The Role of Entomopathogenic Bacillus Thuringiensis: Is It Only Insect Pathogen?

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The use of biopesticides, as a component of integrated pest management (IPM), has been gaining acceptance over the world. An entomopathogenic organism should be highly specific and effective against the target pest and should demonstrate the potential to be successfully processed by continuous production technology.

As an entomopathogenic organism, Bacillus thuringiensis (Bt) fulfills all these requirements. Since its discovery about a century ago, Bt has been used as a biopesticide in agriculture, forestry and mosquito control. Its advantages are specific toxicity against target insects, lack of polluting residues and safety to non-target organisms such as mammals, birds, amphibians and reptiles. Today Bt is the most successful commercial microbial insecticide, comprising about 90% of the biopesticide market. Although several proteins and other compounds produced by Bt contribute to its insecticidal activity, by far the most important components are the proteins that form parasporal crystalline inclusions during sporulation. Transgenic crops based on insecticidal crystal proteins of Bt are now an international industry with revenues of several billion dollars per year.

B. thuringiensis is a Gram-positive, spore-forming, soil bacterium and is the most successful biological control agent that produces distinctly shaped crystals during sporulation. Its life cycle is simple. When nutrients and environmental conditions are sufficient for growth, the spore germinates producing a vegetative cell that grows and reproduces by binary fission. Cells continue to multiply until one or more nutrients, such as sugars, amino acids, or oxygen, become insufficient for continued vegetative growth. Under these conditions, the bacterium sporulates producing a spore and parasporal body, the latter, as noted above, composed primarily of one or more insecticidal proteins in the form of crystalline inclusions. These crystals are composed of proteins, known as insecticidal crystal proteins (ICPs), and also called Cry proteins, which are selectively toxic to different species of several invertebrate phyla. Much of the technology developed to study structure and function of Cry proteins has provided the foundation for genetic engineering of this class of biopesticides [1].

Individual Cry toxin has a defined spectrum of insecticidal activity, usually restricted to a few species in one particular order of Lepidoptera (butterflies and moths), Diptera (flies and mosquitoes), Coleoptera (beetles and weevils), Hymenoptera (wasps and bees), and nematodes, respectively [2]. A few toxins have an activity spectrum that spans two or three insect orders due to the combination of toxins in a given strain.

Endotoxin crystals must be ingested to have an effect. This is the reason sucking insects and other invertebrates such as spiders and mites are not sensitive to Cry proteins used in Bt insecticides or Bt crops. Their mode of action involves several events that must be completed several hours after ingestion in order to lead to insect death. Following ingestion, the crystals are solubilized by the alkaline conditions in the insect midgut and are subsequently proteolytically converted into a toxic core fragment [3]. Under the highly acidic conditions in stomachs of many vertebrates, including humans, Cry and Cyt protein crystals may dissolve, but once in solution they are rapidly degraded to non-toxic peptides by gastric juices, typically in less than 2 min. During proteolytic activation, peptides from the N terminus and C terminus are cleaved from the full protein. Activated toxin binds to receptors (glycoprotein or glycolipid) located on the apical microvillar membranes of epithelial midgut cells [4]. After binding, toxin adopts a conformation allowing its insertion into the cell membrane and form a cation-selective channel. Subsequently, oligomerization occurs, and this oligomer forms a pore or ion channel induced by an increase in cationic permeability within the functional receptors contained on the brush borders membranes [5]. Complete nature of this process still remains unknown [6]; however, it is believed that toxin aggregation occurs at the membrane surface after receptor binding, or alternatively only after the toxin inserts itself into the membrane. Once a sufficient number of these channels have formed, a surplus of cations, K+ for example, enter the cell. This causes an osmotic imbalance within the cell, and the cell compensates by taking in water. This process, referred to as colloid-osmotic induced lysis, continues until the cell ruptures and exfoliates from the midgut microvillar membrane. When a sufficient number of cells have been destroyed, the midgut epithelium loses its integrity. This allows the alkaline gut juices and bacteria to cross the midgut basement membrane, resulting in death, the latter caused by Bt bacteremia and tissue colonization in lepidopteran species.

The new tools of biotechnology are changing the way scientists can address problems in the agriculture. Transgenic technology, involving a wide range of pesticidal genes from Bt, dominates the scenario of agricultural biotechnology. At the same time, Bt technology is also the most vehemently criticized area of agricultural biotechnology. Genetic improvement of Bt strains for the development of novel biopesticides entails increasing their potency against target insects, broadening the insecticidal spectra for specific crop applications, improving persistence on plants, and optimizing fermentation production. The biotechnological applications of Bt as biopesticide have increased in the last ten years and a new way of expanding the biotechnology of Bt has started. The challenge is great and there is still a need for research in different areas. Many of the topics involved in Bt biotechnology are deeply discussed in Bacillus thuringiensis biotechnology book edited by Sansinenea E. in 2012 [7].

Formerly, only the insecticidal properties of B. thuringiensis attracted extensive attention. However, B. thuringiensis has also been shown to produce other active components that are secondary metabolites, some only recently discovered, which have been implicated in various industrial applications. These include industrial production of enzymes
with great interest in detergent and food sectors; the production of primary metabolites such as vitamins and ribonucleosides of secondary metabolites including bacteriocins and biosurfactants and of plant growth promoting formulations. Moreover, recent studies have shown that the aerobic spore formers can produce fine chemicals with interesting biotechnological applications like for example carotenoid pigments and a variety of biopolymers including poly-γ-glutamic and lactic acids. These findings open perspectives for new biotechnological applications of Bacillus and related species.

Among the most important secondary metabolites of Bt are the spore, β-exotoxin (also called thuringiinsin), antibiotics such as zwitermicin, vegetative insecticidal proteins (Vip’s), phospholipases, chitinases, and various proteases [7,8]. In general, these metabolites serve as; (i) competitive weapons used against other bacteria, fungi, amoebae, plants, insects and large animals; (ii) metal transporting agents; (iii) symbiosis effectors between microbes and plants, nematodes, insects and higher animals; (iv) sexual hormones; and (v) as differentiation factors [9]. This wide variability of the structure and activity of the secondary compounds expands the potential industrial importance of the genus Bacillus and its related genera [8]. Besides, Bacillus species form spores that can be easily formulated and have high viability compared with vegetative cells. Finally they are commonly diffused in the environment including soil [8]. The metabolites produced by Bacillus spp. can also affect the microflora in the rhizosphere, providing an environment antagonistic to pathogens, or they can trigger host defense responses [10].

For many years, basic biologists were uninterested in secondary metabolism; study of this type of non-essential metabolism was left to industrial scientists and academic chemists and pharmacognostics. Today, the situation is different. The wide structural variability of these compounds has attracted the curiosity of chemists and the biological activities possessed by natural products have inspired the study of this type of non-essential metabolism. Screening of microbial extracts reveals the biological activities possessed by natural products have inspired the study of this type of non-essential metabolism. Screening of microbial extracts reveals the large structural diversity of natural compounds with broad biological activities, such as antimicrobial, antiviral, immunosuppressive, and antitumor activities, that enable the bacterium to survive in its natural environment. Many economically valuable microbial products are secondary metabolites.

The frontier of expanding knowledge is now secondary metabolism which poses many questions of considerable interest to science: What are the functions of secondary metabolites in nature? How are the pathways controlled? What are the origins of secondary metabolism genes? What are the origins of the resistance genes which producing organisms use to protect themselves from suicide? Are these the same genes as those found in clinically-resistant bacteria? Fortunately, molecular biology has produced tools with which to answer these questions. It is clear that basic mechanisms controlling secondary metabolism are now of great interest to many academic (and industrial) laboratories throughout the world. These findings widen the potential industrial importance of Bacillus spp., particularly of B. thuringiensis, beyond insecticidal usage and may help explain the role of Bacillus spp. in the soil ecosystem.

References

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