REVIEW

Bacillus thuringiensis Growth and Toxicity
Basic and Applied Considerations
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Abstract
Despite the known importance of the composition of culture media and culture conditions on Bacillus thuringiensis growth and toxicity, very few reviews are concerned with this subject. This article reviews some aspects of the microbiology of Bacillus thuringiensis, and how toxicity is affected by the composition of growth media and bioreactor operation.

Index Entries: Bacillus thuringiensis; toxic proteins; medium composition.

1. Introduction
Since its discovery by Ishiwata in 1902, Bacillus thuringiensis (BT) has raised the attention of investigators researching in many different areas of microbiology, entomology, and biochemistry, because of its ability to synthesize a toxic protein, specific against several insects. Early studies were focused on the control of some agricultural pests and field applications, with variable success. Soon after the suggestion that the parasporal crystal was the toxic agent (1,2), studies on the purification and characterization of the toxic component were performed (3). This led to several groups working on the biochemistry and characterization of the parasporal crystal (4,5), together with research on the physiology and biochemistry of BT (6-8). In the last 10 years developments in molecular biology and genetic engineering techniques have led to an increased understanding of the molecular biology of crystal formation (9). Simultaneously, exhaustive studies have been made regarding the mode of action of BT 8-endotoxin (10-12).

Obviously, a great number of reviews and books dealing with different aspects of BT have been published. Among them, it is worthwhile to mention the reviews by Rogoff and Yousten (13) and Bulla et al. (14), related to general aspects of BT; Lüthy et al. (15), with a complete analysis of BT metabolism; Aronson et al. (16), dealing with BT and other insect pathogens; Rowe and Margaritis (17), covering almost all subjects related to biotechnology of BT (including economic aspects); Whiteley and Schnepf (9) and Höfte and Whiteley (18), two excellent reviews on the molecular biology of BT crystal toxin; Priest (19), a short review on mosquito control; and Gill et al. (20), a thorough analysis of BT toxin mode of action. At least three books have been published, dealing with different aspects of BT (21-23). However, despite the known importance of the composition of culture media and culture conditions on BT growth and toxicity, very few reviews are concerned with these subjects (24,25). The purpose of this work is to review some aspects of the microbiology of BT, and how toxicity is affected by the composition of growth media and bioreactor operation.

2. Biology of Bacillus thuringiensis
2.1. Microbiology and Biochemistry

BT is a large Gram-positive, spore-forming bacteria that produces, concomitantly with sporu-

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lation, a proteinaceous parasporal crystal known as δ-endotoxin.

2.1.1. Carbon Metabolism

This microbe is a chemoheterotroph, and aerobically oxidizes carbohydrates to organic acids, which are further oxidized to carbon dioxide. During vegetative growth, sugars are mainly metabolized through the Embden-Meyerhof-Parnas pathway yielding pyruvic and acetic acids, which are further oxidized via the Tricarboxylic Acids cycle enzymes (15). These enzymes are fully derepressed when sporulation begins. As BT shows no α-ketoglutarate dehydrogenase activity, it has been concluded that an incomplete TCA cycle is present in this microorganism (7,26). The latter demonstrated the presence of the γ-aminobutyric acid pathway, which transforms glutamate to succinate, thus circumventing the α-ketoglutarate dehydrogenase step.

The rate of catabolism of pyruvic and acetic acids is lower if amino acids are present in the medium (27) because these compounds may act as carbon and nitrogen sources. In the case of glutamate, it is converted to γ-aminobutyric acid, which is metabolized via succinate.

Another important feature of BT is the strong exoprotease production observed when the acids begin to be metabolized. These exoproteases are different from those produced during vegetative growth. They are neither repressed by ammonia nor stimulated by glutamate (28). In addition, they have different substrate specificities (29). The appearance of exoproteases is an early event recognized to be associated with the initiation of sporulation.

2.1.2. Nitrogen Metabolism

Because of the synthesis of both sporulation specific enzymes and crystal proteins, BT must mobilize, during the sporulation phase, most of the nitrogen assimilated during vegetative growth. More than 50% of the amino acids that are present in the pool of free amino acids are alanine and glutamate. The transfer of nitrogen from glutamate to other metabolites is catalyzed mainly by glutamate oxaloacetate transaminase and, to a lesser extent, by glutamate pyruvate transaminase and glutamate dehydrogenase. The pathways for ammonia assimilation are catalyzed by alanine dehydrogenase and glutamate dehydrogenase (26,30). Although glutamate synthase and glutamine synthetase have been found in Bacillus megaterium (31,32), Bacillus licheniformis (33), and Bacillus subtilis (34), as far as the authors are aware, no report about the presence of this system in BT has been made. Meers et al. (35) found that the expression of these enzymes was very high for B. megaterium (among other bacteria) when the growth was ammonium limited. As the majority of the work on BT has been performed in batch cultures (i.e., excess of all nutrients), it should be very interesting to discover whether these enzymes are present when BT is cultivated under NH₄⁺ limitation.

2.2. Genetics

2.2.1. Toxin Genes of Bacillus thuringiensis

It has been demonstrated that crystal protein genes of BT are present on one or more plasmids (36,37). Studies related to gene expression have demonstrated that these genes are transcribed by a sporulation-specific RNA polymerase (38), and that the control of gene expression is exerted at the transcriptional level (39).

The genes encoding for crystal proteins have been classified by Höfte and Whiteley (18) into four classes and several subclasses (Table 1). The major gene classes are CryI, CryII, CryIII, CryIV, and CytA. All genes belonging to class I encode for polypeptides with molecular weights of ca 130 kDa, which are specific for Lepidoptera larvae. Genes from Lepidoptera- and Diptera-specific CryII group and from CryIII group (Coleoptera-specific) encode for polypeptides of about 70 kDa. The genes from group IV (present in BT var israelensis, Diptera-specific) are quite different from the others: CryIVA encodes for a 134-kDa protein; CryIVB, for a protein of 128 kDa; CryIVC encodes for a protein of 78 kDa; and the product of gene CryIVD is a polypeptide of 72 kDa. BT var israelensis presents also a Cyt gene, which codifies for a 27-kDa polypeptide, with activity against invertebrate and vertebrate cells. A closely related gene is found in BT var morri-