Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **1 (2):** 44-47 (2013)
Research Article

International Journal of Pure & Applied Bioscience

Isolation of Crystal Protein from *Bacillus thuringiensis*

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ABSTRACT

Bacillus thuringiensis (Bt) is a Gram-positive bacterium naturally found in soil, water and grain dust, and can be cultivated in liquid, solid and semi-solid media. The objective of this work was to test the effect of Bt toxin against onsects and mosquitoes. An Infected cotton leaves were used for the isolation of Bt by serial dilution. It is incubated for 1 to 2 days for better growth then this culture is used for staining for the isolation of pure species of BT. Brown, smooth small Rod shaped gram positive bacilli colonies are isolated. After isolation these colonies are subjected to the extraction process for crystal protein extraction. This protein is extracted by repeated centrifugation and then it is purified and used as an insecticidal. When this protein is applied to Insects and mosquitoes it resulted in their death.

Key words: *Bacillus thuringiensis, gram positive, Biopesticides*

INTRODUCTION

Bacillus thuringiensis (*Bt*) is the most successful commercial biopesticide in the biological control market[1,2] accounting for 90% of all biopesticides sold all over the world. *B. thuringiensis* is a Gram positive spore-forming bacterium found naturally in soil, water, dead insects and grain dust. This bacterium is characterized by its ability to produce crystalline inclusions proteins or crystals called endotoxin during sporulation and/or stationary phase. The crystalline inclusions along with the spores have a great potential to control a great number of pest insects belonging to the order Lepidoptera, Diptera and Coleoptera[3]. Fall armyworm, *Spodoptera frugiperda*, is one of the most important corn insect pests in Brazil and its damage may reduce yield production up to 34% [4]. The use of chemical insecticides to control *S. frugiperda* has increased over the years in Brazil, and has generated resistance of this insect to chemicals. Microbial pesticides may become feasible and reliable alternative to control this insect, however *Bt* based biopesticides is limited due to high production costs[5]. *B. thuringiensis* strains have a narrow spectrum of insecticidal activity when compared to conventional chemicals. Regarding specificity *Bt* shows differences even within an order of insects (e.g. Lepidoptera) and dramatic differences in sensitivity are exhibited among species[6]. Differences in sensitivity were found among species, i.e. *Spodoptera* spp. are difficult to control with *Bt* based biopesticide (strain HD1), however *Heliothis virescens* and *Plutella xylostella* are not[7,8] also found that *Bt* was not much efficient against S. *frugiperda*, but these authors used only one available biopesticide at that time (strain HD1).

Bt is a gram positive spore forming bacteria that produces insecticidal Proteins called as beta and delta toxins .Since the early 1900's this Bacteria group has received greater attention for its use as agricultural and Forest pest control. *Bacillus Thuringiensis* forms two types of endospores -rods and cocci. The ability to produce more or less heat resistant spores is restricted, with few exceptions to a group of gram positive motile rods with peritrechous Flagella. *Bacillus thuringiensis* is an example of aerobic spore formers. The

aerobic spore formers live in soil. Many bacilli form chains or Filaments. Synthetic pesticides originate form the living system to sustainability Indicates that these are two types of pesticides, chemical or synthesized Pesticides and natural pesticides. Synthetic pesticides originate from the living system and can either Inhibit, repel or destroy organisms that would otherwise cause economic Loss. It need not necessarily be a pest that only attacks a field crop.Therefore, anything that affects the well being and economics of human is a pest. Microbes transgenics and products derived from plants and animals can definitely from part of bio-pesticides. Crop species are infested by many species of insect and pathogens resulting in heavy losses in yield amounting to 240 billion rupees per annum. Products from biological sources are emerging as a new crop protection strategy. Various types of bio-pesticides and bio control agents are available in the world market. Bt. *(Bacillus thruingiensis)* consists of the largest and most widely commercialized bio-pesticides category. Control of insects can be achived by means other than causing rapid death. The bio-potency of Bt is found to be adversely effected by UV and white light as proved under laboratory studies.Fields studies also showed that evening spray of Bt, is more effective
Against target pests as compared to the day time. It is therefore, advisable to use Bt. In the evening hours during the crop season under north Indian Conditions. In particular, genetically modified technology coupled with bio-Pesticides, like neem and Bt. should be used to increase the production of Cotton, improve the efficiency of production and reduce the environmental impact of agriculture.

MATERIAL AND METHODS

Preparation of Nutrient's agar media as following step:

Firstly we had taken sterilized 1000ml conical flask. ii. Then we had taken 500ml D/W in flask. iii. After that mix properly all components of Nutrient's agar media. iv. Autoclaved 500ml media
for 15 to 20 minutes. Nutrient agar is poured in six Petri plates in laminar air Flow**.** And then in same way nutrient broth is prepared and poured in to six different sterilization flasks.

Preparation of sample-

1 gm infected cotton leaves are crushed properly in 100 ml Distilled water. After crushing vigorously suspension was obtained which was then filtered to remove solid particle from liquid Portion of the extract. Soild particles are discarded and then 1/10, 1/100, 1/1000 Serial dilutions of the leaf extract is prepared.

Inoculation of sample in media-

With the help of sterilized inoculating tube microbial cells suspension is steaked on the media plates. Streaked plates are incubated at 30 degree Celsius for four days to support growth and appearance of bacterial Colonies.

Inoculation of colonies in liquid medium (broth)-

Well separated colonies were observed and picked up with a sterile inoculation needle and were inoculated in the the liquid medium aseptically. Inoculated cultures were then placed on shaker for 1-2 days to allow sufficient growth of culture.

Grame staining-

Staining is done for the observation of presence of bacillus thuringiensis in the culture medium.

Isolation of crystal protein from *bacillus thuringiensis*-

First of all low speed density centrifugation was performed of Culture broth. Then supernatant was collected and solid particles which settled and the bottom were discarded. Again low speed centrifugation of the above collected Supernatant was performed. And again supernatant was collected and solid particles were from bottom discarded. Finally high speed centrifugation of this above collected Supernatant was carried out. Now this time liquid protein in the supernatant was removed and cells of Bacillus Thuringiensis settled at the bottom was collected.

Then cells are disrupted by freezing and drying method just to release crystal protein completely which is used as Bt toxin.

RESULTS AND DISCUSSION

When the isolation of Bt is done on the gram stained culture following observations were seen on slides-

Observation table

PLATE NO.	TIME OF VISIBLE OBSERVATION(hrs.)OF COLONIES	OBSERVATION (WITH NAKED EYES)	OBSERVATION (AFTER GRAM STAINING)	INTERENCE
1.	106.00hrs	Brown ,smooth patches of bacterial colonies	Purple rodes are observed under microscope	Gram positive bacteria (Bt) is present in the sample
2.	130.00hrs	Many cell clumps appeared	DO	DO
3.	118.00hrs	DO	DO	DO
4.	142.00hrs	White and creamy patches appeared showing fungal contamination	Black fungal spores are observed	Sample has been contaminated by fungus
5.	154.00hrs	Isolated single bacterial cell coloy appeared	Purple coloured rods observed	Showing the presence of Bt in the sample
6.	154.00hrs	Do	Purple coloured rods observed	DO

Rod shaped gram positive bacilli are observed and isolated, they are endospore containing cells- ***Bacillus thuringiens.***

Bt toxin that is protein from bacterium, *Bacillus thuringiensis* has been obtained which is then can be stored and preserved by Freezing method for future use as an insecticide.

When field insects as well as mosquitoes and house flies were applied on solution containing isolated crystal protein of Bt then It resulted in their death.

CONCLUSION

The use of Bt and its subspecies for practical pest control has both Advantages and problems. These bacteria produce toxin that is remarkably safe for humans. Domestic animals and non target fauna. On the other hand, *B. thuringiensis* preparations applied to foliage can be washed off by rain or may be inactivated by sunlight. Therefore it is required repeated applications for long term protection against some pest species. While repeated and high does of application may result in development of insect's resistance against Bt.

REFERENCES

1. YANG, X-M.; WANG, S. S. Development of *Bacillus thuringiensis* fermentation and process control from a practical perspective. Biotechnology and Applied Biochemistry, Orlando, **28:** 95-98 (1998)
2. GLARE, T. R.; O'CALLAGHAN, M. *Bacillus thuringiensis*: biology, ecology and safety. Chichester: J. Wiley. 350 (2000)
3. VIDYARTHI, A. S.; TYAGI, R. D.; VALERO, J. R.; SURAMPALLI, R. Y. Studies on the production of *B. thuringiensis* based biopesticides using wastewater sludge as a raw material. Water Research, New York, **36:** 4850-4860 (2002)

4. CARVALHO, R. P. L. Danos, flutuação da população, controle e comportamento de *Spodoptera frugiperda* (J.E.Smith, 1797) e susceptiblidade de diferentes genótipos de milho, em condições de campo. 170 f. Tese (Doutorado) – Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba (1970)
5. DULMAGE, H. T. Production of microbial insecticides by fermentation. In: BURGES, H. D. (Ed.). Microbial control of pests and plant diseases. *London: Academic* Press, 143-222 (1981)
6. BRAVO, A. SARABIA, S. LOPEZ, L. ONTIVIEROS, H. ABARCA, C.ORTIZ, A. LINA, L. VILLALOBOS, F. J. PENA, G. NUÑES-VALDEZ, M. E. SOBERÓN, M. Mortality of first instar (two day old) *Spodoptera frugiperda* larvae with *B. thurinngiensis sv tolworthi* produced in three different media and concentrations (1998)
7. WAQUIL, J. M. VIANA, P. A. LUCENA, A. I. T. CRUZ, I. OLIVEIRA, A. C. Controle da lagarta-do-cartucho em milho com inseticidas químicos e biológicos. Pesquisa Agropecuária Brasileira, Brasilia, DF, **17:** 163-166 (1982)
8. BAUM, A. B.; JOHNSON, T. B.; CARLTON, B. C. *Bacillus thuringiensis* – Natural and recombinant biopesticide products. In: HALL, F. R.; MENN, J. J. (Ed.). Biopesticides: use and delivery. *Totowa: Humana Press,* p. 189-209 (1999)
9. Miller T. L. and Churchill B. W. Substrates for Large-Scale Fermentation, In: Manual of Industrial Microbiology and Biotechnology. (Eds.): Domain, A. L. and Solomon, N. A. American Society for Microbiology, Washington DC, pp. 122-135 (1986)
10. Nickerson K. W. and Bulla L. A. Physiology of Spore-Forming Bacteria Associated With Insects, Minimal Nutrition Requirements for Growth, Sporulation and Parasporal Crystal Formation of *Bacillus thuringiensis*. *Appl. Microbiol*., **28:** 124- 128 (1974)
11. Paul A. A. and Southgate D. A. T. *The Composition of Foods.* 4th edition, *Elsvier, Amsterdam* (1978)
12. Rajalakshmi S., and Shethna Y. I. The Effect of Amino Acids on Growth, Sporulation and Crystal Formation in *Bacillus thuringiensis* var. *thuringiensis*. *J. Ind. Indust. Sci*., **4:** 169-176 (1977)
13. Solomons G. I. *Materials and Methods in Fermentation.* Academic Press, New York, pp. 115-132 (1969)
14. Zabriske D. W., Armiger W. B., Phillips D. H., and Alabno P. A. *Traders Guide to Fermentation Media Formulation*. Traders Protein Division, Traders Oil Mill Fort Worth, TX (1980)
15. Chauhan N. K., Sain M., Mathuriya B. L., Nagar J., Production of biomass from various agro products using entomopathogenic fungi. *Int. J. Pure App. Biosci*, **1** (1): 7-12 (2013)
16. Maheshwari R., Sharma I. R., Soil Status in Relation to Blast Disease in Bundi District of Rajasthan, INDIA. *Int. J. Pure App. Biosci*, **1** (1):13-19 (2013)
17. Datta S., Nama K. S., Paras P., Sharma P., Shaikh N., Nagar J., Antagonistic Activity of Lactic Acid Bacteria from Dairy Products. *Int. J. Pure App. Biosci*, **1** (1): 28-32 (2013)