Bacillus thuringiensis : Fermentation Process and Risk Assessment. A Short Review

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Several factors make the local production of Bacillus thuringiensis *(Bt) highly appropriate for pest control in developing nations. Bt can be cheaply produced on a wide variety of low cost, organic substrates. Local production results in considerable savings in hard currency which otherwise would be spent on importation of chemical and biological insecticides.*

The use of Bt in Brazil has been limited in comparison with chemical insecticides. Although Bt is imported, some Brazilian reserarchers have been working on its development and production. Fermentation processes (submerged and semi-solid) were applied, using by-products from agro-industries.

As the semi-solid fermentation process demonstrated to be interesting for Bt endotoxins production, it could be adopted for small scale local production.

Although promising results had been achieved, national products have not been registered due to the absence of a specific legislation for biological products. Effective actions are being developed in order to solve this gap.

Regardless of the biocontrol agents being considered atoxic and harmless to the environment, information related to direct and indirect effects of microbials are still insufficient in many cases. The risk analysis of the use of microbial control agents is of upmost importance nowadays, and is also discussed.

Key words: *Bacillus thuringiensis* - fermentation process - risk assessment

The growing world population associated with the need of third world countries to increase their incomes in order to pay foreign debts, and stabilize or improve the standard of living of their population have resulted in considerable alterations of distinct ecosystems.

The maintenance of the ecosystems at acceptable standards depends upon the joint effort of all sectors of the society, involving those related to industrial production, agriculture, mining, extractivism, treatment of urban rejects, and even leisure practices.

In the agricultural sector, the main factors responsible for the environmental deterioration refer to the virtual elimination of native vegetation in frointier regions, inadequate soil management and chemical contamination. Public concern with the presence of chemical residues on food items and pest resistance to those products have leaded government and industries to demand or work on the development of less risky alternatives with emphasis on biological pest control.

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The use of insect pathogens as microbial control agents gained increased support at the international level (Dulmage et al. 1990), mainly against agricultural pests. Great success has been obtained with the development of new strains of the bacterial pathogen *Bacillus thuringiensis* (Bt). The use of Bt for microbial control by itself or as a component in a pest management system has shown its practicability and effective action against pest problems (Burges 1981).

BT STUDIES IN BRAZIL

The first use of commercial Bt in Brazil was in 1960 in the Instituto Agronômico de Campinas (IAC), but the state of the art was not sufficient to promote its use, and farmers then did not understand the considerable differences between chemical and biological insecticide action and effectiveness.

As commercial Bt was imported, and still is, economic reasons have restricted its wider use. So, fermentation technology has to aim at the production of these endotoxins at reduced costs.

FERMENTATION ASPECTS

Submerged process - Studies were initiated in 1970, at the State University of Campinas (UNICAMP) to explore the feasibility of producing

endoxin preparations of Bt, using submerged fermentation with cheap liquid by-products as components of the fermentation media. The problems of the medium composition, its price and their influence on the final cost of the product were studied for mini and pilot scale production. The results obtained, generated two industrial patents (Moraes 1976b, 1985) about the fermentation process, using sugar cane molasse and corn steep liquor as sources of nutrient. Besides these studies, some other UNICAMP staff members studied the rheological characteristics of many culture media, the influence of different aeration levels on the growth and sporulation of Bt, the continuous culture technique for examining the effects of growth rate or its changes on sporulation, and finally the effects of different ways of drying on the viability of the spores and the potency of the insecticide obtained. These works have been registered in several dissertations, thesis, and some published papers (Moraes 1973, 1976a, 1981, 1993, Santana 1980, Lonsano 1982, Capalbo 1982, 1989, Capalbo & Moraes 1984, 1986, 1988a,b, Capalbo et al. 1993, Moraes & Capalbo 1986a,b,c, Moraes et al. 1989, 1990, 1991, 1993).

Besides these pioneer works in Brazil, other institutions have developed studies on Bt genetics, safety, formulation techniques, improvement of its stability in the field, and so on.

Based on the promising results obtained in laboratory and pilot scale production of Bt at UNICAMP, the National Research Center for Environmental Impact Assessment (CNPMA) of the Brazilian Agricultural Research Corporation (EMBRAPA), with the cooperation of UNICAMP and the State University "Julio de Mesquita Filho" (UNESP/S.J.R.Preto), initiated in 1985 studies to employ semi-solid fermentation (FSS) process to spare the need of large aerated fermentors.

Semi-solid fermentation process - Semi-solid fermentation means the growth process of microorganisms on solid materials, not in a liquid phase (Hesseltine 1977). In this type of fermentation the substrate may be put on a tray, in a plastic bag or in a flask; after inoculation the microorganism develops on the substrate. The container can be occasionally shaked, to allow the substrate at the bottom to move to the top providing the necessary aeration for growth of the microorganism.

To obtain an economic production of Bt by FSS, the fermentation medium has to be as cheap as possible, and it has to support conveniently the endotoxin production. The selection of the components of the medium is made based on some of the following guidelines: availability of the agroindustrial solid by-product in the region where the work is conducted; consistency of the composition of the by-product; low cost of the by-product (Capalbo 1989).

The presence of ions calcium and magnesium improves the sporulation of most of the sporeformer bacteria (Charney et al. 1951, Kolodziej & Slepecky 1964). So these ions should be naturally present in the substrate or they should be added to the culture medium as a complement. Products rich in mineral salts and not expensive could be good alternatives (Moraes et al. 1989).

The range of incubation temperature and growth conditions, as well as some FSS parameters should be taken in account. For example, periodic humidification must be accomplished in order to avoid dryness of the culture medium.

Among the simpliest fermentation development analysis one could choose counting viable spores, microbiological growing parameters and/or physico-chemical analysis.

Some typical results of FSS fermentation of Bt are shown on Table I.

Another analysis is the biossay which indicates the activity of the product obtained both in submerged and semi-solid fermentation. Typical results attained with Bt, obtained by FSS process, against *Anticarsia gemmatalis* larvae are presented in Table II.

TABLE I

Production of *Bacillus thuringiensis* spores, by semi-solid fermentation process, with periodic humidification

Harvest time (h)	pH	Moisture (%)	CFU[a]/g
0	5.5	50.1	—
48	6.3	46.2	1.8×10^4
168	8.1	54.6	2.0×10^{10}
216	8.3	55.2	$>10^{10}$

a : CFU = colony forming unit

TABLE II

Bioassay of *Bacillus thuringiensis*, obtained by semi-solid fermentation, against *Anticarsia gemmatalis*

CFU[a]/ml applied on the leaf	Mortality (%)
1.9×10^9	100
1.9×10^6	75
2.3×10^{9b}	100
Control[c]	0

a : CFU = colony forming unit
b : standard: commercial product DIPEL (Abbot Laborat., USA)
c : control = only water

The semi-solid fermentation process showed some advantages over the conventional stirred or aerated liquid fermentations because: the medium is relatively simple since only meal plus small amounts of water are needed; the required space occupied by the fermentation equipment is relatively small if compared to the yield of the product; there is no waste of liquid as there is in liquid fermentation; aeration is easily obtained since there are air spaces between each particle of the substrate; the product may be dried and stored at low cost.

Naturally, many problems occured, and some of them have been already solved: the selection of Bt strain that do not produces exotoxins, and the need for monitoring methods and devices for small scale FSS process. However, the monitoring devices, as well as the control of the heat generated by the growth of microorganisms, will require considerable innovation by engineers and technicians when the process is scaled-up.

Other aspects - Brazil is internationally known for the recent intensive activities in the area of biological control. A large portion of those activities refer to the mass production and use of pathogens for pest control. It is recognized that Bt is a desirable agent for insect pest control, specially if it could be developed at low cost and with high specificity.

There are many groups in Brazil that produce Bt in laboratory scale by means of FSS and submerged fermentation. Many problems will arise when they decide to produce Bt in commercial scale, that are related to quality control of the product, standardization, formulation, cost benefit ratio, and registration.

Although the number of successful projects is increasing, Brazil does not have a specific legislation for registration and use of such biological control products, what has certainly negatively affected the greater use of those products. The possibility of increasing such local production and utilization of microbial agents requires some standard models and risk assessment methodologies specific for national biotic environment in order to confirm its harmless.

LEGISLATION AND RISK ASSESSMENT

Specific legislation and protocols for the registration of biological products have to be gradually improved and implemented in Brazil; a rigorous re-evaluation of safety of products already registered must be done; techniques compatible with agricultural sustainability should be developed, facilitating registration of safe pesticides.

Rules for registration of biocontrol products are presently being prepared through a project on risk assessment about the use of biocontrol products, conduted jointly by EMBRAPA/CNPMA, Instituto Biológico/SP, USP/ESALQ, UNESP/Jaboticabal, in cooperation with IBAMA (Brazilian Institute of Environment and Natural Resources).

The protocol for registration of biocontrol agents must be implemented, and the group of researchers from the project is developing many efforts in order to supply some complementary informations. The proposed actions for evaluation of some biocontrol microorganisms will consist of: the bioassays with non-target organisms of this community and with standard test organisms; estimation of indirect effects on arthropod and pathogen populations; capacity to multiply, persist and disseminate; genetic estability of the microbial agent; development of resistance in the target pest; and field validation of detected effects.

As a result of the development of this project, it is expected: to subsidize demands for the development of alternative procedures related to periodic releases of microbial agents; generate information to be used in establishing registration requirements for those agents; establish safety rules in their production and use; indirectly increase the use of biocontrol agents by indicating the relative safety of each one.

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