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Minireview

The hidden lifestyles of *Bacillus cereus* and relatives

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Summary

***Bacillus cereus sensu lato*, the species group comprising *Bacillus anthracis*, *Bacillus thuringiensis* and *B. cereus* (*sensu stricto*), has previously been scrutinized regarding interspecies genetic correlation and pathogenic characteristics. So far, little attention has been paid to analysing the biological and ecological properties of the three species in their natural environments. In this review, we describe the *B. cereus sensu lato* living in a world on its own; all *B. cereus sensu lato* can grow saprophytically under nutrient-rich conditions, which are only occasionally found in the environment, except where nutrients are actively collected. As such, members of the *B. cereus* group have recently been discovered as common inhabitants of the invertebrate gut. We speculate that all members disclose symbiotic relationships with appropriate invertebrate hosts and only occasionally enter a pathogenic life cycle in which the individual species infects suitable hosts and multiplies almost unrestrained.**

Introduction

The *Bacillus cereus* group, a very homogeneous cluster within the *Bacillus* genus, comprises six recognized species: *B. cereus*, *B. thuringiensis*, *B. anthracis*, *B. mycoides*, *B. pseudomycoides* and *B. weihenstephanensis*. These species are closely related, but their precise phylogenetic and taxonomic relationships are still debated. Recent data based on multilocus enzyme electrophoresis (MEE) (Helgason *et al*., 2000) and DNA sequence variations of the 16S–23S internal transcribed spacers (Daffonchio *et al*., 2000) suggested that *B. anthracis*, *B. thuringiensis* and *B. cereus sensu stricto* are members of a single species, *B. cereus sensu lato*. Whereas intensive work has been performed to decipher their genetic relationship (Harrell *et al*., 1995; Helgason *et al*., 2000; Hansen *et al*., 2001; Chen and Tsen, 2002), less attention has been paid to comparing the biological and ecological properties of the three species in their natural environments. The main purpose of this review is to elucidate the ecological and biological properties of *B. cereus*, i.e. the three species *B. anthracis*, *B. thuringiensis* and *B. cereus*, with special focus on interactions with other organisms. Furthermore, to the extent of the limited information available, the species *B. mycoides*, *B. pseudomycoides* and *B. weihenstephanensis* are also included in this analysis.

Properties of *Bacillus anthracis*

Bacillus anthracis is the causative agent of anthrax, which is primarily a disease in mammals, including man (for recent reviews, see Mock and Fouet, 2001). Apart from being one of the oldest known diseases, described as one of the Egyptian plagues in the time of Moses, many of the ecological and epidemiological questions about anthrax are still unanswered. Anthrax has been linked with endemic soil environments long before *B. anthracis* was identified as the causative agent (Rayer, 1850; Davaine, 1863).

The virulence of *B. anthracis* is based on the presence of two virulence plasmids, pXO1 (181.7 kbp) and pXO2 (94.8 kbp). The plasmid pXO1 encodes three toxic factors: the protective antigen (PA), the lethal factor (LF) and the oedema factor (EF) (Bhatnagar and Batra, 2001). These components associate into two bipartite exotoxins, PA-LF and PA-EF. The plasmid pXO2 encodes a poly D glutamic acid capsule enabling the bacterium to withstand phagocytosis. The loss of pXO2 renders the cells incapable of establishing an infection, i.e. the bacterium becomes attenuated, a trait that is the basis of the Sterne vaccine

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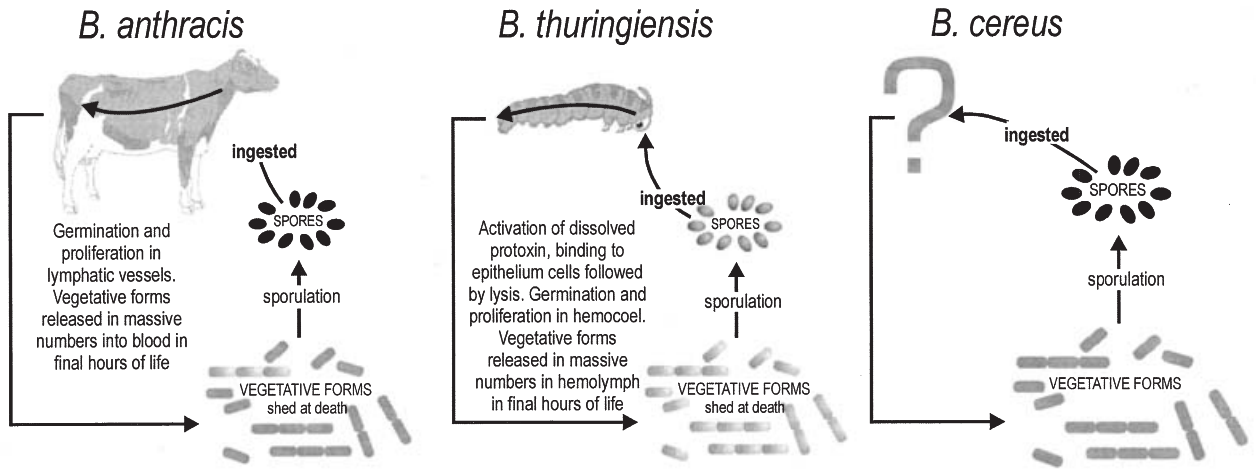

Fig. 1. An illustration of the known pathogenic life cycles of *B. anthracis* and *B. thuringiensis*. Although a human pathogen, *B. cereus* has not been shown to enter a pathogenic life cycle similar to those of *B. anthracis* and *B. thuringiensis*.

strain. Both plasmids have been sequenced recently (Okinaka *et al.*, 1999a,b).

Current models of *B. anthracis* ecology rely on its pathogenicity, i.e. the spores are ingested by herbivores, the animals becomes infected, and the bacteria proliferate in the lymphoid glands concomitantly expressing the exotoxins, which ultimately leads to the death of the animal (see Fig. 1). Once the animal is dead, the vegetative cells of *B. anthracis*, having reached a serum concentration of $>10^7$ cells ml^{-1}, will be outcompeted by anaerobic bacteria from the gastrointestinal tract through antagonistic interactions (Dragon and Rennie, 1995). The environmental fate of the spore is not known in detail. The spores will survive 'indefinitely' in dry and protected environments. However, exposure to sunlight for 4 h has a significant negative effect on the survival of the spores (Lindeque and Turnbull, 1994). Furthermore, photoinduced repair of UV damage is absent in *B. anthracis* spores of the Sterne vaccine strain (Knudson, 1986). A few reports stated that spores could actually germinate in nature when conditions are favourable. Van Ness (1971) reported that soil pH above 6.0 and temperatures above 15.5°C favour outbreaks of anthrax. The term 'incubator areas' has been introduced to describe puddles in which decaying grass and other organic matter constitute the nutrients necessary for the germination of *B. anthracis* spores. However, the study by Van Ness (1971) did not show actual growth of *B. anthracis* in these incubator areas, and other studies indicated that *B. anthracis* has very specific growth requirements, making it very unlikely for the spores to germinate outside a host (Minett and Dhanda, 1941). It is also noteworthy that growth of *B. anthracis* outside a host often leads to loss of virulence caused by loss of the plasmid carrying the capsule gene (pXO2) – an argument that further reduces the incubator area theory. As a consequence, Dragon and Rennie (1995) renamed the areas 'storage areas'.

Tabaniid flies (horse and deer flies from, for instance, the genera *Tabanus* and *Chrysops*) have been reported to disseminate anthrax and to excrete *B. anthracis* in their faeces up to 13 days (the average lifetime of adult tabaniid flies) after initial feeding on animals infected with anthrax (Khrisna Rao and Mohiyudeen, 1958). These flies have also shown ability to transmit anthrax even after subsequent feeding on uninfected hosts (Krinsky, 1976). In an experiment with radioactive-labelled blood from an impala carcass, Braack and De Vos (1990) were able to show that the faeces of carrion-feeding blowflies (*Diptera*, Family Calliphoridae) were deposited in the vicinity of the carcass on leaves and twigs. The kudu antelopes in South Africa normally eat leaves and twigs and could therefore be more at risk of acquiring anthrax disseminated this way. Moreover, these browsers are normally severely affected in anthrax epizootic episodes. In laboratory experiments, stable flies and mosquitoes have been shown to transmit *B. anthracis* after feeding on infected animals (Turell and Knudson, 1987). Also, faeces samples collected from scavengers in the Etosha National Park in Namibia revealed *B. anthracis* spores in more than half the samples (Lindeque and Turnbull, 1994), indicating a possible route of dissemination. Furthermore, the same study showed a rapid decline in shed, vegetative bacilli, and failed to demonstrate multiplication of *B. anthracis* in the environment.

Properties of *Bacillus thuringiensis*

Bacillus thuringiensis is generally regarded as an insect

pathogen, because of its ability to produce large crystal protein inclusions (δ-endotoxins) during sporulation, the only feature that can distinguish *B. thuringiensis* from *B. cereus* (Baumann *et al*., 1984). These inclusions, which constitute up to 25% of the dry weight of the sporulated cells (Agaisse and Lereclus, 1995), are responsible for the biopesticide activity of the bacterium and its target specificity (van Rie *et al*., 1990) (see Fig. 1). The genes encoding the insecticidal proteins are generally located on large transferable plasmids (Kronstad *et al*., 1983; González and Carlton, 1984). The *B. thuringiensis* denomination actually comprises a considerable number of isolates covering a broad range of toxins active against larvae from different insect orders, especially *Lepidoptera*, *Diptera* and *Coleoptera*. At present, more than 235 delta-endotoxin gene sequences have been described (Crickmore *et al*., 2002), and 82 different serotypes have been reported (Lecadet *et al*., 1999). The numbers of delta-endotoxin genes are thought to grow steadily as most *B. thuringiensis* strains carry more than one delta-endotoxin gene. Furthermore, several *B. thuringiensis* strains are known to produce vegetative insecticidal proteins (VIPs). Unlike the δ-endotoxins, the expression of which is restricted to sporulation, VIPs are expressed in the vegetative stage of growth starting at mid-log phase as well as during sporulation.

Although *B. thuringiensis* is an insect pathogen, the ecology of the bacteria is still somewhat of an enigma. According to Martin and Travers (1989), *B. thuringiensis* is a ubiquitous soil microorganism, but it is also found in environmental niches, including phylloplane and insects. Descriptions of natural epizootic episodes are very rare but were reported in the first observation of *B. thuringiensis* by Ishiwata (Milner, 1994), in water mills (Vankova and Purrini, 1979), in a corn crop (Porcar and Caballero, 2000) and in mosquito breeding habitats (Damgaard, 2000). In addition to being organized into a structured parasporal crystal, the δ-endotoxins can also be embedded in the spore wall. Du and Nickerson (1996) found that germination of spores of *B. thuringiensis* ssp. *kurstaki* HD-73 with Cry1Ac embedded in the spore coat could be activated by alkaline conditions, whereas selected Cry-negative *B. thuringiensis* ssp. *kurstaki* HD-73 could not. Furthermore, cry^+ spores could bind to toxin receptors in brush border membrane preparations, a binding that also stimulated spore germination (Du and Nickerson, 1996). This phenomenon may, in part, explain the evolutionary advantage of possessing δ-endotoxins, namely the ability for *B. thuringiensis* to germinate faster than *B. cereus* and thus have a greater chance to proliferate and dominate in an insect gut, even in the absence of the crystalline δ-endotoxins. It is important to note, however, that δ-endotoxins have, *per se*, no apparent antimicrobial effect for enhancing colonization efficacy (Koskella and Stotzky, 2002).

Several facts and/or premises on the ecological niche occupied by *B. thuringiensis* have been reported: (i) *B. thuringiensis* does not grow in soil, but is deposited there by insects (Glare and O'Callaghan, 2000); (ii) *B. thuringiensis* may grow in soil when nutrient conditions are favourable (Saleh *et al*., 1970); and (iii) it occupies the same niche as *B. cereus*; (iv) vegetative *B. thuringiensis* proliferates in the gut of earthworms, leather jacket larvae and in plant rhizospheres (Hendriksen and Hansen, 2002); (v) multiplication of *B. thuringiensis* occurs in insects weakened by the presence of other pathogens (Eilenberg *et al*., 2000), and (vi) germinating *B. thuringiensis* ssp. *israelensis* were found in excreted food vacuoles of protozoa (Manasherob *et al*., 1998).

These different possibilities are not mutually exclusive. It is conceivable that *B. thuringiensis* is a natural inhabitant of the intestinal systems of certain insects, with or without provoking disease and eventually death. Thus, the bacterium is able to be released in soil and can subsequently proliferate when conditions are propitious. Hansen and Salamitou (2000) hypothesized that *B. thuringiensis* is a natural inhabitant of the digestion system of many invertebrates. As such, if the animal is diseased, the *B. thuringiensis* present in the digestion system can start to grow in the dying/dead carcass. As nutrients become limited, sporulation occurs, along with the production of δ-endotoxins. These spores and toxins can then contribute to a local epizootic in dense populations of target organisms. The presence of *B. thuringiensis* in the intestine of mammals is transient, indicating that the food of these animals has varying contents of *B. thuringiensis* (Swiecicka *et al*., 2002). Along the same lines, long-term sheep feeding with *B. thuringiensis*-based biopesticide preparations ($\approx 10^{12}$ spores daily for 5 months) did not harm the animals (Hadley *et al*., 1987). Furthermore, recent studies of faecal samples from greenhouse workers did not show adverse effects after exposure to *B. thuringiensis* (Jensen *et al*., 2002)

Rhizoid-growing and psychrotolerant bacteria

Rhizoid growth is characterized by the production of colonies with filaments or root-like structures that may extend several centimetres from the site of inoculation. Relatively few data are available on the rhizoid-growing bacteria *B. mycoides* and *B. pseudomycoides*, and more specifically on their ecology. As for the other members of the *B. cereus* group, they have been isolated from various environmental niches, including manured soils (Klimanek and Greilich, 1976), activated sludge, arthropod guts (C. Vannieuwenburgh and J. Mahillon, unpublished results) or plant rhizosphere, where they are thought to have antagonistic activity against fungal species (Pandey *et al*., 2001). Similarly, inhibition of the pathogen *Listeria monocytogenes*

by putative *B. mycoides* has also been reported in silage (Irvin, 1969). Although the rhizoid growth is characteristic of *B. mycoides*, non-rhizoid variants have been described; in a study of environmental isolates of *B. mycoides* by von Wintzingerode *et al*. (1997), it was found that fatty acid analysis identified the majority of the isolates as *B. mycoides* even though they lacked the characteristic rhizoid growth. Even less information has been gathered on *B. weihenstephanensis*, which regroups part of the psychrotolerant *B. cereus* isolates (Lechner *et al*., 1998; Stenfors and Granum, 2001), except for their wide distribution in natural habitats (von Stetten *et al*., 1999).

The hidden life cycle of *B. cereus*

One major consequence of the lack of knowledge on the ecology of *B. cereus* is that pleomorphism of *B. cereus* has not been given much attention. This could result partly from the general notion of modern microbiologists that bacteria only occasionally show slight morphological variation. In the very early days of microbiology, the study of microorganisms was almost exclusively restricted to microscopical observations and, hence, surprisingly detailed observations were made then.

Bacillus cereus is a well-known food poisoning bacterium. *B. cereus* causes two distinct types of food poisoning, characterized either by diarrhoea and abdominal pain (diarrhoeal syndrome) or by nausea and vomiting (emetic syndrome). The latter has often been associated with fried rice. Apart from food poisoning cases, there are only a few reports on intestinal carriage of *B. cereus*. Turnbull and Kramer (1985) reported seasonal changes in the isolation of *B. cereus* ranging from 24.3% in the winter to 43% in the summer from faecal samples from 120 school children. Ghosh (1978) reported the presence of *B. cereus* in 100 samples from 711 adults (14%). Both papers stated that, because of the omnipresence of *B. cereus* in many food products, the bacteria are inevitably ingested in small numbers and thus contribute to the transitory intestinal flora.

A place to look for this bacterium in its natural niche is the gut microflora of invertebrates. In certain arthropods, the 'intestinal stage' of *B. cereus* has been shown to be filamentous, the so-called *Arthromitus* stage. In fact, this filamentous stage of the bacterium was discovered in different soil-dwelling arthropods as early as 1849 (Leidy, 1849). The filamentous forms of *B. cereus* have been studied in continuous cultures (Wahren *et al*., 1967) and have lately been proposed as the normal intestinal stage of *B. cereus sensu lato* in soil-dwelling insects (Margulis *et al*., 1998). Furthermore, colonization of mosquito larvae and various soil-dwelling pests by *B. cereus* has been observed (Feinberg *et al*., 1999; Luxananil *et al*., 2001; Wenzel *et al*., 2002) (see Fig. 1).

Other circumstantial evidence supports the data on *B. cereus* colonization of insect gut systems. In aphids, Dasch *et al*. (1984) reported that the introduction of penicillin had little effect on growth and, as evident from Table 1, the majority of the members of the *B. cereus* group are known to produce β-lactamases. In one case, the symbiont of *Cletus signatus* (a hemipteran insect) is identified as *B. cereus* var. *signatus* (Singh, 1974). A high frequency of vegetative *B. cereus* and *B. mycoides* has been found in the gut of the earthworm *Lumbricus terrestris* (B. M. Hansen and N. B. Hendriksen, unpublished results).

Gene transfer in the environment

Interestingly, earthworms are known to contribute to gene transfer activity with gut passage being a prerequisite for DNA transfer (Daane *et al*., 1996; Thimm *et al*., 2001). Other insects have been shown to promote gene transfer, and transfer of *B. thuringiensis* plasmids has been observed in lepidopteran larvae (Jarrett and Stephenson, 1990; Thomas *et al*., 2000; 2001). It is therefore tempting to envisage the continuous exchange of *B. thuringiensis* plasmids, as these phenotype/virulence plasmids are easily transferable by transduction, mobilization or conjugation (Reddy *et al*., 1987; Green *et al*., 1989; Stepanov *et al*., 1989; Jensen *et al*., 1996). The actual exchange of DNA is further corroborated by the data presented in Table 1, e.g. the replicon of the virulence plasmid pXO2 is almost identical to the replicon found on the conjugative plasmid pAW63 from *B. thuringiensis* ssp. *kurstaki* HD-73 (Wilcks *et al*., 1999). Other genotypical features such as the presence of genetic markers for phospholipase C and non-haemolytic enterotoxin genes characteristic of *B. cereus* further substantiate the close relationship among these species. Furthermore, serotyping of *B. thuringiensis* has revealed that the number of cross-reacting H-antigens among *B. cereus* strains is increasing (Lecadet *et al*., 1999).

However, the case of *B. anthracis* has its own particularities. It now seems likely that *B. anthracis* does not simply stem from the superposition of the virulence genes borne by the pXO1 and pXO2 plasmids on the chromosomic background of an opportunistic bacteria, *B. cereus*. Recent studies have indeed indicated that the 'emergence' of *B. anthracis* as a specialized animal and human pathogen has most probably proceeded through a stepwise, reciprocal adaptation between its chromosomal and extrachromosomal genomes (Mignot, 2002). This has resulted in a finely tuned gene regulation of different operons and regulons, such as those involved in sporulation/germination, haemolytic activity, capsule formation or exotoxin expression. These complex genomic cross-talks are thought to be mediated by an arsenal of gene regulators, among which are the plasmid-encoded PagR and AtxA

Table 1. Selected phenotypic, genotypic and ecological features of *B. anthracis*, *B. thuringiensis* and *B. cereus*.

	B. anthracis	*B. thuringiensis*	*B. cereus*
Phenotype			
Penicillin resistant (β-lactamase production)	11% of tested *B. anthracis* strains showed resistance to penicillin G (Cavallo *et al.*, 2002)	Yes	1% of tested strains showed no resistance to ampicillin (Rusul and Yaacob, 1995)
Haemolytic activity[a] (on sheep erythrocytes)	Weak haemolysis by some strains of *B. anthracis* (Drobniewski, 1993; Guttmann and Ellar, 2000)	Yes	Few haemolysis-negative mutants have been isolated
Motility	Isolated monoflagellar *B. anthracis* have been described (Liang and Yu, 1999). Occasional motile strains (Brown and Cherry, 1955)	Spontaneous flagella-minus mutants of *B. thuringiensis* can be readily isolated	4% of tested strains showed no motility (Logan and Berkeley, 1984). Occasional isolation of non-motile variants (Brown and Cherry, 1955)
Crystalline parasporal inclusions	No	6% of tested strains showed no inclusions (Logan and Berkeley, 1984)	No
Mucoid colony (capsule synthesis)	Yes	No	No
Gamma phage sensitivity	Several rare *B. anthracis* are refractory (Abshire *et al.*, 2001)	No	*B. cereus* ATCC4342 susceptible (Abshire *et al.*, 2001)
Chitinase activity	Activity was not found in *B. anthracis* Guttmann and Ellar, 2000)	Yes	Yes
Genotype			
pXO1	Yes	Sequence homology to pBtoxis of *Bt* ssp *israelensis* (Berry *et al.*, 2002) IS*231* from *Bt* ssp. *finitimus* found in pXO1 (Okinaka *et al.*, 1999b) *B. thuringiensis* ssp. *kurstaki* (ATCC 33679) shows high homology to an unknown ORF in pXO1 (base numbers 121815–122327) (Okinaka *et al.*, 1999b; Pannucci *et al.*, 2002)	*B. cereus* (ATCC 43881) shows high homology to an unknown ORF of pXO1 (co-ordinates 121815–122327) (Okinaka *et al.*, 1999b; Pannucci *et al.*, 2002)
pXO2	Growth of *B. anthracis* outside a host often leads to loss of pXO2	The replicons of pAW63 from *Bt* ssp. *kurstaki* are almost identical to that of pXO2 (Wilcks *et al.*, 1999)	
Phospholipase C	+ (Mignot *et al.*, 2001; G. B. Jensen, unpublished results)	+	+
*nhe*A gene (accession no. Y19005)	+ (Mignot *et al.*, 2001; G. B. Jensen, unpublished results)	+	+
Ecology			
Host range (toxin specific)	Vertebrates	Invertebrates Specific toxins are only active against a limited number of related invertebrate hosts	Not known
Distribution	Worldwide, but many areas not yet studied	Worldwide, but lack of success in isolating in Antarctica (Wasano *et al.*, 1999)	Worldwide
Prevalence in hosts	Endemic in Africa/Asia	Generally low natural levels of infection, occasional epidemics among mosquitoes and insects in stored product environment (Milner, 1994)	Present in invertebrates (gut system), but not regarded as a disease of invertebrates

a. Note that at least four distinct haemolytic protein complexes can participate in the haemolytic activity.

(Guignot *et al*., 1997; Mignot *et al*., 2001; Mignot, 2002). For instance, the pleiotrophic regulator PlcR that regulates several virulence functions in *B. cereus* (Gohar *et al*., 2002) is inactive in *B. anthracis* because of a nonsense mutation. The introduction of a functional PlcR in *B. anthracis* activates several *B. cereus*-like virulence functions, which are not normally expressed in *B. anthracis* (Mignot *et al*., 2001). This is in agreement with the data of Bonventre (1965), who found that, in contrast to *B. cereus*, filtrates from liquid cultures of *B. anthracis* were not toxic to animal tissue culture cells.

Table 1 lists the textbook characteristics of each member of the *B. cereus* group together with exceptions found in the literature. These data are intended to display both the close relationship among the species and, subsequently, the possible pitfalls of data misinterpretation. Thus, *B. anthracis* seems to constitute a narrow group of highly similar strains, which have only recently been distinguished genetically (Jackson *et al*., 1999; Ticknor *et al*., 2001). Consequently, and as the most significant differences are plasmid encoded, it seems appropriate to (p)reserve the name *B. anthracis* for *B. cereus* strains possessing the pXO1 and pXO2 plasmids. Likewise, emetic *B. cereus* strains constitute a narrow group of bacteria, most of which belong to the *B. cereus* H-1 serotype. Furthermore, strains that produce the emetic toxin do not show expression of enterotoxins and starch hydrolytic activity (Agata *et al*., 1996; Pirttijarvi *et al*., 2000).

Conclusion

The presence of *B. anthracis* in both vultures and various biting insects reveals multiple routes of recycling of *B. anthracis*. Whether there is *de facto* colonization of the intestinal systems of both the vultures and the insects or the observations cited here resulted from transient exposures resulting from feeding habits is still debatable. However, the carnivorous nature of the *Tabanus* larvae may equip the adult fly with an intestinal flora comprising any member(s) of the *B. cereus* group and, although much of the data on anthrax transmission by tabaniid flies is experimental, the importance of tabaniid flies in natural outbreaks is conceivable. According to previously presented data, *B. cereus* can enter a filamentous stage in which it colonizes a variety of insects. In this context, it is suggested, as illustrated in Fig. 2, that members of the *B.*

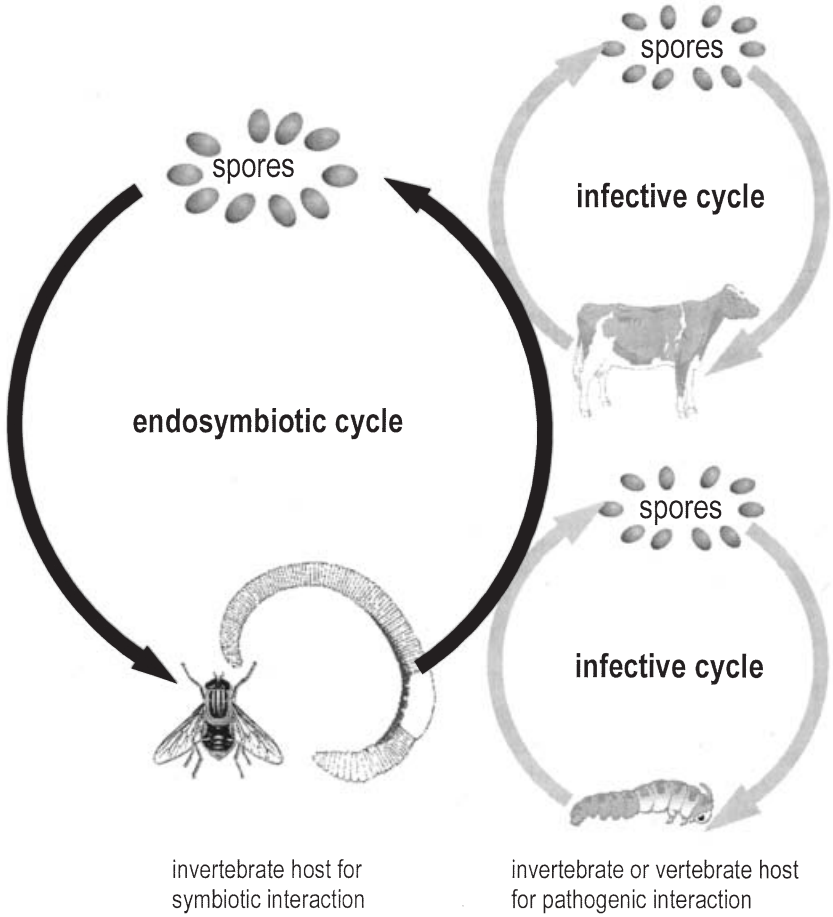

Fig. 2. A supposed model in which the members of the *B. cereus* group experience two life cycles: one type in which the bacteria live in a symbiotic relation with their invertebrate host(s) and another, more infrequent life cycle, in which the bacteria can multiply rapidly in another infected insect host or a mammal.

cereus group experience two types of life cycles: one in which the bacteria live in a symbiotic relation with their invertebrate host(s) and another, more infrequent life cycle, in which the bacteria can multiply rapidly in another and infected host (invertebrate or vertebrate). The relationship between the two types of life cycle has not yet been documented experimentally, but some indications exist. In the case of a pathogenic relationship, the invertebrate host from the symbiotic relationship becomes the vector of the disease.

For example, a recent study showed that female mosquitoes are attracted to culture filtrates of *B. thuringiensis* for ovipositioning (Poonam *et al*., 2002). It is possible that these and other insects could have a preference for ovipositioning in areas where *B. thuringiensis* is frequently located, i.e. soil (Martin and Travers, 1989), activated sludge (Mizuki *et al*., 2001), water (Ichimatsu *et al*., 2000; Maeda *et al*., 2000) and the 'storage areas' mentioned earlier, subsequently giving the larvae a possibility of being fitted with an intestinal flora consisting of members of the *B. cereus* group. These bacteria can then provide their host with enhanced capabilities, for instance degrading cellulose (Wenzel *et al*., 2002).

Further studies on the ecology of *B. anthracis*, *B. cereus* and *B. thuringiensis* will hopefully not only shed light on the working models proposed here. They will also enable us to set up better controlling programmes that could cope with different objectives. One objective is to avoid *B. anthracis* outbreaks especially in risk areas. Other objectives are to improve the biotechnological use of *B. thuringiensis* and consequently obtain better control of insect pests.

Although experimental evidence is still missing, it is likely that the rhizoid-growing bacteria share part of the horizontal gene pool of the *B. cereus senso lato* group, using plasmid conjugation, phage transduction or DNA transformation. Consequently, it remains to be seen whether, and how, these still cryptic bacteria participate, directly or indirectly, in the various life cycles of the other members of the *B. cereus group*.

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