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BACILLUS THURINGIENSIS DEVELOPMENT FROM 1971 TO 1996: CASES OF A RESEARCH GROUP IN BRAZIL

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ABSTRACT

Brazil is internationally known for its activities in mass production and use of biocontrol agents. Batch studies on *Bacillus thuringiensis* fermentation were initiated in 1970, at the State University of Campinas (UNICAMP) to explore the feasibility of producing endotoxin preparations, using cheap liquid by-products as fermentation substrates. The culture media composition, its price, and their influence on the final cost were studied for mini- and pilot-scale production. The results obtained generated two industrial patents on the fermentation process, using sugar cane molasses and corn steep liquor as sources of nutrients. Besides these studies, others such as rheological characteristics of many culture media and the culture broths, the influence of aeration and agitation levels on growth and sporulation, the continuous technique affecting growth and sporulation rate, the effects of different ways of drying on the viability of the spores and the potency of the insecticide obtained, as well as the solid state fermentation were developed. These studies were done as Master and Ph.D. theses, and some results were published from 1971 to 1996.

KEY WORDS: *Bacillus thuringiensis* case studies, fermentation processes, biopesticide, endotoxin preparations, agroindustrial residues, waste water residues, low cost substrates.

INTRODUCTION

The first use of a *Bacillus thuringiensis* (*Bt*) biopesticide was registered in Brazil, as a result of applications of the imported product by researchers of the Centennial Agricultural Institute of Campinas in the state of S. Paulo. However, Brazilian studies about *Bt* started in 1970 with the initiation of a Master of Science thesis (Moraes, 1973).

Since then, many researches have been developed. They had as subject processes of both submerged and solid state fermentation. The engineering parameters, agitation and aeration in submerged fermentation were studied (Moraes et al., 1981). After separation of the culture broth, the thermobacteriological indexes z and D in the drying process, with conventional and spray dryers were determined (Arruda, 1993).

Studying several parameters and variables, pH, aeration rate, agitation rate, spore and crystal formation, sugar consumption, as well as different culture media and some reactors verified the possibility of producing *Bt*, using cheap raw material available in this country (Moraes et al., 1981; Moraes, 1993). Several different residues and waste water, mainly from agroindustries, were studied (Moraes, 1976a; Capalbo 1982; Capalbo and Moraes, 1988; Capalbo, 1989; Moraes et al., 1990, 1991; Capalbo et al., 1993). The fluid behavior of the fermentative broth was studied and the rheological aspects were compared with other fluids obtained from fermentative processes (Lozano, 1982).

Both the endotoxin or spore-crystal complex toxin and the exotoxin or thermostable toxin were produced and bioassayed (Moraes, 1981).

Two patents* were deposited at the National Institute of Industrial Property (Moraes, 1976b, Moraes, 1985).

MATERIALS AND METHODS

Microorganisms

Bacillus thuringiensis var. *kurstaki*, *B. thuringiensis* var. *thuringiensis* and *B. thuringiensis* subsp. *israelensis.*

Fermentors

Mini one liter fermentor, 5, 14, 20, 250 liter fermentors.

Substrates

Sugar cane molasses, corn steep liquor, monosodium glutamate waste water, coconut waste water, rice grits and husks, pulp and paper industry residues, cassava flour, cassava waste, cassava waste water, and so on.

Insect pests

Anticarsia gemmatalis, Plodia interpunctella, Musca domestica, Chrysomya megacephala, Chrysomya sp., *Ceratitis capitata, Drosophila melanogaster.*

Growth and sporulation patterns

Followed by measuring biomass (optical density), sugar consumption (spectrophotometrically), spore formation (spore count or dipicolinic acid determination), pH variation during the fermentative process, specific bioassay.

Bioassays

In the studies of application of the toxins produced, which were conducted against pests of soybean (*Anticarsia gemmatalis*), stored rice (*Plodia interpunctella*), the endotoxins were employed. The action of the thermostable exotoxins was studied against *Musca domestica, Chrysomya* sp., *Drosophila melanogaster, Ceratitis capitata* (Moraes, 1981; Capalbo, 1982; Capalbo, 1989; Arruda, 1993).

*The second patent won in 1985 the State of S. Paulo Governor Prize, and was exposed at the Women's Inventions Exposition, held in Geneva, Switzerland in 1986, organized by the World Inventors Property Organization.

CONCLUSIONS

The results of the research on *Bt* and its applications developed in Brazil, showed the potential of producing this powerful bioinsecticide regionally, using many types of agricultural residues. This is because Brazil is a very big country and has different climates, soils, humid and arid areas, where one can find low cost substrates to develop a regional, inexpensive production.

By using cheap substrates — agricultural residues and agroindustrial waste water, with a good carbon–nitrogen balance (as well as vitamins and mineral salts composition), it was possible to compose low cost culture media to be used in the process, whether in submerged or solid state fermentation.

Brazilian losses with agricultural products amount to almost forty percent of the total production. The process is available to technology transfer, to be produced in several states of this big country that is Brazil, or countries where these types of residues are available and insect pests pose a problem.

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REFERENCES

Arruda, R.O.M. 1993. Studies of *Bacillus thuringiensis* viability determination after drying processes. M.Sc. Thesis, University of Campinas (UNICAMP), Campinas/SP, Brazil. 85 pp.

Capalbo, D.M.F. 1982. Contribuiçio ao estudo da fermentaçao contínua de *B. thuringiensis*. M.Sc. Thesis, UNICAMP, Campinas/SP, Brazil. 81 pp.

Capalbo, D.M.F. 1989. Desenvolvimento de um processo de fermentaçao semi-sólida para obtençao de *Bacillus thuringiensis* Berliner. Ph.D. Thesis, UNICAMP, Campinas/SP, Brazil. 159 pp.

Capalbo, D.M.F. and Moraes, I.O. 1988. Production of proteic protoxin by *Bacillus thuringiensis* by semi-solid fermentation. In: *Simpósio Anual da Academia de Ciências do Estado de S. Paulo, 12., Campinas/SP, Brazil, Anais*. pp. 46–55.

Capalbo, D.M.F., Moraes, I.O. and Arruda, R.O.M. 1993. Development of a bioreactor for semi-solid purposes: bacterial insecticide fermentations. In: Developments in Food Engineering. Edit. Y.T. Matsuno. Blackie, London. pp. 606–608.

Lozano, D.C. 1982. Studies on rheology of fermentation broths. M.Sc. Thesis. UNICAMP, Campinas/SP, Brazil. 74 pp.

Moraes, I.O. 1973. Bacterial insecticide production using submerged fermentation. M.Sc. Thesis. UNICAMP, Campinas/SP, Brazil. 70 pp.

Moraes, I.O. 1976a. Studies of submerged fermentation to get bacterial insecticide in minifermentors. Ph.D. thesis. UNICAMP, Campinas/SP Brazil. 77 pp.

Moraes, I.O. 1976b. Processo de fermentaçao submersa para produçao de um inseticida bacteriano. Patente BR PI 7 608 688.

Moraes, I.O. 1981. Production, separation and bioassay of the thermostable exotoxin of *Bacillus thuringiensis*. Tese de Livre Docência. UNICAMP, Campinas/SP, Brazil. 100 pp.

Moraes, I.O. 1985. Processo de produçao de toxina termoestável de *Bacillus thuringiensis*. Patente BR PI 8 500 663.

Moraes, I.O. 1993. Production and utilization of *B. thuringiensis* for crop protection. In: The Biopesticide *Bacillus thuringiensis* and its Applications in Developing Countries. Edit. H.S. Salama, O.N. Morris and E. Rached. Al Ahram Community Press, Cairo, Egypt. pp. 227–232.

Moraes, I.O., Capalbo, D.M.F., Del Bianchi, V.L. and Sifuentes, L.E. 1991. Technical aspects of the use of wastewaters to get useful products through fermentation process. In: *Cong. Lat. Am. de Transferencia de Calor y Materia. 4. La Serena, Chile. Actas. Univ. La Serena*. pp. 93–96.

Moraes, I.O., Capalbo, D.M.F. and Moraes, R.O. 1990. Solid and liquid waste utilization in fermentation processes to get bacterial insecticides. *Engineering and Food — Advanced Processes* 3:785–792.

Moraes, I.O., Santana, M.H.A. and Hokka, C.O. 1981. The influence of oxygen concentration on bacterial insecticide production. *Advances in Biotechnology* 1:75–79.