andalousiensis	37	4AW1
argentinensis	58	4BV1
asturiensis	53	4BQ1
azorensis	64	4CB1
balearica	48	4BK1
brasilensis	39	4AY1
cameroun	32	4AF1
canadensis	5a,5c	4H1-4H2
chanpaisis	46	4BH1
colmeri	21	4X1
coreanensis	25	4AL1
dakota	15	4R1
darmstadiensis	10a,10b	4M1-4M3
entomocidus/subtoxicus	6	4I1-4I5
finitimus	2	4B1-4B2
fukuokaensis	3a,3d,3e	4AP1
galleriae	5a,5b	4G1-4G6
graciosensis	66	4CD1
guiyangiensis	43	4BC1
higo	44	4AU1
huazhongensis	40	4BD1
iberica	59	4BW1
indiana	16	4S2-4S3
israelensis	14	4Q1-4Q8
japonensis	23	4AT1
jegathesan	28a,28C	4CF1
jinghongiensis	42	4AR1
kenyae	4a,4c	4F1-4F4
kim	52	4BP1
konkukian	34	4AH1
kumamtoensis	18a,18b	4W1
kurstaki	3a,3b,3c	4D1-4D21
kyushuensis	11a,11c	4U1
leesis	33	4AK1
londrina	10a,10c	4BF1
malayensis	36	4AV1

Serovar	Serotype	BGSC No.
mexicanensis	27	4AC1
monterrey	28a,28b	4AJ1
morrisoni	8a,8b	4K1-4K3
muju	49	4BL1
navarrensis	50	4BM1
neoleonensis	24a,24b	4BE1
nigeriensis	8b,8d	4AZ1
novosibirsk	24a,24c	4AX1
ostriniae	8a,8c	4Z1
oswaldocruzi	38	4AS1
pakistani	13	4P1
palmanyolensis	55	4BS1
pingluonsis	60	4BX1
pirenaica	57	4BU1
poloniensis	54	4BR1
pondicheriensis	20a,20c	4BA1
pulsiensis	65	4CC1
rongseni	56	4BT1
roskildiensis	45	4BG1
seoulensis	35	4AQ1
shanongiensis	22	4AN1
silo	26	4AG1
sooncheon	41	4BB1
sotto/dendrolimus	4a,4b	4E1-4E4
sumiyoshiensis	3a,3d	4AO1
sylvestriensis	61	4BY1
thompsoni	12	4O1
thuringiensis	1	4A1-4A9
tochigiensis	19	4Y1
toguchini	31	4AD1
tohokuensis	17	4V1
tolworthi	9	4L1-4L3
toumanoffi	11a,11b	4N1
vazensis	67	4CE1
wratislaviensis	47	4BJ1
wuhanensis	none	4T1
xiaguangiensis	51	4BN1
yunnanensis	20a,20b	4AM1
zhaodongensis	62	4BZ1

E. COLI CLONES OF *B. THURINGIENSIS CRY* GENES

Indexed by BGSC Accession Number

BGSC No.	Original Code	Reference	Description
ECE52	JM103(pOS4101)	Ge et al. (1989) PNAS 86:4037	*cry1Aa* cloned in pKK223-3; in *E. coli* JM103; ampicillin resistant
ECE53	JM103(pOS4201)	Ge et al. (1989) PNAS 86:4037	*cry1Ac* cloned in pKK223-3; in *E. coli* JM103; ampicillin resistant
ECE54	JM103(pOS4301)	Ge et al. (1989) PNAS 86:4037	*cry1Ab* cloned in pKK223-3; in *E. coli* JM103; ampicillin resistant
ECE125	DH5α(pSB607)	Donor: Dean DH Source: Yamamoto T	*cry1Ca* cloned in pTZ19R; in *E. coli* DH5α; ampicillin resistant
ECE126	DH5α(pSB304.2)	Donor: Dean DH Source: Yamamoto T	*cry2Aa* cloned in pTZ19R; in *E. coli* DH5α; ampicillin resistant
ECE127	pSB1103	Donor: Dean DH Source: Yamamoto T	*cry1Ea* cloned in pTZ19R; in *E. coli* DH5α; ampicillin resistant
ECE128	pSB1407	Donor: Dean DH Source: Yamamoto T	*cry1Ba* cloned in plasmid; ampicillin resistant
ECE129	pSB1507	Donor: Dean DH Source: Yamamoto T	*cry1Da* cloned in plasmid; ampicillin resistant
ECE130	pSB1501	Donor: Dean DH Source: Yamamoto T	*cry9Aa* cloned in pSB1402; in *E. coli* DH5α; ampicillin resistant
ECE131	JM103(pOS4601)	Wu and Dean (1996) J Mol Biol 255:628	*cry3Aa* cloned in pKK223-3; in E. coli JM103; ampicillin resistant

Indexed by Cloned Gene

Gene Name	BGSC No.
cry1Aa	ECE52
cry1Ab	ECE54
cry1Ac	ECE53
cry1Ba	ECE128
cry1Ca	ECE125
cry1Da	ECE129
cry1Ea	ECE127
cry2Aa	ECE126
cry3Aa	ECE131
cry9Aa	ECE130

Note: The right to use these genes for profit may be protected by U.S. and foreign patents. Please read the "Important Notice" on page 4 before ordering or using these clones.

BACILLUS CEREUS STRAINS BY BGSC CODE

BGSC No.	Original Code	Reference	Description
6A1	T	Halvorson HO, source and donor	Wild type isolate
6A2	T-HT	Halvorson HO, source and donor	Wild type isolate
6A3	NRRL-569	Halvorson HO, source and donor	Wild type isolate; host for transducing phages CP51 and CP54
6A4	6A3 StrepR	Dean DH, source and donor	Streptomycin-resistant mutant of NRRL-569
6A5	ATCC 14579	(1980) Int. J. Syst. Bacteriol. 30:256	Wild type isolate. Type strain of *B. cereus.*
6E1	GP7	Bernhard K, et al. (1978) J Bacteriol 133:897	Contains naturally occurring plasmids, pBC15 and pBC16; resistant to tetracycline.
6E2	GP11	Bernhard K, et al. (1978) J Bacteriol 133:897	Contains naturally occurring plasmids, pBC17 and pBC18; resistant to kanamycin.
6S1	T-HW3	Zytkovicz TH, Halvorson HO (1972) Spores V, p 59	*dpa*
6S2	T-HT-8	Halvorson HO, source and donor	*dpa ger*

BACILLUS CEREUS GENOMIC LIBRARY

The *Bacillus* Genetic Stock Center is pleased to announce the availability of a high quality genomic library for *Bacillus cereus*. The library is described in an upcoming publication, still in press at the time this catalog was printed: Rondon, M. R., S. J. Raffel, R. M. Goodman, and J. Handelsman. 1999. Toward functional genomics in bacteria: analysis of gene expression in *Escherichia coli* from a bacterial artificial chromosome library of *Bacillus cereus.* Proc. Natl. Acad. Sci. USA **96:** (in press). We thank these members of the University of Wisconsin Plant Pathology *B. cereus* group for their generosity.

The library was constructed in a bacterial artificial chromosome vector in *E. coli*, a system that offers some distinct advantages over YAC vectors. First, BAC libraries appear to be quite stable. Secondly, many bacterial genes will be expressed in *E. coli*. Indeed, Rondon, *et al.* were able to detect six of ten *B. cereus*-specific activities they screened for among the *E. coli* clones. Thirdly, BAC clones can be manipulated rapidly and easily for electrophoretic analysis, DNA sequencing, and mutagenesis.

The BGSC will distribute copies of the library **at no cost** to serious researchers at academic, government, and other non-profit organizations. Interested parties at for-profit companies are encouraged to contact the BGSC to discuss distribution options. As individual clones are better characterized, they will be made available separately as well.

Description of Library

DNA source:	*Bacillus cereus* UW85
E. coli host strain:	DH10B
Cloning vector:	pBeloBAC11 (see Shizuya, *et al.*1992. Proc. Natl Acad Sci USA 89:8794; Kim, *et al.* 1996. Genomics 34:213)
Number of clones:	323
Insert size range:	40 kb - >175 kb
Average insert:	98 kb
Genome coverage:	5.75 fold
P that a given 1 kb gene is included:	~99.7%

BACTERIOPHAGES OF *B. CEREUS* & *B. THURINGIENSIS*

Indexed by BGSC Accession No.

BGSC No.	Original Code	Reference	Description
1P25	Bace-11	Ackermann HW, DuBow MS (1987) in: Viruses of Prokaryotes--II. Natural Groups of Bacteriophages. CRC Press, Boca Raton, FL	
1P26	Bam35-c	Ackermann HW, *et al.* (1987) Can J Microbiol 24:986	Phage with icosahedral head, a double capsid, and spikes at vertices; chloroform sensitive
1P27	Bam35-v	Ackermann HW, *et al.* (1987) Can J Microbiol 24:986	Phage with icosahedral head, a double capsid, and spikes at vertices; chloroform sensitive
1P28	TP-15	Ackermann HW, *et al.* (1994) Arch Virol 135:333; Reynolds *et al.* 1988. J Gen Microbiol 134:1577	Small temperate phage with 51 nm head, 184 nm tail; genome 33-35 kb
1P29	B1715V1	Ackermann HW, *et al.*(1995) Res Microbiol 146:643; Ackermann HW, *et al.* (1994) Arch Virol 135:333	Phage with long tail, single fiber; forms clear or veiled plaques of 0.5 mm; genome size 78 kb
1P30	Bastille	Ackermann HW, *et al.*(1995) Res Microbiol 146:643	Phage with capsomers, double base plate; forms clear, irregular plaques of 0.5-1.5 mm
1P31	Bat 1	Ackermann HW, DuBow MS (1987) in: Viruses of Prokaryotes--II. Natural Groups of Bacteriophages. CRC Press, Boca Raton, FL	
1P32	Bat 5	Ackermann HW, DuBow MS (1987) in: Viruses of Prokaryotes--II. Natural Groups of Bacteriophages. CRC Press, Boca Raton, FL	
1P33	Bat 7	Ackermann HW, DuBow MS (1987) in: Viruses of Prokaryotes--II. Natural Groups of Bacteriophages. CRC Press, Boca Raton, FL	
1P34	Bat 10	Ackermann HW, *et al.*(1995) Res Microbiol 146:643	Phage with elongated head; forms clear expanding plaques 0.1-0.5 mm
1P35	Bat 11	Ackermann HW, DuBow MS (1987) in: Viruses of Prokaryotes--II. Natural Groups of Bacteriophages. CRC Press, Boca Raton, FL	
1P36	Bat 18	Ackermann HW, DuBow MS (1987) in: Viruses of Prokaryotes--II. Natural Groups of Bacteriophages. CRC Press, Boca Raton, FL	
1P37	CP-54Ber	Lecadet M-M, *et al.* (1980) J Gen Microbiol 121:203	Transducing phage with hexagonal icosahedral head; tail composed of core with contractile sheath, base plate with peripheral knobs; cold labile; stability improved by 10% DMSO, 2 mM $MgSO_4$
1P38	DP7	Ackermann HW, *et al.*(1995) Res Microbiol 146:643	Phage with capsomers, double base plate; forms clear, irregular plaques of 0.5-2.5 mm
1P39	GP-10	Ackermann HW, *et al.*(1995) Res Microbiol 146:643	Phage with elongated head; forms clear, often overgrown plaques of 1.0-1.5 mm
1P40	mor1	De Barjac, H., *et al.* (1974) CRS Acad Sci Ser D 279:1939	
1P41	PK1	Ackermann HW, *et al.*(1995) Res Microbiol 146:643	Phage with capsomers, double base plate; forms clear plaques of 0.5-1.0 mm, frequently with halo
1P42	P400	Ackermann HW, DuBow MS (1987) in: Viruses of Prokaryotes--II. Natural Groups of Bacteriophages. CRC Press, Boca Raton, FL	
1P43	Tb10	Ackermann HW, *et al.* (1994) Arch Virol 135:333; Ackermann HW, *et al.*(1995) Res Microbiol 146:643	Phage with transverse tail disks; forms clear, expanding plaques of 1-2 mm; genome size 42.5 kb

BACTERIOPHAGES BY ORIGINAL CODE

Original Code	BGSC No.
B1715V1	1P29
Bace-11	1P25
Bam35-c	1P26
Bam35-v	1P27
Bastille	1P30
Bat 1	1P31
Bat 10	1P34
Bat 11	1P35
Bat 18	1P36
Bat 5	1P32
Bat 7	1P33
CP-54Ber	1P37
DP7	1P38
GP-10	1P39
mor1	1P40
P400	1P42
PK1	1P41
Tb10	1P43
TP-15	1P28

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

MEDIA FOR GROWTH AND SPORULATION

2×SG Medium

Leighton, T. J. and R. H. Doi. 1971. J. Biol. Chem. **246:**3189-3195.

Although most strains of *B. thuringiensis* or *B. cereus* grow reasonably well in familiar media such as LB or Nutrient broth, it is often possible to obtain higher growth densities and sporulation frequencies with more specialized media. We have used a *B. subtilis* sporulation medium with very good results.

Per liter of distilled water:

Difco Nutrient broth	16.0 g
KCl	2.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Agar (if desired)	17.0 g

Adjust the pH to 7.0 with addition of 1 M NaOH. Autoclave. Cool to 55˚C and add:

1 M $Ca(NO_3)_2$	1.0 ml
0.1 M $MnCl_2 \cdot H_2O$	1.0 ml
1 mM $FeSO_4$	1.0 ml
50% (w/v) glucose, filter sterilized	2.0 ml

HCO Medium

Lecadet, M.-M., M. O. Blondel, J. Ribier. 1980. J. Gen. Microbiol. 121:203-12.

A semi-defined medium for growth and sporulation in *B. thuringiensis berliner* and many other strains.

Per liter of distilled water:

Casamino acids	7.0 g
KH_2PO_4	6.8 g
$MgSO_4 \cdot 7H_2O$	0.12 g
Agar	17 g

Adjust the pH to 7.2. Autoclave. Cool to 55˚C and add from sterile stocks to a final concentration of:

$MnSO_4 \cdot 4H_2O$	0.0022 g
$ZnSO_4 \cdot 7H_2O$	0.014 g
$Fe_2(SO_4)_3$	0.02 g
$CaCl_2 \cdot 4H_2O$	0.018 g
Glucose	3 g

BP Medium

A complete medium for growth and sporulation. Substitute 7.0 g of Bactopeptone (Difco) for the casamino acids in HCO medium.

MA18 Medium

A minimal medium for growth and sporulation. Replace the casamino acids in CHO with a mixture of 18 amino acids, each to a final concentration of 20 ˚g/ml: alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, tyrosine, threonine, tryptophan, serine, and valine.

NBY Medium

Thorne C. B. 1968. J. Virol. 2:657-662.

Per liter of distilled water:

Nutrient broth	8 g
Yeast extract	3 g
Agar (if desired)	15 g

NBYS Medium

Lecadet, M.-M., M. O. Blondel, J. Ribier. 1980. J. Gen. Microbiol. 121:203-12.

A complete medium for *B. thuringiensis berliner* and many other strains; useful for phage propagation.

Per liter of distilled water:

Nutrient broth	8 g
Yeast extract	3 g
KCl	0.4 g
Agar (if desired)	15 g

Autoclave. Cool to 55˚C and add from sterile stock solutions:

1.2% $MgSO_4 \cdot 7H_2O$	1 ml
0.1% $MnSO_4 \cdot 4H_2O$	1 ml
0.014% $FeSO_4 \cdot 7H_2O$	1 ml
7.5% $CaCl_2 \cdot 2H_2O$	1 ml

PA Medium

Thorne C. B. 1968. J. Virol. 2:657-662.

For assaying phage CP-54Ber and related phages. Gives 30% higher efficiency of plating that NBY.

Per liter of distilled water:

Nutrient broth	8 g
NaCl	5 g
$MgSO_4 \cdot 7H_2O$	0.2 g
Agar (if desired)	15 g

Adjust pH to 5.9. Autoclave. Cool to 55˚C and add from sterile stock solutions:

5% $MnSO_4 \cdot 4H_2O$	1 ml
15% $CaCl_2 \cdot 2H_2O$	1 ml

G-Tris Medium

Aronson, A. I., *et al.* 1971. J. Bacteriol. 106:1016-1025.

G-Tris is a general purpose growth and sporulation medium for *B. cereus* and *B. thuringiensis.*

The following stocks are made up and sterilized in concentrated form, then mixed with sterile distilled water to the following **final concentrations**:

Tris-HCl (pH 7.6)	0.01	M
$FeSO_4 \cdot 7H_2O$	0.00005	%
$CuSO_4 \cdot 5H_2O$	0.0005	%
$ZnSO_4 \cdot 7H_2O$	0.0005	%
$MnSO_4 \cdot H_2O$	0.005	%
$MgSO_4$	0.02	%
$CaCl_2 \cdot 2H_2O$	0.008	%
K_2HPO_4	0.05	%
$(NH_4)_2\ SO_4$	0.2	%
glucose, filter sterilized	0.1	%

CDGS Medium

Nakata, H. M. (1964) J. Bacteriol. 88:1522.

CDGS is a chemically defined growth and sporulation medium developed for *B. cereus* T. It is also useful for many *B. thuringiensis* strains, although the partial auxotrophic requirements can vary from strain to strain.

For CDGS, use the recipe for G-Tris with the following modifications:

In the place of Tris-HCl (pH 7.6), use 0.1 M potassium phosphate buffer, pH 6.4.
In the place of Yeast extract, use the following amino acid mixture to the following final concentrations:

L-glutamic acid	1.84	µg/ml
L-leucine	0.80	µg/ml
L-valine	0.30	µg/ml
L-threonine	0.168	µg/ml
L-methionine	0.07	µg/ml
L-histidine	0.05	µg/ml

ELECTROPORATION OF *B. THURINGIENSIS* AND *B. CEREUS*

A major advance in the genetics of *Bacillus cereus* and *B. thuringiensis* came in 1989 when several labs independently applied electroporation technology to transform vegetative cells with plasmid DNA (Belliveau and Trevors; Bone and Ellar; Lereclus *et al.*; Mahillon *et al.*; Masson *et al.*; Schurter *et al.*). By comparison, previous transformation techniques were much slower, more labor intensive, and less efficient. The protocols developed at this early stage varied in the cell preparation methods, electroporation buffer components, and electric pulse parameters (see the accompanying table), but each could achieve frequencies of 10^2-10^5 transformants per microgram of plasmid DNA with a wide variety of hosts and vectors.

Macaluso and Mettus (1991) added the important observation that some *B. thuringiensis* strains restrict methylated DNA. Plasmids isolated from *B. megaterium* or Dcm^- strains of *E. coli* transformed *B. thuringiensis* with much higher frequencies than did DNA isolated from *B. subtilis* or Dcm^+ strains of *E. coli*. Their data also provided evidence that several restriction systems exist within *B. thuringiensis*. The use of unmethylated DNA with the Macaluso and Mettus protocol allows transformation frequencies as high as 3×10^6 /μg of DNA to be achieved (see Protocol 1).

Belliveau, B. H. and J. T. Trevors. 1989. Transformation of *B. cereus* vegetative cells by electroporation. Appl. Environ. Microbiol. **55:**1649-1652.

Bone, E. J. and D. J. Ellar. 1989. Transformation of *Bacillus thuringiensis* by electroporation. FEMS Letts. **58:**171-178.

Lereclus, D., O. Arantès, J. Chaufaux, and M.-M. Lecadet. 1989. Transformation and expression of a cloned δ-endotoxin gene in *Bacillus thuringiensis.* FEMS Letts. **60:**211-218.

Macaluso, A. and A. M. Mettus. 1991. Efficient transformation of *Bacillus thuringiensis* requires nonmethylated plasmid DNA. J. Bacteriol. **173:**1353-1356.

Mahillon, J., W. Chungjatupornchai, J. Decock, S. Dierickx, F. Michiels, M. Peferoen, and H. Joos. 1989. Transformation of *Bacillus thuringiensis* by electroporation. FEMS Letts. **60:**205-210.

Masson, L., G. Préfontaine, and R. Brousseau. 1989. Transformation of *Bacillus thuringiensis* vegetative cells by electroporation. FEMS Letts. **60:**273.

Schurter, W., M. Geiser and D. Mathé. 1989. Efficient transformation of *Bacillus thuringiensis* and *B. cereus* via electroporation: transformation of acrystalliferous strains with a cloned delta-endotoxin gene. Mol. Gen. Genet. **218:**177-181.

Electroporation Protocol

Adapted from:

Macaluso and Mettus. 1991. J Bacteriol 173:1353-1356.

Materials:

culture of *B. thuringiensis* host on solid medium
plasmid DNA, prepared from *B. megaterium* or *E. coli dcm*$^-$ host
100 ml stock of BHIG (Brain Heart Infusion + 0.5% glycerol), sterile
10 ml of BHIG in a 250 ml flask, sterile
95 ml of BHIG in a 1 or 2 liter flask, sterile
150 ml of EB (0.625 M sucrose, 1 mM $MgCl_2$), sterile and chilled on ice
two sterile centrifuge bottles
sterile cryovials for storage of electro-competent cells, if desired
0.4 mM electroporation cuvettes (BioRad)
BioRad GenePulser® with Pulse Controller®
sterile test tubes
selective agar plates (LB agar plus appropriate antibiotic)

Protocol:

Day 1

1. Inoculate 10 ml of BHIG in a 250 ml flask with a single *Bt* colony.
2. Incubate at 30°C with moderate shaking overnight.

Day 2

Cell Preparation

1. Dilute 5 ml of overnight culture into 95 ml BHIG in 1 or 2 liter flask.
2. Incubate 1 hr at 30°C with vigorous shaking.
3. Pellet cells by centrifugation (at an appropriate speed for your machine)
4. Suspend in 100 ml cold EB. *Note: Be careful to keep cells cold from this point forward until the electric pulse has been delivered during transformation.*
5. Repeat centrifugation.
6. Suspend in 50 ml cold EB.
7. Store 2 ml aliquots of the cell suspension in cryovials and store in -70°C for later use, if desired.

Transformation

1. If using frozen cells, thaw them on ice. Transfer 0.8 ml aliquot of cell suspension to a 0.4 cm cuvette; mix in up to 10 µl DNA.
2. Chill on ice 5 minutes.
3. Apply a single pulse at a setting of 2.5 kV, 25 µF, 5 Ω.
4. Dilute cell suspension with 1.6 ml BHIG.
5. Incubate at 30°C with moderate shaking for 1 hr.
6. Plate dilutions on selective agar plate and incubate overnight.

Comparison of published methods for electroporation of *B. cereus* and *B. thuringiensis*

Step	Belliveau *et al.*	Bone and Ellar	Lereclus *et al.*	Macaluso *et al.*	Mahillon *et al.*	Masson *et al.*	Schurter *et al.*
Starter culture	10 ml LB, 16 hr, 37°C, 100 rpm	Streak cells on LB agar; inc. ON at R.T.		BHIG (BHI + 0.5% glycerol), OH, 30°C, with shaking	20 ml 2×LB, ON, 37°C, 180 rpm		10 ml LB, ON, 27°C, 50 rpm
Culture	2 ml inoculum into 10 ml LB; 2.5 hr, 37°C, 100 rpm, final OD_{600} = 1.0	Inoculate LB from plate; 30°C, 200 rpm, final OD_{600} = 0.5	1 liter BHI, 37°C, with shaking, final OD_{600} = 2.0	dilute 1:20 in BHIG; 1 hr, 30°C, with shaking	Inoculate 4 ml into 400 ml pre-warmed 2×LB; 3-4 hr, 37°C, 180 rpm	1 liter NB, final OD_{600} = 0.5	dilute 1:100 into LB; 30°C, 250 rpm, final OD_{550} = 0.2
Washes	pellet, 1 ml ice cold EB repeat repeat	pellet, cold H_2O repeat pellet, cold EB	none	pellet, EB	pellet, 200 ml H_2O pellet, 30 ml H_2O	pellet, EB; 4 cycles	pellet, 1/40 volume EB
EB composition	10 mM HEPES, pH 7.0	1 mM HEPES, pH 7.0; 10% glycerol	40% PEG 6000 (w/v)	0.625 M sucrose, 1 mM $MgCl_2$	30% PEG 1000 (w/v)	10% glycerol	400 mM sucrose, 1 mM $MgCl_2$, 7 mM phosphate buffer, pH 6.0
Final Suspension	pellet, 4 ml ice cold EB	pellet, cold EB to 10^9-10^{10} CFU/ml	pellet, 10 ml cold EB	pellet, 0.5 vol EB	pellet, EB to 5 ml/g	pellet, 2 ml EB (to 10^{10} CFU/ml)	pellet, 1/40 volume EB
Mixture with DNA	0.8 ml cells + 30 µl DNA (0.5 µg); chill 5 min	0.1-0.2 ml cells + 1-5 µl DNA (0.1-0.5 µg); chill 1 min	0.2 ml + 1 µg DNA in TE; chill	0.8 ml + <10 µl DNA; chill on ice 5 min	0.1 ml cells + DNA	40 µl cells + DNA	0.8 ml cells + DNA; incubate 4°C 10 min
Cuvette	0.4 cm	0.4 cm	0.2 cm	0.4 cm	0.4 cm (?)	0.2 cm	0.4 cm (?)
Pulse	1.5 kV, 3 µF (no pulse controller used)	2.5 kV, 25 µF, 200 Ω	2.5 kV, 25 µF, 1000 Ω	1.3 kV, 25 µF, 5 Ω	1.4 kV, 25 µF, 400 Ω	0.5 kV, 25 µF (no pulse controller)	1.3 kV, 25 µF (no pulse controller)
Dilution	into 7.2 ml LB	immediately with 1 ml LB	into 2 ml BHI	on ice 5 min; dilute into 1.6 ml BHIG	with 1.9 ml LB	with 1 ml SOC	with 1.2 ml LB
Recovery/Expression	1 hr, 37°C, 60 rpm	1 hr, 30°C	1 hr, 37°C, shaking	1 hr, 30°C, shaking	90 min, 37°C, shaking	1 hr, 37°C, shaking	1 hr, 30°C, 250 rpm
Recipients	*B. cereus* 569	*Bt* serovars *morissoni, aizawai, kurstaki, israelensis*	5 of 7 strains transformable	9 *Bt* strains	17 of 21 *Bt* strains transformable	*Bt kurstaki*	"Bt" HD1 cryB
Frequencies	10^2-10^4 /µg	10^4-10^5 /µg	10^2-10^5 /µg	up to 3×10^6 /µg	10^2-10^5 /µg depending on strain, plasmid	$3\text{-}4\times10^5$ /µg	10^6-10^7 /µg
Notes		Co-transformation of pC194, pUB110 at 10^2		Plasmids from *B. megaterium* or Dcm- *E. coli* more efficient than from Dcm+ or *B. subtilis* At least 4 classes of *Bt* restriction systems	used pC194, pE194, and specialized vectors		frequency curve linear over 5ng-5µg DNA; recipient may not actually have been derived from HD1

GENERALIZED TRANSDUCTION WITH CP-54BER

Adapted from: Lecadet, M.-M., M. O. Blondel, J. Ribier. 1980. J. Gen. Microbiol. 121:203-12.

Assaying Phage CP54-Ber

1. Prepare spores in one of the complete growth and sporulation media described in the previous pages (2×SG, NBYS, BP). Wash them in sterile distilled water, store them at 4°C for 3-4 days, and wash them again. Heat to 65°C for 15 min. Cool. Store at 4°C.
2. Mix 0.1 ml of a suitable phage dilution with 3 ml of soft PA agar (0.5% agar) and 10^8 spores.
3. Pour the soft agar, spore, and phage mixture on PA agar (1.5% agar).
4. Incubate at 35°C overnight. Plaques are turbid on *B. thuringiensis berliner* 1715, clear on *B. cereus* 6A3 (NRRL-569).

Propagating Phage CP54-Ber

1. Mix 0.1 ml of phage dilution with 3 ml of soft NBY agar (0.5% agar) and 10^8 spores.
2. Pour the soft agar, spore, and phage mixture on NBY agar (1.5% agar).
3. Incubate at 35°C overnight.
4. Flood plate with 3 ml NBY broth. Take up broth and soft agar layer in a 10 ml pipet.
5. Pellet agar, cells by low speed centrifugation (5,000 × *g*)
6. Filter the lysate through 0.8 micron Millipore filter.

Transduction with Phage CP54-Ber

1. Inoculate a single colony of the recipient strain into 10 ml NBYS and incubate at 30˜C with shaking for 5-6 hours ($OD_{650} \approx 3.5$).
2. Centrifuge at 5,000 × *g* for 15 min to pellet cells.
3. Suspend cells in original volume of fresh broth. Cell titer will be $\approx 4 \times 10^8$ cfu/ml.
4. Mix 0.8 ml of recipient culture with 0.1-0.2 ml of phage lysate, diluted to give a multiplicity of infection of ≥ 2.
5. Incubate at 35°C for 30 min with mild shaking
6. Plate 0.1 ml aliquots on selective media. Select for antibiotic resistance or base or vitamin prototrophy on HCO; select for amino acid prototrophy on MP18 with the appropriate amino acid omitted.

CONJUGATION-LIKE MATING WITH *B. THURINGIENSIS*

Many strains of *B. thuringiensis* are able to transfer plasmids to other *B. thuringiensis* or *B. cereus* strains by a conjugation-like process first reported by González and Carlton (5). At least eight self-transmissable plasmids have since been identified in *B. thuringiensis* (table 1). A number of other uncharacterized conjugation systems have been detected as well (6).

The best studied conjugative plasmid is pXO16, the 200 kb plasmid of *B. thuringiensis* subsp. *israelensis.* In contrast to the mating systems in other bacterial species, pXO16-mediated conjugation does not involve pili and is not induced by pheromones. Instead, mixtures of donor and recipient strains form large clumps (1). The clumping mechanism is protease sensitive, and there is some evidence that a specific S-Layer protein found in donor strains may be required for plasmid transfer (1, 9). Scanning electron micrographs show direct connections between cells in mating cultures but not in monocultures of donor or recipient strains (2). Plasmid transfer can be detected within 3.5-4 min after mixing donor and recipient cultures. Transfer of DNA is rapid, on the order of 1 kb per second. Recipients can become donors after 40 minutes, probably the time required for a mating protein to be incorporated into the S-Layer. Virtually every cell in the donor population is potent, and when equal numbers of donors and recipients are mixed, virtually every recipient receives pXO16 (3). A variety of plasmids, including the *B. cereus* plasmid pBC16, the *Staphylococcus aureus* plasmids pC194 and pE194, as well as cryptic *B. thuringiensis* plasmids, can be mobilized at plasmid-dependent frequencies in the range of 10^{-2}-10^{-5} transconjugants per donor cell (2). Not every *B. thuringiensis* strain can serve as a recipient in pXO16-mediated mating. Among BGSC strains, 4D4, 4H2, 4K1, 4N1, 4O1, 4P1, 4T1, 4U1, 4Q7, and 6A5 are known to be recipients. BGSC strains 4B2, 4C3, 4E4, 4G5, 4I4, 4J4, 4L3, 4R1, 4S2, and 4Y1 are known not to be recipients (6).

Protocol--Mating *Bacillus thuringiensis* subsp. *israelensis* with a recipient (Agr$^-$) strain

1. Grow donor and recipients separately at 30°C in LB containing appropriate antibiotics.
2. Dilute each culture 1:100 into fresh, prewarmed LB and continue incubation.
3. At late log phase ($OD_{600} \approx 0.5$-1.0), add 250 µl per OD_{600} unit of each culture to a prewarmed 7 ml aliquot of LB in a small flask or large test tube.
4. Incubate at 30°C with moderate shaking (180 rpm) for 3 hr.
5. Plate on appropriate agar to select for transconjugants.

Table 1. Conjugative plasmids of *Bacillus thuringiensis*

Plasmid	Host Strain	Description	Recipients	Ref.
pAW63	*Bt kurstaki* HD73 (BGSC 4D4)	Contains no *cry* genes. Transfer nearly 100% efficient in mating experiments. Mobilized plasmids must have functional *mob* (*pre*) gene	*Bti*, *B. cereus*, *B. subtilis*, *B. licheniformis*, *B. sphaericus*	8
pHT73	*Bt kurstaki* HD73 (BGSC 4D4)	Similar to pAW63, but bears a *cry* gene. Less efficient than pAW63 at mobilizing pBC16		8
pXO11	*Bt thuringiensis* 4042A	Does not bear *cry* gene	*B. thuringiensis*, *B. cereus*, *B. anthracis* strains	4
pXO12	*Bt thuringiensis* 4042A	Bears *cry* gene	*B. thuringiensis*, *B. cereus*, *B. anthracis* strains	4
pXO13	*Bt morrisoni* 4049	Does not bear *cry* gene	*B. thuringiensis*, *B. cereus*, *B. anthracis* strains	7
pXO14	*Bt alesti*YAL	Does not bear *cry* gene	*B. thuringiensis*, *B. cereus*, *B. anthracis* strains	7
pXO15	*Bt morrisoni* 4049	Does not bear *cry* gene	*B. thuringiensis*, *B. cereus*, *B. anthracis* strains	7
pXO16	*Bt morrisoni* 4049	Transfer nearly 100% efficient in mating experiments. Able to mobilize theta-form plasmids and rolling circle plasmids lacking the *mob* or *pre* gene.	*B. cereus*, *B. anthracis* strains; *B. thuringiensis* strains with Agr$^-$ phenotype	6, 7

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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

Table 2. Sequences of the PCR primers in Table 1.

Primer	Sequence (5'→3')	Ref
05091/2	GCCGTCTAGAGGATCCTTGTGTTGAGATA	8
13091/1	GCCGGAATTCAAGCTTATGAAACTAAAGAATCCAGA	8
7/8(-)	YYTCTAAWYCYTGACTACTT	11
7/8(+)	YCRDTTYCGYAGAGARATGA	11
CJ1	TTATACTTGGTTCAGGCCC	6
CJ10	AAAGATCTGGAACACCTTT	6
CJ11	CAAACTCTAAATCCTTTCAC	6
CJ12	CTGCAGCAAGCTATCCAA	6
CJ13	ATTTGAATTGTCAAGGCCTG	6
CJ14	GGAACCAAGACGAACTATTGC	7
CJ15	GGTTGAATGAACCCTACTCCC	7
CJ16	TGAGGATTCTCCAGTTTCTGC	7
CJ17	CGGTTACCAGCCGTATTTCG	7
CJ18	ATATGGAGTGAATAGGGCG	7
CJ19	TGAACGGCGATTACATGC	7
CJ2	TTGGAGCTCTCAAGGTGTAA	6
CJ3	CAGCCGATTTACCTTCTA	6
CJ4	AACAACTATCTGTTCTTGAC	6
CJ5	CTCTTATTATACTTACACTAC	6
CJ6	GTTAGATTAAATAGTAGTGG	6
CJ7	TGTAGCTGGTACTGTATTG	6
CJ8	CTTCATCACGATGGAGTAA	6
CJ9	CATAATTTGGTCGTTCTGTT	6
CJI-1	TGTAGAAGAGGAAGTCTATCCA	7
CJI-2	TATCGGTTTCTGGGAAGTA	7
CJIII20	TTAACCGTTTTCGCAGAGA	7
CJIII21	TCCGCACTTCTATGTGTCCAAG	7
CJIIIA23	CCCCGTCTAAACTGAGTGT	7
CJIIIB24	AACGAAAGATTCTGCTCC	7
CJIIIC25	CCTATTCTTTCATTTTGACC	7
CJIIICg26	AGTGGAGAGTTTACGGTAGCC	7
CJIIIcte 22	CAATCCCAGTGTTTACTTGGAC	7
CJIIID27	CGAAATACGAAATACTATGAG	7
CJIIIE28	TGACAAGTACTGGATTCTGCAA	7
CJIIIE29	GTTGTTGATGAGGTTCCCCTT	7
CoI1A	GTCCGCTGTATATTCAGGTG	5
CoI1B	CACTTAATCCTGTGACGCCT	5
CoI2A	AGGTGCCAACTAACCATGTT	5
CoI2B	GATCCTATGCTTGGTCTAGT	5
CR3c	ATAAGCCCAATATCATG	1
CR8	AAGTAAAGATTTCTGGG	1
DA5c	TCAAAAGGTGTGGCAAG	1
Dip1A	CAAGCCGCAAATCTTGTGGA	5
Dip1B	ATGGCTTGTTTCGCTACATC	5
Dip2A	GGTGCTTCCTATTCTTTGGC	5
Dip2B	TGACCAGGTCCCTTGATTAC	5
EE-11A(d)	CCGAACCTACTATTGCGCCA	2
EE-11A(r)	CTCCCTGCTAGGATTCCGTC	2
EE-2Aa(r)	GAGATTAGTCGCCCCTATGAG	2
EE-2Ab(r)	TGGCGTTAACAATGGGGGGAGAAAT	2
EE-2Ac(r)	GCGTTGCTAATAGTCCCAACAACA	2
EE-3Aa(r)	TGGTGCCCCGTCTAAACTGAGTGT	2
EE-3Ba(r)	ACGAAAGATTCTGCTCCTAT	2
EE-3C(r)	ATTTTGGTACCTCCTGTACCCACC	2
EE-4A(d)	GGGTATGGCACTCAACCCCACTT	2
EE-4B(d)	GAGAACACACCTAATCAACCAACT	2
EE-7Aa(d)	GCGGAGTATTACAATAGAATCTATCC	2
EE-8A(d)	GAATTTACTCTATACCTTGGCGAC	2
EE-8B(d)	GACCGCATCGGAAGTTGTGAG	2
EE-8C(d)	GGTGCTGCTAACCTTTATATTGATAG	2
gral-cry1(d)	CTGGATTTACAGGTGGGGATAT	4
gral-cry1(r)	TGAGTCGCTTCGCATATTTGACT	4
gral-cry11(d)	TTAGAAGATACGCCAGATCAAGC	4

Primer	Sequence (5'→3')	Ref
gral-cry11(r)	CATTTGTACTTGAAGTTGTAATCCC	4
gral-cry13(d)	CTTTGATTATTTAGGTTTAGTTCAA	4
gral-cry13(r)	TTGTAGTACAGGCTTGTGATTC	4
gral-cry8(d)	ATGAGTCCAAATAATCTAAATG	4
gral-cry8(r)	TTTCATTAATGAGTTCTTCCACTCG	4
gral-cyt(d)	AACCCCTCAATCAACAGCAAGG	4
gral-cyt(r)	GGTACACAATACATAACGCCACC	4
gral-nem(d)	TTACGTAAATTGGTCAATCAAGCAAA	4
gral-nem(r)	AAGACCAAATTCAATACCAGGGTT	4
I(-)	MDATYTCTAKRTCTTGACTA	9
I(+)	TRACRHTDDBDGTATTAGAT	9
IAa	TTCCCTTTATTTGGGAATGC	9
IAb	CGGATGCTCATAGAGGAGAA	9
IAc	GGAAADTTTCTTTTTAATGG	9
IAd	ACCCGTACTGATCTCAACTA	9
IAe	CTCTACTTTTTATAGAAACC	11
IA's	CAATAGTCGTTATAATGATT	9
II(-)	AACTCCATCGTTATTTGTAG	11
II(+)	TAAAGAAAGTGGGGAGTCTT	11
IIA	TCTCATAGGGGCGACTAATC	11
IIB	TGATATAGGTGCATCTCCGT	11
III(-)	AASTKAGWKGTWGAAGCATA	11
III(+)	AAACHGAAYTAACAAGAGAC	11
K3un2	GCTGTGACACGAAGGATATAGCCAC	10
K3un3	CCTCCTGTAAATCCTGGTCCT	10
K5un2	AGAACCAGGATTTACAGGAGG	10
K5un3	CAATGCGTACCTTACAATTGTTTAAGTAAT	10
Lep1A	CCGGTGCTGGATTTGTGTTA	5
Lep1B	AATCCCGTATTGTACCAGCG	5
Lep2A	CCGAGAAAGTCAAACATGCG	5
Lep2B	TACATGCCCTTTCACGTTCC	5
OX7as	CTGAGGTATTTTGTGGA	1
RB-19	GGGACTGCAGGAGTGAT	3
SB-1	TGCATAGAGGCTTTAAT	3
SB-2	TCGGAAAATGTGCCCAT	3
spe-cry8A(d)	ATGAGTCCAAATAATCTAAATG	4
spe-cry8A(r)	TCTCCCCATATATCTACGCTC	4
spe-cry8B(d)	ATGAGTCCAAATAATCTAAATG	4
spe-cry8B(r)	GAACATCTCGTAAGGCTC	4
spe-cry8C(d)	ATGAGTCCAAATAATCTAAATG	4
spe-cry8C(r)	GGTACTCGATTGTCCAGT	4
spe-cry9A(d)	ATGAGTCCAAATAATCTAAATG	4
spe-cry9A(r)	TCTCCCCATATATCTACGCTC	4
spe-cry9B(d)	TCATTGGTATAAGAGTTGGTGATAGAC	4
spe-cry9B(r)	CCGCTTCCAATAACATCTTTT	4
spe-cry9C(d)	CTGGTCCGTTCAATCC	4
spe-cry9C(r)	CCGCTTCCAATAACATCTTTT	4
U3-18c	AATTGCTTTCATAGGCT	3
U8-15c	CAGGATTCCATTCAAGG	3
Un1(d)	CATGATTCATGCGGCAGATAAAC	2
Un1(r)	TTGTGACACTTCTGCTTCCCATT	2
Un2(d)	GTTATTCTTAATGCAGATGAATGGG	2
Un2(r)	CGGATAAAATAATCTGGGAAATAGT	2
Un3(d)	CGTTATCGCAGAGAGATGACATTAAC	2
Un3(r)	CATCTGTTGTTTCTGGAGGCAAT	2
Un4(d)	GCATATGATGTAGCGAAACAAGCC	2
Un4(r)	GCGTGACATACCCATTTCCAGGTCC	2
Un7,8(d)	AAGCAGTGAATGCCTTGTTTAC	2
Un7,8(r)	CTTCTAAACCTTGACTACTT	2
V(-)	AGGATCCTTGTGTTGAGATA	11
V(+)	ATGAAACTAAAGAATCCAGA	11
VI(-)	TRAATYCTATTRAACAATCCTA	11
VI(+)	TAYGGTTTTAAAKKTGCTGG	11

Note: For degenerate primers, B=C+G+ T; D=A+G+T; H=A+C+T; K=G+T; M=A+C; R=A+G; Y=T+C

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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

NAME	ORIGINAL	ACCESSION NUMBER(S)	CODING REGION	REF.
Cry1Bd1	CryE1	U70726	842 ... 4534	12
Cry1Be1		AF077326, I90319, I90731	1 ... 3681	80
Cry1Ca1	CryIC	X07518	47 ... 3613	46
Cry1Ca2	CryIC	X13620, A10218	241 ... >2711	97
Cry1Ca3	CryIC	M73251, I76416, I76777	1 ... 3570	88
Cry1Ca4	CryIC	A27642	234 ... 3800	122
Cry1Ca5	CryIC	X96682	1 ... >2268	115
Cry1Ca6	CryIC	X96683	1 ... >2268	115
Cry1Ca7	CryIC	X96684	1 ... >2268	115
Cry1Cb1	CryIC(b)	M97880, I83311	296 ... 3823	49
Cry1Da1	CryID	X54160, A15537, A27640	264 ... 3758	45
Cry1Da2		I76776, I76415	1 ... 3495	82
Cry1Db1	prtB	Z22511	241 ... 3720	57
Cry1Ea1	CryIE	X53985	130 ... 3642	123
Cry1Ea2	CryIE	X56144	1 ... 3513	8
Cry1Ea3	CryIE	M73252, I15489, I15490, I21415, I21416	1 ... 3513	91
Cry1Ea4		U94323	388 ... 3900	48
Cry1Ea5		A15535	54 ... 3566	9
Cry1Eb1	CryIE(b)	M73253, A27529, I73894	1 ... 3522	90
Cry1Fa1	CryIF	M63897, I76417	478 ... 3999	14
Cry1Fa2	CryIF	M73254, I76778	1 ... 3525	89
Cry1Fb1	prtD	Z22512	483 ... 4004	57
Cry1Fb2	CryINA67-1	AB012288	1 ... 3780	67
Cry1Fb3		AF062350		114
Cry1Fb4		I73895		83
Cry1Ga1	prtA	Z22510	67 ... 3564	57
Cry1Ga2	CryIM	Y09326	692 ... 4210	104
Cry1Gb1	CryH2	U70725	532 ... 4038	12
Cry1Ha1	prtC	Z22513	530 ... 4045	57
Cry1Hb1		U35780	728 ... 4195	55
Cry1Ia1	CryV	X62821	355 ... 2511	117
Cry1Ia2	CryV	M98544	1 ... 2157	36
Cry1Ia3	CryV	L36338	279 ... 2435	108
Cry1Ia4	CryV	L49391	61 ... 2217	56
Cry1Ia5	CryV159	Y08920	524 ... 2680	102
Cry1Ia6	CryV101	AF076953	1 ... 2160	137
Cry1Ib1	CryV465	U07642	237 ... 2393	108
Cry1Ic1		AF056933	1 ... 2160	79
Cry1Ja1	ET4	L32019, I38759, I70137	99 ... 3519	26
Cry1Jb1	ET1	U31527	177 ... 3686	124
Cry1Jc		I90318, I90730		80
Cry1Ka1		U28801	451 ... 4098	54
Cry2Aa1	CryIIA	M31738	156 ... 2054	20
Cry2Aa2	CryIIA	M23723	1840 ... 3738	131
Cry2Aa3		D86064	2007 ... 3911	98
Cry2Aa4		AF047038	10 ... 1908	71
Cry2Aa5		AJ132464	<1 ... 1860	135
Cry2Aa6		AJ132465	<1 ... 1860	135
Cry2Aa7		AJ132463	<1 ... >1611	135
Cry2Ab1	CryIIB	M23724	1 ... 1899	131
Cry2Ab2	CryIIB	X55416	874 ... 2775	17
Cry2Ac1	CryIIC	X57252	2125 ... 3990	133
Cry3Aa1	CryIIIA	M22472, I01210, I02147	25 ... 1956	42
Cry3Aa2	CryIIIA	J02978	241 ... 2172	101
Cry3Aa3	CryIIIA	Y00420	566 ... 2497	44
Cry3Aa4	CryIIIA	M30503	201 ... 2132	68
Cry3Aa5	CryIIIA	M37207	569 ... 2500	23
Cry3Aa6	CryIIIA	U10985	569 ... 2500	2
Cry3Ba1	CryIIIB2	X17123, I25973	25 ... >1977	109
Cry3Ba2	CryIIIB	A07234	342 ... 2297	94
Cry3Bb1	CryIIIBb	M89794	202 ... 2157	25
Cry3Bb2	CryIIIC(b)	U31633	144 ... 2099	24
Cry3Bb3		I15475	<1 ... >1291	95
Cry3Ca1	CryIIID	X59797, I15474, I90312	232 ... 2178	60
Cry4Aa1	CryIVA	Y00423	1 ... 3540	129
Cry4Aa2	CryIVA	D00248, E01676	393 ... 3935	103

NAME	ORIGINAL	ACCESSION NUMBER(S)	CODING REGION	REF.
Cry4Ba1	CryIVB	X07423, X05692	157 ... 3564	16
Cry4Ba2	CryIVB	X07082	151 ... 3558	120
Cry4Ba3	CryIVB	M20242	526 ... 3930	134
Cry4Ba4	CryIVB	D00247, E01905	461 ... 3865	103
Cry5Aa1	CryVA(a)	L07025	1 ... >4155	110
Cry5Ab1	CryVA(b)	L07026	1 ... >3867	72
Cry5Ac1		I34543	1 ... >3660	85
Cry5Ba1	PS86Q3	U19725, I34523	1 ... >3735	85
Cry6Aa1	CryVIA	L07022, I13734, I15529	1 ... >1425	73
Cry6Ba1	CryVIB	L07024, I13735	1 ... >1185	72
Cry7Aa1	CryIIIC	M64478, A07236	184 ... 3597	59
Cry7Ab1	CryIIIC(b)	U04367	1 ... >3414	84
Cry7Ab2	CryIIIC(c)	U04368	1 ... >3414	84
Cry8Aa1	CryIIIE	U04364, I25971	1 ... >3471	31
Cry8Ba1	CryIIIG	U04365, I25972	1 ... >3507	69
Cry8Ca1	CryIIIF	U04366	1 ... 3447	75
Cry9Aa1	CryIG	X58120	5807 ... 9274	112
Cry9Aa2	CryIG	X58534	385 ... >3837	34
Cry9Ba1	CryX	X75019	26 ... 3488	105
Cry9Ca1	CryIH	Z37527	2096 ... 5569	58
Cry9Da1	N141	D85560	47 ... 3553	5
Cry9Da2		AF042733	<1 ... >1937	130
Cry9Ea1		AB011496	211 ... 3660	70
Cry9-like		AF093107	<1 ... >1917	130
Cry10Aa1	CryIVC	M12662	941 ... 2965	119
Cry11Aa1	CryIVD	M31737	41 ... 1969	21
Cry11Aa2	CryIVD	M22860	<1 ... 235	3
Cry11Ba1	jeg80	X86902, A49087,	64 ... 2238	19
Cry11Bb1	94 kDa	AF017416	97 ... 2346	77
Cry12Aa1	CryVB	L07027	1 ... >3771	72
Cry13Aa1	CryVC	L07023	1 ... 2409	99
Cry14Aa1	CryVD	U13955	1 ... 3558	86
Cry15Aa1	34kDa	M76442	1036 ... 2055	11
Cry16Aa1	cbm71	X94146	158 ... 1996	6
Cry17Aa1	cbm72	X99478	12 ... 1865	6
Cry18Aa1	CryBP1	X99049	743 ... 2860	136
Cry19Aa1	Jeg65	Y07603	719 ... 2662	96
Cry19Ba1		D88381	626 ... 2671	47
Cry20Aa1	86kDa	U82518	60 ... 2318	62
Cry21Aa1		I32932	1 ... 3501	81
Cry21Aa2		I66477	1 ... 3501	29
Cry22Aa1		I34547	1 ... 2169	85
Cry23Aa1	CryET33	AF038048		22
Cry24Aa1		U88188	1 ... 2022	51
Cry25Aa1		U88189	1 ... 2028	51
Cry26Aa1		AF122897		132
Cry27Aa1		AB023293		132
Cry28Aa1		AF132928		132
Cyt1Aa1	CytA	X03182	140 ... 886	126
Cyt1Aa2	CytA	X04338	509 ... 1255	128
Cyt1Aa3	CytA	Y00135	36 ... 782	27
Cyt1Aa4	CytA	M35968	67 ... 813	32
Cyt1Ab1	CytM	X98793	28 ... 777	118
Cyt1Ba1		U37196, I17395, I44773, I90047	1 ... 795	87
Cyt2Aa1	CytB	Z14147	270 ... 1046	53
Cyt2Ba1	"CytB"	U52043	287 ... 655	38
Cyt2Ba2		AF020789	<1 ... >469	37
Cyt2Ba3		AF022884	<1 ... >469	37
Cyt2Ba4		AF022885	<1 ... >469	37
Cyt2Ba5		AF022886	<1 ... >471	37
Cyt2Ba6		AF034926	<1 ... >472	37
Cyt2Bb1		U82519	416 ... 1204	15

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PESTICIDAL ACTIVITY OF CRY AND CYT PROTEINS

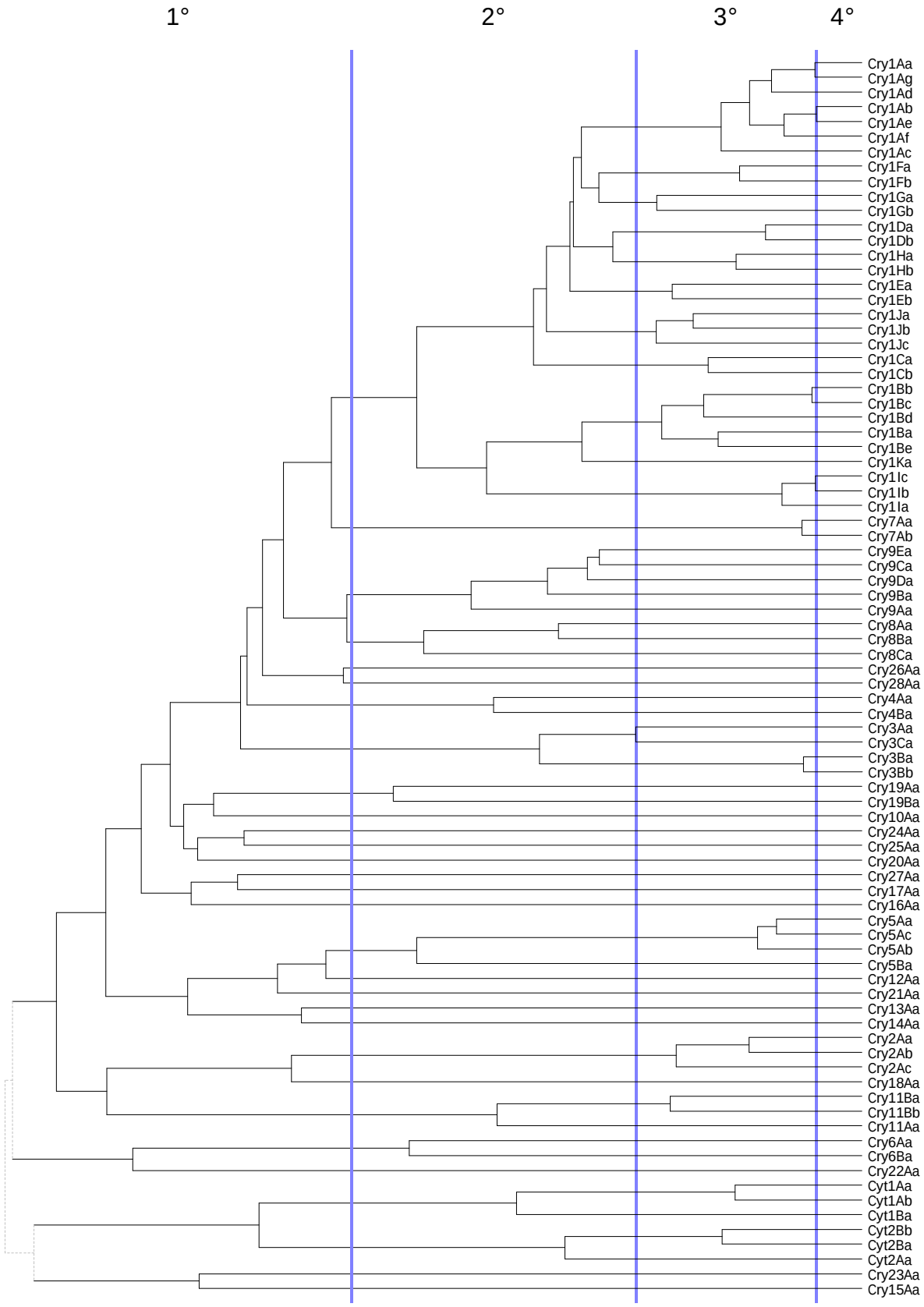
Cry proteins exhibit toxicity to insects and other invertebrates. Typically, a given Cry protein has a fairly narrow range of target organisms against which it is effective. The mode of action of Cry proteins is complex. It is thought to involve solubilization and proteolytic processing in the target 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)

NAME	SOURCE STRAIN	KNOWN TOXICITY
Cry1Ea1	*B.t. darmstadiensis* HD-146	*Spodoptera littoralis, Spodoptera exempta* (Lepidoptera: Noctuidae); *Manduca sexta* (Lepidoptera: Sphingidae)
Cry1Eb1	*B.t. aizawai*	Source strain is toxic to *Trichoplusia ni, Spodoptera exigua* (Lepidoptera: Noctuidae); *Plutella xylostella* (Lepidoptera: Plutellidae)
Cry1Fa1	*B.t. aizawai* EG6346	*Plutella xylostella* (Lepidoptera: Plutellidae); *Heliothis virescens, Spodoptera exigua, Spodoptera littoralis* (Lepidoptera: Noctuidae); *Ostrinia nubilalis* (Lepidoptera: Pyralidae)
Cry1Ia1	*B.t. kurstaki* INA-02, 4835	*Spodoptera littoralis* (Lepidoptera: Noctuidae); *Bombyx mori* (Lepidoptera: Bombycidae); *Plutella xylostella* (Lepidoptera: Plutellidae); *Ostrinia nubilalis* (Lepidoptera: Pyralidae); *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)
Cry1Ib1	*B.t. entomocidus* BP465	*Plutella xylostella* (Lepidoptera: Plutellidae)
Cry1Ja1	*B. thuringiensis* EG5847	*Helicoverpa zea, Heliothis virescens, Pseudoplusia includens, Spodoptera exigua, Spodoptera frugiperda, Trichoplusia ni* (Lepidoptera: Noctuidae); *Plutella xylostella* (Lepidoptera: Plutellidae)
Cry1Jb1	*B. thuringiensis* EG5092	*Pseudoplusia includens*, *Trichoplusia ni* (Lepidoptera: Noctuidae); *Ostrinia nubilalis* (Lepidoptera: Pyralidae); *Plutella xylostella* (Lepidoptera: Plutellidae)
Cry1Ka1	*B.t. morrisoni* BF190	*Artogeia rapae* (Lepidoptera: Pieridae)
Cry2Aa1	*B.t. kurstaki* HD-1, HD-263	*Sciropophaga incertulas, Chilo suppressalis, Ostrinia nubilalis* (Lepidoptera: Pyralidae); *Lymantria dispar* (Lepidoptera: Lymantriidae); *Helicoverpa armigera, Heliothis virescens, Trichoplusia ni* (Lepidoptera: Noctuidae); *Aedes aegypti* (Diptera: Cuclidae)
Cry2Ab1	*B.t. kurstaki* HD1	*Manduca sexta* (Lepidoptera: Sphingidae)
Cry2Ac1	*B. thuringiensis* S1	*Heliothis virescens, Trichoplusia ni* (Lepidoptera: Noctuidae); *Manduca sexta* (Lepidoptera: Sphingidae)
Cry3Aa1	*B.t. san diego, B.t. tenebrionis*	*Haltica tombacina*, *Leptinotarsa decemlineata, Pyrrhalta luteola* (Coleoptera: Chrysomelidae); *Hypera brunneipennis, Otiorhynchus sulcatus, Anthonomus grandis* (Coleoptera: Curculionidae); *Tribolium castaneum*, *Tenebrio molitor* (Coleoptera: Tenebrionidae)
Cry3Ba1	*B.t. tolworthi* EG2838	*Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)
Cry3Bb1	*B.t. kumamotoensis* EG4961	*Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)
Cry3Ca1	*B.t. san diego*	*Pyrrhalta luteola* (Coleoptera: Chrysomelidae)
Cry4Aa1	*B.t. israelensis* 4Q2-72	*Anopheles stephensi, Aedes aegypti, Culex pipiens* (Diptera: Cuclidae)
Cry4Ba1	*B.t. israelensis* 4Q2-72	*Aedes aegypti* (Diptera: Cuclidae)
Cry5Aa1	*B. thuringiensis* PS17A	*Caenorhabditis elegens*, *Pratylenchus* spp. (plant parasitic nematodes)
Cry5Ab1	*B. thuringiensis* PS7	*Fasciola hepatica* (liver fluke); *Caenorhabditis elegens*, *Pratylenchus* spp. (plant parasitic nematodes)
Cry6Aa1	*B. thuringiensis* PS52A1	*Pratylenchus* spp., *Panagrellus redivivus* (plant pathogenic nematodes)
Cry6Ba1	*B. thuringiensis* PS52A1	*Pratylenchus* spp. (plant pathogenic nematode)
Cry7Aa1	*B. thuringiensis* BTS137J	*Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)
Cry8Aa1	*B.t. kumamotoensis* PS50C	*Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)
Cry8Ba1	*B.t. kumamotoensis* PS50C	*Cotinis* spp. (Coleoptera: Scarabaeidae)
Cry8Ca1	*B.t. japonensis* strain Buibui	*Anomala cuprea* (Coleoptera: Scarabaeidae)
Cry9Aa1	*B.t. galleriae* 11-67	*Galleria mellonella* (Lepidoptera: Pyralidae)
Cry9Ca1	*B.t. tolworthi* H9	*Agrotis segetum, Helicoverpa armigera, Heliothis virescens, Mamestra brassicae, Spodoptera exigua, Spodoptera littoralis* (Lepidoptera: Noctuidae); *Manduca sexta* (Lepidoptera: Sphingidae); *Ostrinia nubilalis* (Lepidoptera: Pyralidae); *Plutella xylostella* (Lepidoptera: Plutellidae); *Bombyx mori* (Lepidoptera: Bombycidae); *Choristoneura fumiferana* (Lepidoptera: Tortricidae)
Cry10Aa1	*B.t. israelensis* ONR60A	*Aedes aegypti* (Diptera: Cuclidae)
Cry11Aa1	*B.t. israelensis* HD-567	*Anopheles stephensi, Aedes aegypti, Culex pipiens* (Diptera: Cuclidae)
Cry11Ba1	*B.t. jegathesan* 367	*Anopheles stephensi, Aedes aegypti, Culex pipiens* (Diptera: Cuclidae)
Cry11Bb1	*B.t. medellin*	*Anopheles albimanus, Aedes aegypti, Culex quinquefasciatus* (Diptera: Cuclidae)

NAME	SOURCE STRAIN	KNOWN TOXICITY
Cry12Aa1	*B. thuringiensis* PS33F2	*Pratylenchus* spp. (plant pathogenic nematode)
Cry13Aa1	*B. thuringiensis* PS63B	nematodes
Cry14Aa1	*B.t. sotto* PS80JJ1	*Diabrotica* (Coleoptera:); nematodes
Cry15Aa1	*B.t. thompsoni* HD-542	*Manduca sexta* (Lepidoptera: Sphingidae)
Cry16Aa1	*Clostridium bifermentans malaysia* CH18	*Anopheles stephensi, Aedes aegypti, Culex pipiens* (Diptera: Cuclidae)
Cry19Aa1	*B.t. jegethesan*	*Anopheles stephensi, Culex pipiens* (Diptera: Cuclidae)
Cry20Aa1	*B.t. fukuokaensis*	*Aedes aegypti* (Diptera: Cuclidae)
Cry21Aa1	*B.t. higo*	*Culex pipiens molestus* (Diptera: Cuclidae)
Cry22Aa1		hymenopterans
Cry23Aa1		*Tribolium castaneum,* (Coleoptera: Tenebrionidae); *Popillia japonica* (Coleoptera: Scarabaeidae)
Cyt1Aa1	*B.t. israelensis* IPS82	*Anopheles stephensi, Aedes aegypti, Culex pipiens* (Diptera: Cuclidae)
Cyt1Ab1	*B.t. medellin* 163-131	*Anopheles stephensi, Aedes aegypti, Culex pipiens* (Diptera: Cuclidae)
Cyt2Aa1	*B.t. kyushuensis*	*Anopheles stephensi, Aedes aegypti, Culex pipiens* (Diptera: Cuclidae)
Cyt2Bb1		*Aedes aegypti* (Diptera: Cuclidae)

Phylogram of Cry and Cyt Holotype Sequences



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.

- **What is the mode of action of Cry proteins?**

  Rajamohan, F., M. K. Lee, D. H. Dean. 1998. *Bacillus thuringiensis* insecticidal proteins: molecular mode of action. Progress in Nucleic Acid Research and Molecular Biology **60:**1-27.

  Dean, D H., F.Rajamohan, M. K.Lee, S. J.Wu, X. J.Chen, E.Alcantara, S. R.Hussain. 1996. Probing the mechanism of action of *Bacillus thuringiensis* insecticidal proteins by site-directed mutagenesis—a minireview. Gene **179:**111-117.

- **What is the relationship between structure and function of Cry proteins?**

  Thompson, M. A., H. E. Schnepf, J. S. Feitelson. 1995. Structure, function and engineering of *Bacillus thuringiensis* toxins. Genet. Engineer. **17:**99-117.

- **How do media and fermentation conditions affect Cry protein synthesis in *Bacillus thuringiensis*?**

  Avignone-Rossa, C., and C. F. Mignone. 1995. *Bacillus thuringiensis* growth and toxicity. Basic and applied considerations. Mol. Biotechnol. 4:55-71.

  Yang, X. M., and S. S. Wang. 1998. Development of *Bacillus thuringiensis* fermentation and process control from a practical perspective. Biotechnol. Appl. Biochem. **28:**95-8.

- **How are plants engineered to express Cry proteins?**

  Estruch, J. J., N. B. Carozzi, N. Desai, N. B. Duck, G. W. Warren, M. G. Koziel. 1997. Transgenic plants: an emerging approach to pest control. Nature Biotechnol. **15:**137-141.

- **How do *Bacillus thuringienis* subsp. *israelensis* products contribute to the control of mosquitoes and other biting flies?**

  Priest, F. G. 1992. Biological control of mosquitoes and other biting flies by *Bacillus sphaericus* and *Bacillus thuringiensis*. J. Appl. Bacteriol. **72:**357-369.

- **How might insect resistance to Cry proteins in transgenic plants be delayed or prevented?**

  McGaughey, W. H., F. Gould, W. Gelernter. 1998. Bt resistance management. Nature Biotechnol. **16:**144-146.

  Roush, R. T., A. M. Shelton. 1997. Assessing the odds: the emergence of resistance to Bt transgenic plants. Nature Biotechnol. **15:** 816-817.

- **What are the public health considerations for use of *Bacillus thuringiensis* and related organisms?**

  Drobniewski, F. A. 1994. The safety of Bacillus species as insect vector control agents. J. Appl. Bacteriol. 76:101-109.

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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