Paenibacillus popilliae（乳化病）

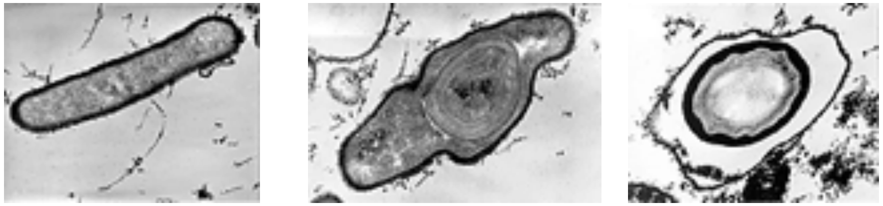
Paenibacillus popilliae

Emended description of *Paenibacillus lentimorbus* (Dutky 1940) comb. nov.
Paenibacillus lentimorbus (len.ti.mor«bus. L. adj. *lentus* slow; L. n. *morbus* disease; M.L. n. *lentimorbus* the slow disease).

This description is based on this work and the studies of Dutky (1940), Gordon *et al*. (1973) and Rippere *et al*. (1998).
Cells are rods, 0±5±0±7 lm by 1±8±7 lm which stain Gram-variable or -negative in exponentialphase cultures.
Ellipsoidal spores are located centrally or terminally in swollen sporangia and may or may not be accompanied by a refractile parasporal body visible by phase-contrast microscopy. The type strain does not produce a parasporal body.
May be isolated from haemolymph of infected larvae of the Japanese beetle (*Popillia japonica* Newman) and related larvae.

Paenibacillus larvae **：American foulbrood**

アメリカ腐蛆病　（ミツバチの病気）



Paenibacillus alvei **： Foulbrood**

腐蛆病　（ミツバチの病気）

Paenibacillus popilliae (lentimorvus) **:Milky disease**

乳化病　（コガネムシの病気）

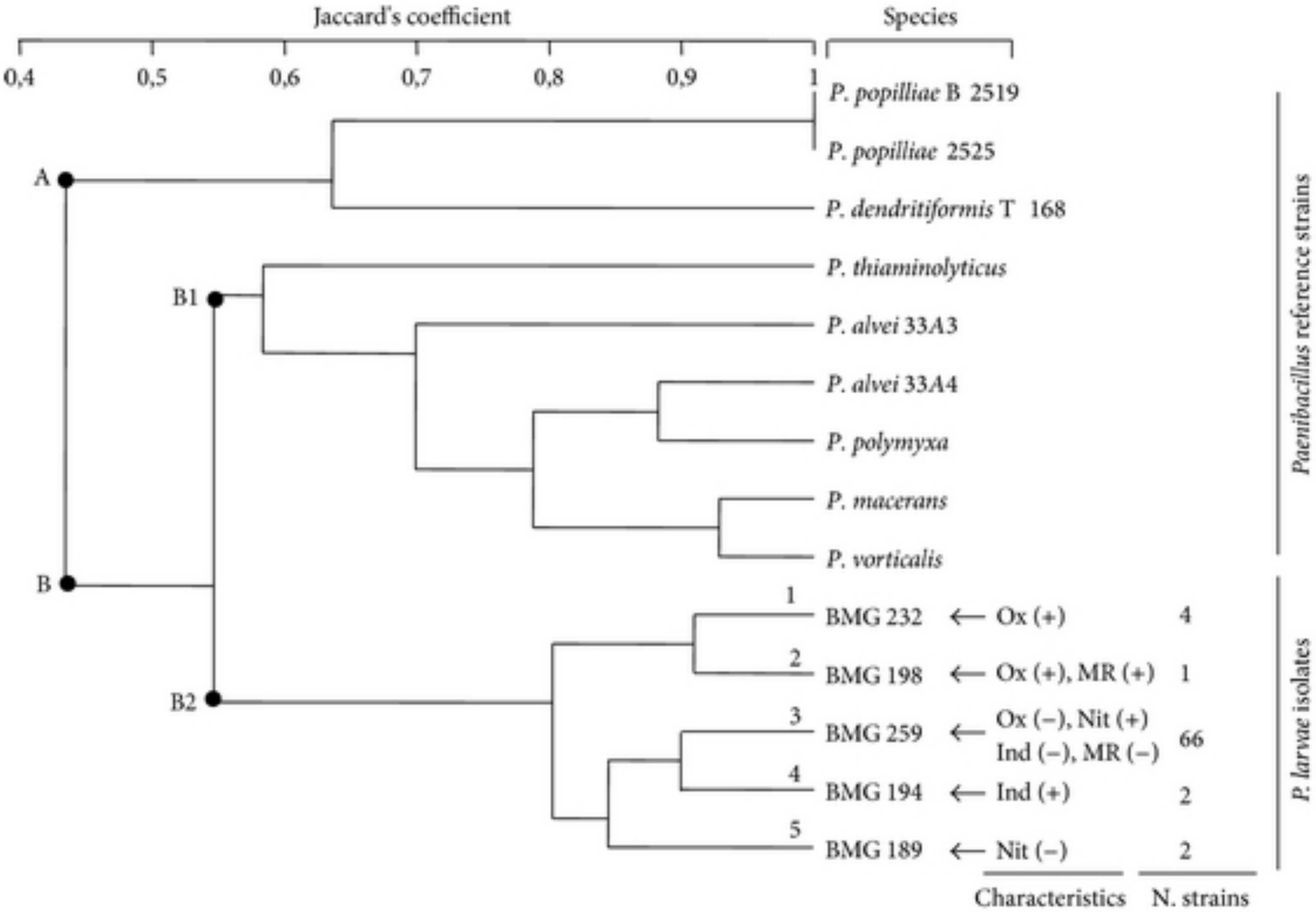
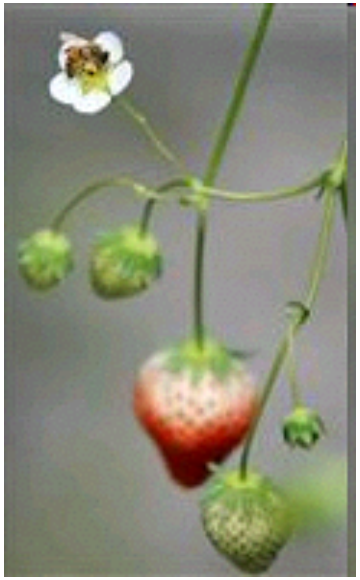


Figure 2: Dendrogram showing the biochemical profile relationship between *P. larvae* isolates and *Paenibacillus* reference strains. Ox: oxidase; Nit: nitrate reduction; MR: methyl red; Ind: indol; +: positive response; −: negative response.

ミツバチ失踪　農薬？伝染病？環境変化？

http://headlines.yahoo.co.jp/hl?a=20090410-00000091-san-bus_all



ミツバチが昨年から激減する謎の現象が起きている。農林水産省は寄生ダニが発生する病気などで大量死したのではないかと推測しているが、真相は分かってい ない。農家では、イチゴやメロンなどの受粉作業にミツバチを使っているため、栽培に深刻な影響も出ており、果物の価格高騰につながりかねない。「自然環境 が変化しているのか」と不安を募らせる関係者も出ている。

イチゴの花の受粉を行うミツバチ

　ミツバチ激減の原因を農水省は「寄生ダニや農薬で大量に死んだからではないか」と推測する。実際にミツバチが大量死する例もあり、大量の幼虫が死ぬ腐蛆（ふそ）病など伝染病の可能性も指摘されている。

International Journal of Systematic Bacteriology (1999), 49, 531–540

Transfer of *Bacillus lentimorbus* and *Bacillus popilliae* to the genus *Paenibacillus* with emended descriptions of *Paenibacillus lentimorbus* comb. nov. and *Paenibacillus popilliae* comb. nov.

Bertil Pettersson, Karen E. Rippere, Allan A. Yousten and Fergus G. Priest

Almost complete 16S rRNA gene sequences were generated for the type strains of the obligate insect pathogens *Bacillus lentimorbus* and *Bacillus popilliae* and a second strain of *Bacillus popilliae* (NRRL B-4081) received as '*Bacillus popilliae* var. *melolonthae* '. A phylogenetic tree was constructed which grouped these strains into a well defined subcluster within the genus *Paenibacillus*. *Bacillus popilliae* NRRL B-4081 occupied an intermediate position between the type strains of *Bacillus lentimorbus* and *Bacillus popilliae* but with a marked clustering to the latter. The phylogenetic assignment of these strains to *Paenibacillus* is in contrast to earlier studies which placed these bacteria in the genus *Bacillus*, close to *Bacillus subtilis*. Indeed, the rRNA sequences generated in this study share less than 88% similarity to the deposited sequences for *Bacillus popilliae* ATCC 14706T and *Bacillus lentimorbus* ATCC 14707T. The results obtained by using different tree algorithms, bootstrap analysis, branch lengths and verification by signature nucleotide analysis supported the reclassification of these species in the genus *Paenibacillus* as *Paenibacillus lentimorbus* comb. nov. and *Paenibacillus popilliae* comb. nov.

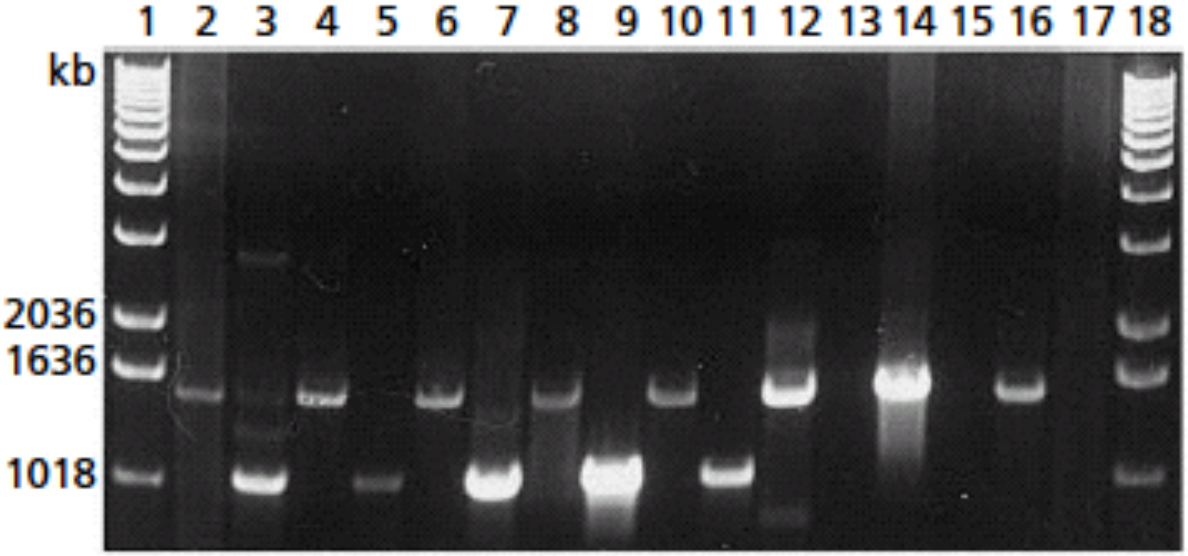


Fig. 1. Agarose gel electrophoresis of PCR products generated with control primers (even lanes) and a *Paenibacillus*-specific primer (odd lanes) for various endospore-forming bacteria. Lanes: 1, size markers (1 kb ladder); 2 and 3, *Paenibacillus alvei* DSM 29^{T}; 4 and 5, *Paenibacillus chibensis* NRRL B-142^{T}; 6 and 7, *Bacillus popilliae* ATCC 14706^{T}; 8 and 9, *Bacillus lentimorbus* ATCC 14707^{T}; 10 and 11, *Bacillus popilliae* NRRL B-408; 12 and 13, *Bacillus subtilis* NCIMB 3610^{T}; 14 and 15, *Bacillus sphaericus* 2362; 16 and 17, *Brevibacillus borstelensis* HRS 23; 18, size markers.

Journal of Insect Biotechnology and Sericology 82, 45-48 (2013)

Draft Genome Sequence of *Paenibacillus popilliae* ATCC 14706^T

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Kousuke Tashiro[2], Shin-ichiro Asano[4], Chisa Yasunaga-Aoki[1] and Susumu Shimizu[1]

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(Received March 29, 2013; Accepted June 10, 2013)

Key words: *Paenibacillus popilliae*, draft genome sequence, entomopathogenic bacteria

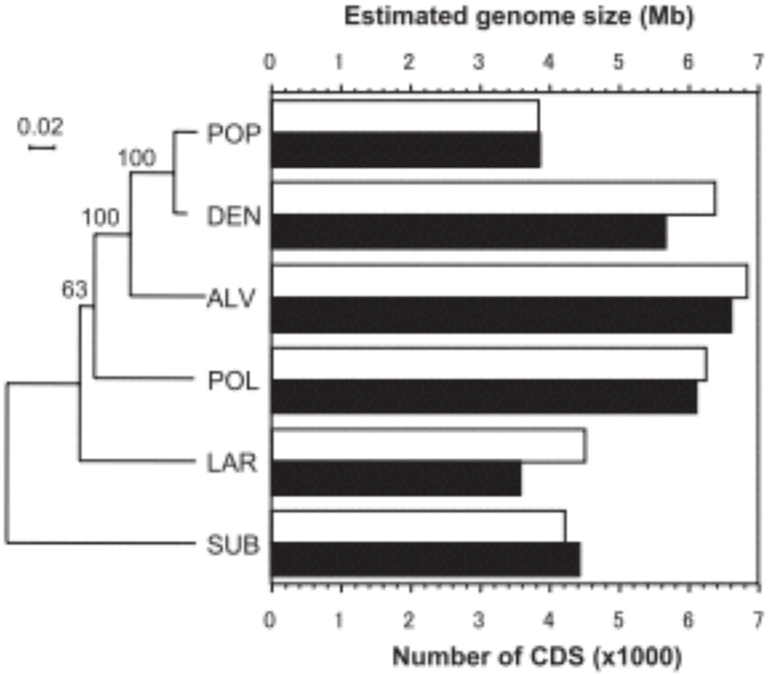


Fig. 4. Comparison of estimated genome size and number of CDS among *Paenibacillus* species. Estimated genome size (open column) and number of CDS (closed column) are shown in the graph. Since the genome of *P. polymyxa* SC2 has been completely sequenced, total genome size and total CDS composed of chromosome and plasmid were used in this analysis. Phylogenetic relationship of *Paenibacillus* species based on amino acid sequences of DNA gyrase subunit A is also shown. *Bacillus subtilis* subsp. *subtilis* 168 was used as the outgroup. The number for each interior branch is the percent bootstrap value (1,000 resamplings). Abbreviation of organisms: POP, *P. popilliae* ATCC 14706^T; DEN, *P. dendritiformis* C454; ALV, *P. alvei* DSM 29^T; POL, *P. polymyxa* SC2; LAR, *P. larvae* subsp. *larvae* BRL2300-10; SUB, *B. subtilis* subsp. *subtilis* 168.

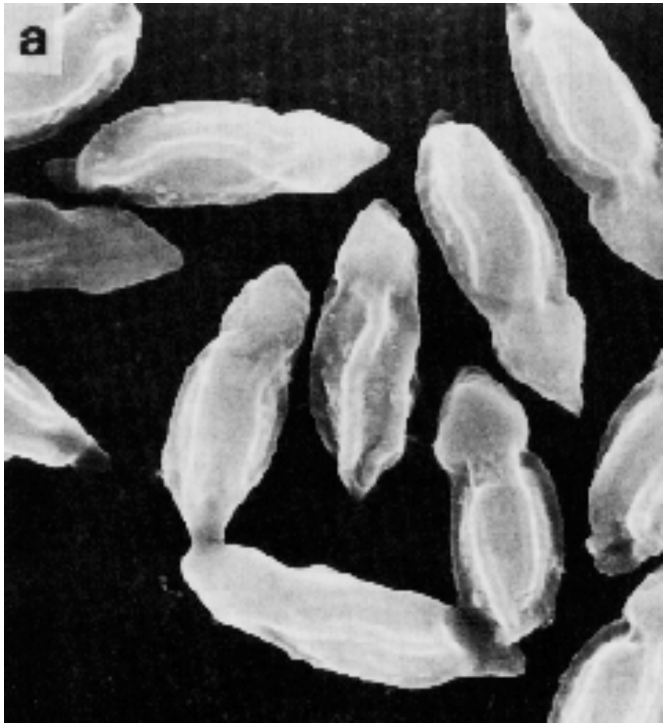
Table 4. Distinctive phenotypic properties of *Paenibacillus lentimorbus*, *Paenibacillus popilliae* and related paenibacilli

Data from Gordon *et al.* (1973), Priest *et al.* (1988), Shida *et al.* (1997) and Rippere *et al.* (1998). +, > 90 % strains positive; –, < 10 % strains positive; v, 11–89 % strains positive; NT, not tested.

Character[a]	*P. alvei*	*P. apiarius*	*P. curdlanolyticus*	*P. kobensis*	*P. larvae* subsp. *larvae*	*P. larvae* subsp. *pulvifaciens*	*P. lentimorbus*	*P. popilliae*	*P. thiaminolyticus*
Spore shape	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval	Oval
Swollen sporangia	+	+	+	+	+	+	+	+	+
Parasporal crystal	–	–	NT	NT	–	–	v	+	–
Anaerobic growth	+	+	–	–	+	+	+	+	+
Catalase	+	+	+	+	–	–	–	–	+
Oxidase[a]	+	–	–	–	NT	–	–	–	+
Nitrate reduction	–	+	+	+	v	v	–	–	+
Acid from:									
Arabinose	–	–	+	+	–	–	–	–	v
Glucose	+	+	+	+	+	+	+	+	+
Mannitol	–	–	–	–	–	+	–	–	–
Xylose	–	–	+	+	–	–	–	–	–
Production of:									
Acetylmethylcarbinol	+	–	–	–	v	v	–	–	–
Indole	+	–	–	–	+	+	–	–	+
Decomposition of:									
Tyrosine	v	+	NT	NT	–	–	–	–	+
Hydrolysis of:									
Casein	+	+	–	–	+	+	–	–	+
Starch	+	+	+	+	–	–	–	–	+
Use of citrate:	–	+	–	–	–	–	–	–	+
Growth in:									
Nutrient broth	+	+	+	+	–	+	–	–	+
2% NaCl	+	+	NT	NT	+	+	+	–	+
5% NaCl	v	+	–	–	–	–	NT	NT	v
0·001 % Lysozyme	+	+	+	+	+	+	+	+	–
Growth at:									
50 °C	–	–	–	–	–	–	–	–	–
pH 5·6	–	+	NT	NT	–	–	–	–	–

[a] Based on reactions of type strains only for *Paenibacillus lentimorbus* and *Paenibacillus popilliae*.

Paenibacillus popilliae　の特徴



•偏性病原体である

　特定の昆虫の体内でのみ増殖する

　人工培養ができない

•胞子形成時に結晶タンパク質を産生

　殺虫活性に関与しているのか？

•種類が少ない

　10亜種以下

•特異的殺虫活性

　鞘翅目（コガネムシ類）にのみ活性

•*Bacillus*属でない

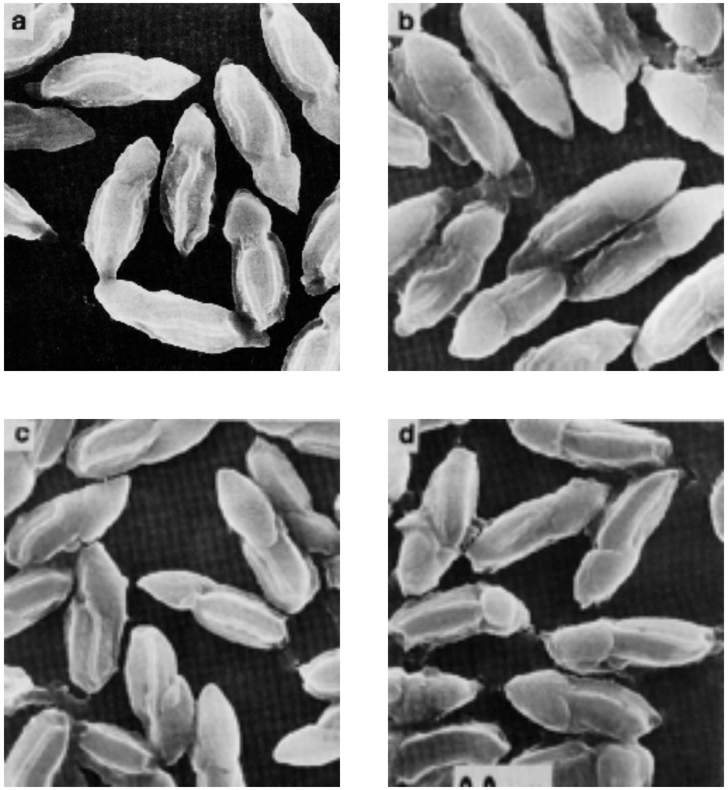
　*Paenibacillus*属

•遺伝子情報を含めほとんど情報が少ない

Popilliae japonica **Newman**
Japanese beetle
マメコガネ

Anomala daimiana **Harold**
cherry chafer
サクラコガネ

Anomala rufocuprea **Motschulsky**
soybean beetle
ヒメコガネ



Scanning electron micrographs of *B. popilliae* var. *popilliae* and the newly isolated strains.
a: reference strain, b: var. *popilliae* Sakura,
c: var. *popilliae* Hime, d: var. *popilliae* Mame

千葉県工業試験場／千葉県農業試験場

セマダラコガネ幼虫に発生した乳化病

Host range of three *B. popilliae* isolates bioassayed against six 3rd-instar beetle larvae

Insect species	var. *popilliae* Mame	var. *popilliae* Hime	var. *popilliae* Sakura
Popilliae japonica マメコガネ	50.5%[a]	35%	30%
Anomala daimiana サクラコガネ	5%	10%	35%
Anomala rufocuprea ヒメコガネ	0%	40%	20%
Mimela testaceipes スジコガネ	6.7%	20%	6.7%
Blitopertha orientalis セマダラコガネ	40%	58.3%	73.3%
Heptophylla picea ナガチャコガネ	0%	0%	0%

[a] **Percentage of inifection**

One million spores were injected per larvae

ALIGNMENT OF 16S rRNA sequences isolated from *B. popilliae*

```
: sakura       GATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCG  343
: ATCC 14706   GATGGACCTACGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCG  254
: ATCC 14707   GATGGACCTACGGCGCATTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACGATGCG  263
               ********  ** ****************** ******************* ********


  sakura       TAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACG  402
  ATCC 14706   TAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACG  314
  ATCC 14707   TAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACG  323
               ************************************************************


  sakura       GGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAA
  ATCC 14706   GGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGCAA
  ATCC 14707   GGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGCAA
               ********************************** **
```

1: *B. popilliae* var. *popilliae* Sakura
2: *B. popilliae* ATCC 14706   (*Paenibacillus popilliae*, Int J Syst Bacteriol(1999),49,531-540)
3: *B. lentimorbus* ATCC 14707 (*Paenibacillus lentimolbus*, Int J Syst Bacteriol(1999),49,531-540)

乳化病のクレイド

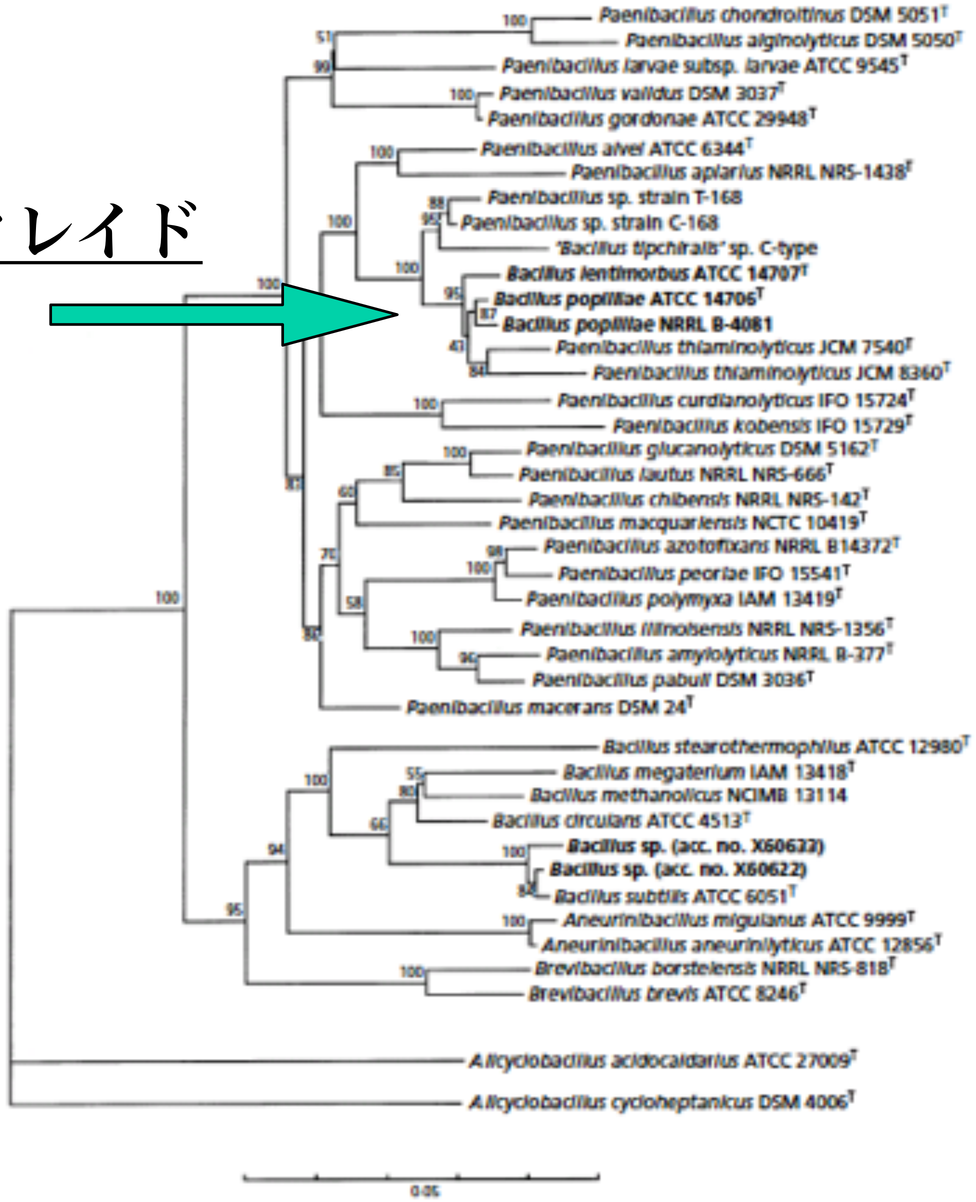


Fig. 2. Phylogenetic tree, based on neighbour-joining (Saitou & Nei, 1987), derived from an alignment comprising 16S rRNA gene sequences, ranging from position 45 to 1430 (*E. coli* numbering; Brosius *et al.*, 1978) from *Paenibacillus* species and selected members of closely related genera. *Alicyclobacillus acidocaldarius* ATCC 27009^T served as outgroup.

Oligonucleotide primers of *cry18A* gene screening

Name	Sequence	Position
BpF1	5'-atgaacaataattttaatgg	743- 762
BpR1	5'-ctaataaagaggagtgtc	2946-2963
BpF2	5'-ttattattacctttatttgc	1466-1485
BpR2	5'-ccattcatcggcattaatgat	1526-1546
BpF3	5'-aaatgccgttttgttgtg	209- 226
BpR3	5'-gtaaattaacttctgc	355- 370
BpF4	5'-gcagaagttaatttac	355- 370
BpR4	5'-tgaaattgaaagttccc	631- 647

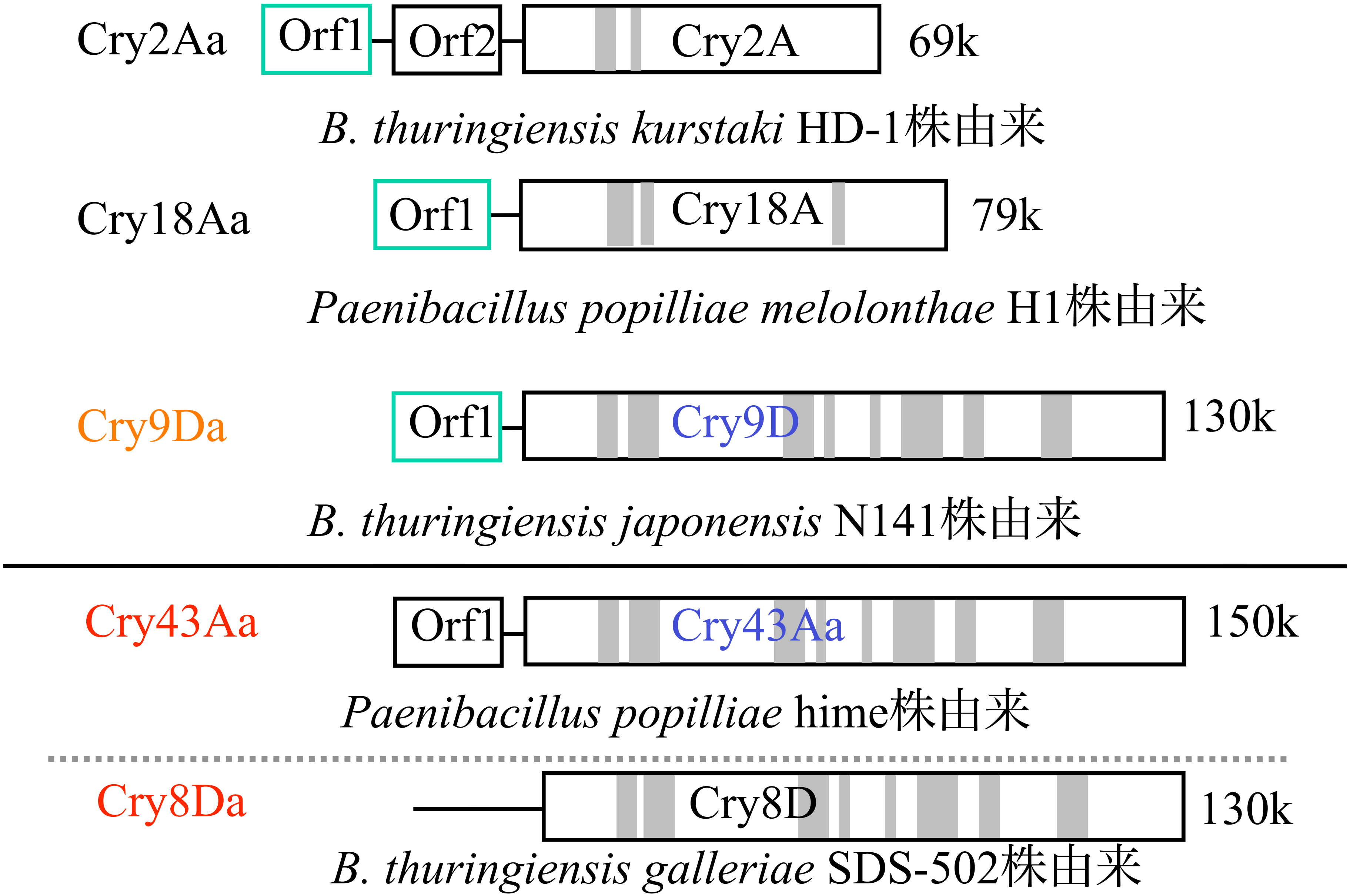
Cloning and analysis of the first *cry* gene from *Bacillus popilliae*.

Zhang J, **Hodgman TC**, **Krieger L**, **Schnetter W**, **Schairer HU**.

Zentrum für Molekulare Biologie der Universität Heidelberg, Germany.

J Bacteriol. 1997 Jul;179(13):4336-41.

diseased larva of the common cockchafer (*Melolontha melolontha* L.)



Cry2Aa Orf1 Orf2 Cry2A 69k

B. thuringiensis kurstaki HD-1株由来

Cry18Aa Orf1 Cry18A 79k

Paenibacillus popilliae melolonthae H1株由来

Cry9Da Orf1 Cry9D 130k

B. thuringiensis japonensis N141株由来

Cry43Aa Orf1 Cry43Aa 150k

Paenibacillus popilliae hime株由来

Cry8Da Cry8D 130k

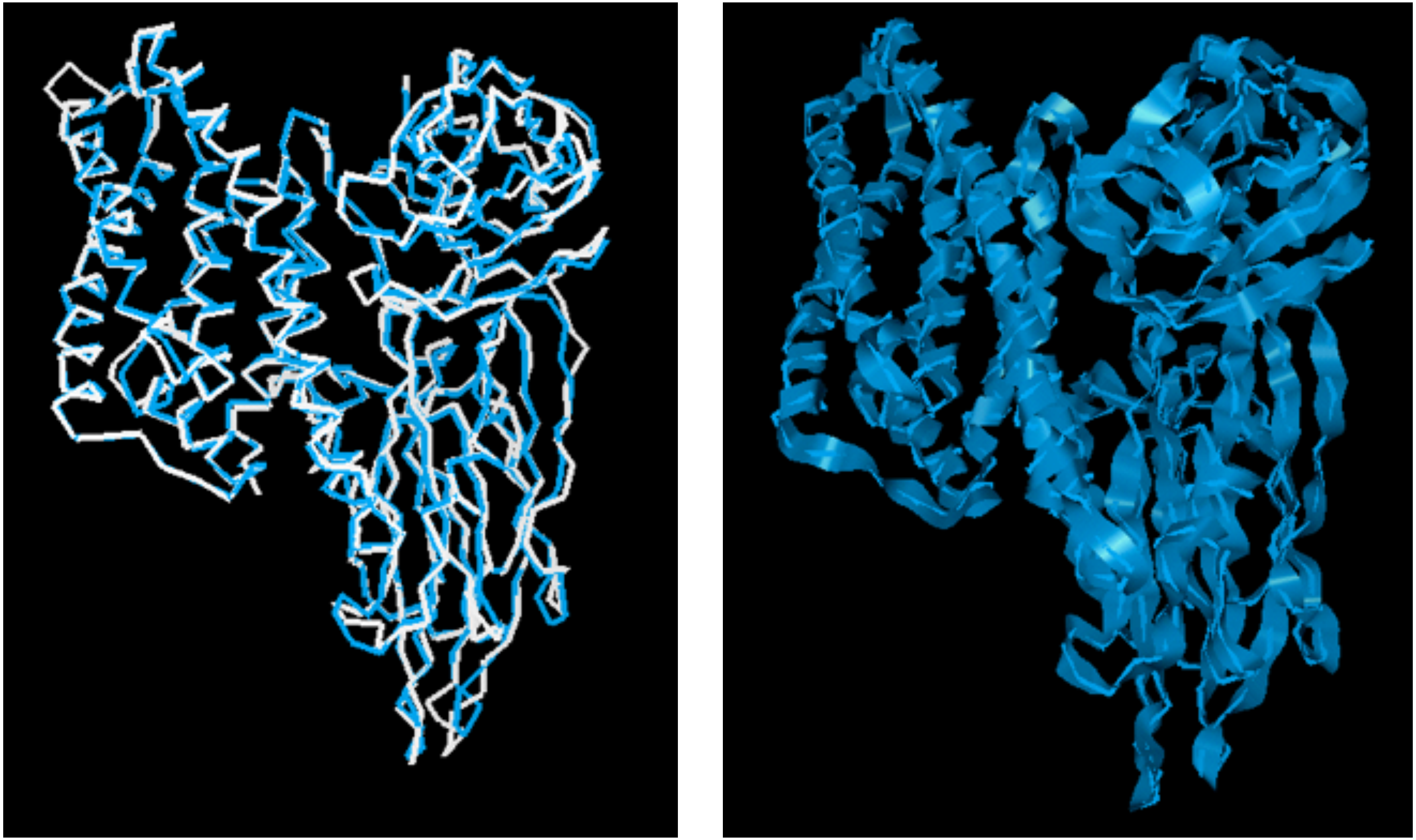
B. thuringiensis galleriae SDS-502株由来

Crystal proteinの構造比較とコガネムシ殺虫活性の関連について

```
Bp1:   TTCCTAGTCCGCATGGATTGTGAACTCTGCATCGTTATTGTAAACTCAAAGATTGGTGT
       :::: : ::::::::::::: :::::: :::::: : ::::::::: ::::: ::::: :
cry18: TTCCGA-TCCCGCATGGATT-TGAACT-TGCATC-TCATTGTAAAC-CAAAG-TTGGTTT
       230        240        250        260        270         280
       ATCTACTGAATGGTTTAGCGGCTCGTAACCGAAACATGCAAAAAAACAGTAAAAGTTGAT
       :::: ::::::::: : : ::::::::::::::::::::: :::::: ::::::::::::
       ATCTACTGAATGGTGTCG-GGCTCGTAACCGAAACATGCAGAAAAACGGTAAAAGTTGAT
          290       300        310       320       330       340
       GACTATGGTAATGCAGAAGTTAATTTACACGTGTTAAAAGTAAAAGGATGTATCCCTTTT
       :::: :::: ::::::::::::::::::::::::::::: ::::::::::::::::::::
       GACTGTGGTCATGCAGAAGTTAATTTACACGTGTTAAAAATAAAAGGATGTATCCCTTTT
            350       360       370       380       390       400
       ATTGCTAATGTAGAAGTGAAACCAATTTATGAACAAACAGGATGTTCCTCTGATCCCCAT
       ::::::::::::::::::::::::::  ::::::::::::::::::::::::::::::::
       ATTGCTAATGTAGAAGTGAAACCAATAAATGAACAAACAGGATGTTCCTCCGATCCCCAT
              410       420       430       440       450       460
       TCATAAAGAAATTACAATTTGTTGCACAGAAAGTATCTGTTGTAGATCATGTACTGAAA
       ::: ::::::::: : ::::: ::::::::::::::::::: :::::::::::::::::
       TCACAAAGAAATTTCTATTTGCTGCACAGAAAGTATCTGTTATAGATCATGTACTGAAA
               470       480       490       500        510
       TGCAGTCTGGAAACCAGCCCCCACTCATCATTTGG
       :::::: :::::::::::::::::::::::: :::
       TGCAGTGTGGAAACCAGCCCCCACTCATCATGTGG
          520       530       540
```

鞘翅目昆虫に活性を有する*cry*遺伝子

cry3Aa1	M22472	Herrnstadt et al 1987	Gene 57 37-46	25-1956
cry3Aa2	J02978	Sekar et al 1987	PNAS 84 7036-7040	241-2175
cry3Ba1	X17123	Sick et al 1990	NAR 18 1305-1305	25-1977
cry3Ba2	A07234	Peferoen et al 1990	EP 0382990	342-2297
cry3Bb1	M89794	Donovan et al 1992	AEM 58 3921-3927	202-2157
cry3Bb2	U31633	Donovan et al 1995	USP 5378625	144-2099
cry3Ca1	X59797	Lambert et al 1992	Gene 110 131-132	232-2178
cry7Aa1	M64478	Lambert et al 1992	AEM 58 2536-2542	184-3597
cry7Ab1	U04367	Payne & Fu 1994	USP 5286486	1-3414
cry8Aa1	U04364	Foncerrada et al 1992	EP 0498537	1-3471
cry8Ba1	U04365	Michaels et al 1993	WO 93/15206	1-3507
cry8Ca1	U04366	Ogiwara et al. 1995	Curr Micro 30 227-235	1-3447
cry8Da1	AB089299	Yamamoto & Asano 2002	Biological Control	1-3435
cry9Da1	D85560	Asano et al 1997	unpublished	47-3553
cry18Aa1	X99049	Zhang et al 1997	J. Bacteriol. 179 4336-4341	743-2863
cry43Aa2	AB176668	Nozawa et al 2004		



Cry3Aトキシン３次元構造より予測されたCryMame１の構造

▬ :Cry3Aa

▬ :CryMame1

バチルス・ポピリエによるコガネムシ幼虫の防除

木村　雅敏，江原　岳，伊藤　佳代子，西橋　秀治

Biological Control of Bacillus Popilliaeagainst Larvae of Scarabaeid Beetles

KIMURAMasaharu, EHARAGaku, ITOKayoko and NISIHASHI Hideji

生きた幼虫体内で胞子化させる方法

Bpは幼虫体液中では容易に胞子化させることがで きる。幼虫に胞子を摂食させる，あるいは体液に注射 することで感染させ，胞子の発芽→増殖→胞子化を起こさせる。幼虫1頭当たりで得られる胞子数は幼虫の種類・齢期によって異なるが，マメコガネ（Popillia japonica）終令幼虫で5×109個1)，ヨーロッパコフキコガネ（Rhopaea verreauxi）の終令幼虫で1.5×1010個である9)。幼虫が好む食物に胞子を混ぜ込み，餌として食べさせると高い感染率が得られる。

現在米国で販売されているBp胞子製剤のMilky Spore Powder（商品名）はこの方法により製造されている。

液体培地で胞子化させる方法

著者等は液体培養で殺虫活性を持つ胞子生産に成功した。市販されている数十種類のペプトン類から胞子化に有効なペプトンを見出し，さらにグルタミン酸の添加により液体培地中での胞子化が可能となった。また培地中のアミノ酸濃度に着目し，濃度を高めピルビン酸を添加することで培養液1 mlあたり0.5〜1.0×109個/mlの胞子を得ることができた。

Paenibacillus popilliae vs *Bacillus thuringiensis*

Paenibacillus popilliae

- 偏性病原体である
  - 特定の昆虫の体内でのみ増殖する
  - 人工培養が難しい
- 胞子形成時に結晶タンパク質を産生
  - 殺虫活性に関与している
- 種類が少ない
  - 10亜種以下
- 遺伝子情報を含めほとんど情報がない
  - 安全性・環境に与えるインパクト
- 特異的殺虫活性
  - 鞘翅目（コガネムシ類）にのみ活性

Bacillus thuringiensis

- グラム陽性の土壌細菌
  - 土壌・死亡昆虫・植物の葉・養蚕農家の塵芥
- 胞子形成時に殺虫性結晶タンパク質を産生
  - 比較的容易に培養が可能
- 種類が豊富である
  - Ｈ抗血清による分類で50亜種以上
- 特異的殺虫活性
  - 鱗翅目・鞘翅目・双翅目昆虫および線虫
- *B.cereus*菌との関係
  - エンテロトキシン・βエクソトキシン
- 定着性因子がない
  - 昆虫体内では増殖しない