Ecological Mysteries: is *Bacillus thuringiensis* a Real Insect Pathogen?

Guillem Marco1, Manuel Porcar1,2

1. Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València. Apartado Postal 22085, 46071 Valencia, Spain
2. Fundació General de la Universitat de València, Spain

Corresponding author email: manuel.porcar@uv.es; Authors

Received: 17 Jan., 2012
Accepted: 26 Jan., 2012
Published: 29 Jan., 2012

This is an open access article published under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract *Bacillus thuringiensis* (*Bt*) can kill insects and multiply in their bodies, but it can also grow in semi-synthetic media; is found in environments were insects are absent; and has been reported to require midgut-associated bacteria for toxicity. We propose here a novel life cycle for *Bt* combining insect-based and insect-independent life cycles.

Keywords Bacillus thuringiensis (*Bt*); Insecticidal crystal proteins (Cry); Microbial ecology; Insect pathogenicity

Background

The bacterium *Bacillus thuringiensis* (*Bt*) has been successfully used as a biopesticide for decades and, from 1996 onwards, also as a source of genes for the construction of transgenic plants (*Bt* crops) protected against insect pests. However, because of a lack of a consistent linkage between insects and *Bt*, the real life cycle of this well-known bacterium is still unclear. It is obvious that *Bt* can kill insects and multiply in their bodies, but *Bt* can also grow very well on simple semi-synthetic media, including LB. Moreover, *Bt* strains have been recovered from environments were insects are scarce or absent; found to be nonpathogenic towards the species they were originally isolated from and yet display activity against insects or other invertebrate species they will certainly rarely encounter (Martin and Travers, 1989); and, last but not least, reported to require midgut-associated bacteria to display insecticidal activity in some cases (Broderick et al., 2006) but not in others (Johnston and Crickmore, 2009). We believe that the answer to the question the title of this letter raises lies on the analysis of the reports on the ability of *Bt* to survive and eventually multiply, in the presence or absence of insects.

*Bt* usually displays a dual biological activity involving toxicity and pathogenicity. The parasporal insecticidal crystal (Cry) proteins synthesized during sporulation are specifically toxic against a wide range of insects (see: http://www.glfc.cfs.nrcan.gc.ca/bacillus). The mode of action of Cry proteins, which involves solubilisation in the insect midgut, activation by digestive enzymes and binding to specific receptors located on the host cell surface, evidences a strong selection pressure towards insecticidal activity. Moreover, spores are known to synergize this activity, since they can enter the insect body and produce septicaemia. The importance of each of these effects (toxicity and pathogenicity), shows a large variation depending on the target species (Suzuki et al., 2004). The toxicity, rather than the pathogenicity, of *Bt* has been exploited for six decades through *Bt*-based biopesticides that can be sprayed on the field and have a direct impact on insect populations without significant bacterial *in situ* multiplication. But epizootics (epidemics) in insects caused by *Bt* are known to occur on the field, and the most powerful mosquitocidal strain, *Bt* var *israelensis* (De Barjac, 1978), was in fact isolated from a stagnant pond showing mass mosquito mortality.

Beyond insects, an alternative insect-free cycle has been proposed. Indeed, *Bt* strains have been reported to multiply on the surface of the leaves and, more interestingly, even to colonize plantlets from the soil (Bizzarri and Bishop, 2008). A comparison between crystal- and non-crystal-producing strains revealed that both exhibited similar ability in terms of leaf colonization, indicating that insecticidal parasporal crystals was not
a mitigating factor for Bt to grow in the absence of insects. Such soil-plant life cycle might not exclude insects, since multiplication in the bodies as well as in the frass of surviving insects has been reported (Bizzarri and Bishop, 2008; Prabhakar and Bishop, 2009), and this would certainly be a supplementary source of spores to re-inoculate soil and then plants through young leaflets colonization. Additionally, since colonized plants are at least partially protected against phytofagous insects (Prabhakar and Bishop, 2009), a plant symbiont-like role for Bt can also be considered.

The analysis of these and similar reports, taken together, lead us to define a new life cycle for Bt that combines the insect-based and insect-independent cycles mentioned above. The proposed cycle, shown in Figure 1, integrates plants, frass and soil as sub-cycles of a main insect-centered cycle. But the key question still remains unanswered: is Bt a real insect pathogen? We think that, beyond the reported epizootics outbreaks, a major argument proving that Bt is indeed an insect pathogen is the elegant specificity of the arsenal of Bt insecticidal toxins active on Lepidoptera, Coleoptera, Diptera and also other invertebrates such as nematodes, which is an obvious adaptive character. But the well-documented insect-free plant-based cycles, horizontal gene transfer phenomena such as Bt Cyt genes identified in the aphid pathogen phytopathogenic bacterium Dickeya dadantii (Grenier et al., 2006), or the ease with which Bt can be grown in the lab strongly reveal that Bt is, additionally to an insect pathogen, also an opportunistic non-pathogen microorganism able to persist and/or multiply on a range of substrates including feces or plant surfaces from which it might re-enter its basic insect-centered life cycle.

Multiplication can be carried out in insects, but also in frass or plant surfaces. Soil may play a central role as a reservoir of spores. The presence of B. thuringiensis on plants can originate from colonization of leaflets from soil, spreading of frass on the leaves from living insects or from decomposition of spore-rich insect bodies, particularly after epizootics outbreaks. Notice that aquatic strains such as B. thuringiensis var. israelensis may display a variation of this with a simpler insect (i.e. mosquito larvae)-freshwater cycle.

Figure 1 Proposed life cycle for Bacillus thuringiensis

Authors' contributions
GM and MP had contributed equally to the work reviewing bibliography and writing and revising the article.

Acknowledgements
We thank two anonymous reviewers for their help in preparation of manuscript.

References
Bizzarri M.F. and Bishop A.H., 2008, The ecology of Bacillus thuringiensis on the Phylloplane: colonization from soil, plasmid transfer, and interaction with larvae of Pieris brassicae, Microb. Ecol., 56(1): 133-139 http://dx.doi.org/10.1007/s00248-007-9331-1 PMid:17973155
Broderick N.A., Raffa K.F., and Handelsman J., 2006, Midgut bacteria required for Bacillus thuringiensis insecticidal activity, Proc. Natl. Acad. Sci., USA, 103(41): 15196-15199 http://dx.doi.org/10.1073/pnas.0604865103 PMid:17005725 PMCid:1622799
De Barjac H., 1978, A new variety of Bacillus thuringiensis very toxic to mosquitoes: B. thuringiensis var. israelensis serotype 14, C.R. Acad. Sci. Hebld. Seances Acad. Sci. D., 286: 797-800 PMid:1786699
Grenier A.M., Duport G., Pagès S., Condemine G., and Rahbé Y., 2006, The phytopathogen Dickeya dadantii (Erwinia chrysanthemi 3937) is a pathogen of the pea aphid, Appl. Environ. Microbiol., 72(3): 1956-1965 http://dx.doi.org/10.1128/AEM.72.3.1956-1965.2006 PMid:16517643 PMCid:1393189
Johnston P.R., and Crickmore N., 2009, Gut bacteria are not required for the insecticidal activity of Bacillus thuringiensis toward the tobacco hornworm, Manduca sexta, Appl. Environ. Microbiol., 75(15): 5094-5099 http://dx.doi.org/10.1128/AEM.00966-09 PMid:19525273 PMCid:2725506
Martin P.A., and Travers R.S., 1989, Worldwide abundance and distribution of Bacillus thuringiensis isolates, Appl. Environ. Microbiol., 55(10): 2437-2442 PMid:16348022 PMCid:203101
Prabhakar A., and Bishop A.H., 2009, Effect of Bacillus thuringiensis naturally colonising Brassica campestris var. chinensis leaves on neonate larvae of Pieris brassicae, J. Invertebr. Pathol. 100(3): 193-194 http://dx.doi.org/10.1016/j.jip.2009.02.001 PMid:19232351
Suzuki M.T., Lereclus D., Arantes O.M., 2004, Fate of Bacillus thuringiensis strains in different insect larvae, Can. J. Microbiol., 50(11): 973-975 http://dx.doi.org/10.1139/w04-087 PMid:15644915