

## **Production of *Bacillus thuringiensis* S<sub>6</sub> biomass using different cheap nitrogen sources**

**Harish Dhingra**

*Department of Science, Faculty of Arts, Science and Commerce, Mody Institute of Technology & Science Rajasthan, India*

**Kamla Chaudhary**

*Department of Biotechnology and Molecular Biology, CCS Haryana Agricultural University, Hisar, India*

---

**Abstract:** In recent years, *Bacillus thuringiensis* is receiving increasing attention for its use in integrated pest management programs for agricultural and forest insect pests and insect vectors of human and other mammalian transmissible diseases. As the overuse and negative impacts of chemical pesticides on soil, water, human health, wildlife and ecological balance have led to the use of microbial insecticides against insect pests of order Lepidoptera, Coleoptera, Diptera and Hemiptera, an attempt has been made to develop low cost Bt formulations for the management of *Helicoverpa armigera*. Agro-industrial residues and their byproducts can be used for large scale production of *B. thuringiensis* biomass. Different low cost agro-industrial based nitrogen sources such as cotton seed meal, soya meal, sunflower meal, urea, ammonium sulfate and potassium nitrate were evaluated for Bt biomass production. Among the different nitrogen sources evaluated, maximum biomass of *B. thuringiensis* isolates was achieved in the medium containing 1.0% cotton seed meal in basal medium. The biomass production in this medium was equivalent to that achieved in LB medium after 48h of growth.

**Key Words:** *Bacillus thuringiensis*, biomass, growth.

---

## Introduction

The American bollworm, *Helicoverpa armigera* (lepidoptera) is one of the most serious pests of different crops in many parts of the world and is controlled by the use of chemical insecticides. Recently, there have been a few reports on the development of resistance by *H. armigera* to chemical insecticides due to their indiscriminate and excessive use. Therefore, there is a need to explore potential microbial insecticides so that losses due to attack by *H. armigera* could be minimized. The use of *Bacillus thuringiensis* based insecticides offers various advantages over harmful chemical insecticides being target specific, biodegradable and economical.

*Bacillus thuringiensis*, a gram positive spore forming bacterium, is the most widely used agent in controlling insect pests of various crop plants. The bacterium produces a parasporal crystal protein inclusion during sporulation which is activated by alkaline proteases in the midgut of the susceptible insect. These crystal proteins cause death of the susceptible insect due to leakage in the midgut of the insect. *B. thuringiensis* is pathogenic to various lepidopteran, coleopteran and dipteran pests without any adverse effect on the non-target insects and on higher organisms (Obeidat *et al.* 2004). *B. thuringiensis* (Bt) is highly effective against the American boll worm, *Helicoverpa armigera* at the larval stage. Bioinsecticides containing *B. thuringiensis* are target specific and can minimize the possibility of development of resistance in insects. The media for the production of Bt biomass contain nitrogenous sources and energy producing carbohydrate sources which are easily assimilated by bacteria. For optimal growth, *B. thuringiensis* also require a variety of inorganic sources which function physiologically as protein, nucleic acid and co-enzyme constituent and as cofactor of numerous enzyme reactions and bacterial cytochromes (Morris *et al.* 1997). But media ingredients especially nitrogen sources like tryptone, peptone, casein etc. are too expensive to use in large scale production of *B. thuringiensis*. Therefore, there is a need to substitute less expensive agricultural products or agro-industrial based by-products. The cost of fermentation ingredients is especially important since they will determine the cost benefit ratio of the final product. Therefore, the present investigation was undertaken to investigate the effect of different agro-industrial based nitrogen sources on biomass production of *B. thuringiensis*.

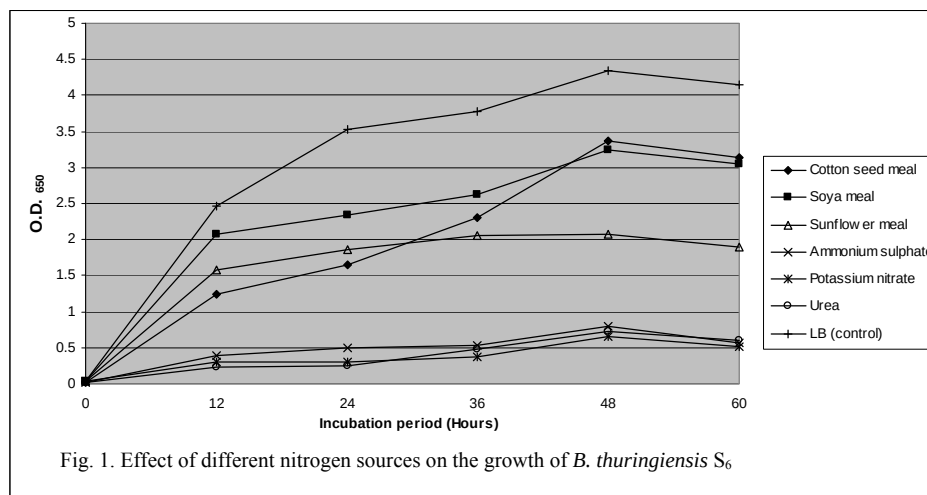
## Materials and Methods

The native isolates of *Bacillus thuringiensis* were obtained from Microbial Biotechnology lab of BMB department, CCS HAU, Hisar and maintained on LB agar slants with 20% glycerol at 4°C. For biomass production of *B. thuringiensis* subsp. *kurstaki*, different agro-industrial by-products viz. cotton seed meal, soya meal, sunflower meal, urea, ammonium sulfate and potassium nitrate were used as nitrogen sources. Cottonseed meal and sunflower meal were obtained from local grocery market. Soya meal, urea, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and KNO<sub>3</sub> were obtained from Himedia. For preparation of potato extract, 250g of potatoes were boiled in one liter of water and extract was used in the growth medium. The basal medium used for the

growth of *B. thuringiensis* isolates was Morris *et al.* (1997) medium with modification which contained Potato extract 10ml/l, Peptone 2.0g/l, Dextrose 1.5g/l, Yeast extract 2.0g/l,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.03g/l,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  0.02g/l,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  0.02g/l, NaCl 5.0g/l, Tween 60 1.0ml/l along with agro-industrial based nitrogen source. Tween 60 was used as a surfactant to increase bacterial cell membrane permeability and consequent uptake of nutrients from the fermentation medium. All media were sterilized at 15 lb/sq inch for 20 min. *B. thuringiensis* isolate S<sub>6</sub> was inoculated into these production media and incubated at 30°C on a rotary shaker. Samples were withdrawn at an interval of 12h, serially diluted and plated on LB agar plates. The plates were incubated for 24h at 30°C and then total numbers of colony forming units (cfu) were calculated. For optimizing the amount of nitrogen source in the media, different concentrations of nitrogen source were used in the production medium.

## Results and Discussion

Different nitrogen sources were evaluated for the production of Bt biomass. Different media were inoculated with *B. thuringiensis* isolate S<sub>6</sub> and growth was monitored at an interval of 12h for 60 hours. It was observed that *B. thuringiensis* isolate attained maximum growth after 48 hours of inoculation in all the media evaluated. The medium containing cotton seed meal produced maximum biomass followed by medium containing soya meal as the nitrogen source (Figure 1). The growth was comparatively very low in medium containing potassium nitrate, urea and ammonium sulfate as the nitrogen sources (Figure 1).



To optimize the concentration of cotton seed meal, its different concentrations were used in production medium. *B. thuringiensis* isolates showed maximum growth after 48h of growth ( $1.3 \times 10^{10}$  cells/ml) when 10g/l of cottonseed meal was added in the production media (Table 1). By increasing or decreasing the level of cottonseed

meal, there was a decrease in cell number. This indicated that optimum concentration of cottonseed meal for maximum biomass production was found to be 10g/l. This medium containing 10g/l of cotton seed meal produced Bt biomass which was comparable to that produced in LB medium (control) (Harish, 2006).

Tab. 1. Effect of cotton seed meal concentration on production of *Bacillus thuringiensis* S<sub>6</sub> biomass

| Cotton seed meal<br>(g/l) | Biomass production*       |       |       |       |       |       |
|---------------------------|---------------------------|-------|-------|-------|-------|-------|
|                           | Incubation period (hours) |       |       |       |       |       |
|                           | 0                         | 12    | 24    | 36    | 48    | 60    |
| 2                         | 4.133                     | 5.008 | 7.283 | 8.546 | 9.568 | 9.170 |
| 4                         | 4.133                     | 5.146 | 7.380 | 8.832 | 9.584 | 9.278 |
| 6                         | 4.158                     | 5.322 | 8.008 | 8.842 | 9.651 | 9.292 |
| 8                         | 4.146                     | 5.342 | 8.033 | 8.871 | 9.695 | 9.459 |
| 10                        | 4.114                     | 5.544 | 8.049 | 8.919 | 9.783 | 9.465 |
| 12                        | 4.107                     | 5.544 | 7.989 | 8.913 | 9.783 | 9.387 |
| LB (control)              | 4.120                     | 5.651 | 7.342 | 8.919 | 9.681 | 9.204 |

\* log number of total viable cells

\*\* potato extract was added @10ml/l in all the media except LB

Designing a cheap, industrial fermentation medium is one of the most important aspects of process development. An important aspect of reducing fermentation costs can be substitution of high cost media ingredients by agro-industrial residues and by-products. *B. thuringiensis* strains are known to utilize a large number of different carbon and nitrogen sources for growth and delta-endotoxin production (Schisler *et al.* 2004). Various agricultural nitrogen sources like deoiled and expeller cakes of mustard, cottonseed, groundnut, corn steep liquor have been used to design low cost fermentation media in batch culture process (Johnson *et al.* 1994). High spore yield was reported with a medium containing cheese whey, soya bean milk and molasses (Alves *et al.* 1997). *B. thuringiensis* also require, for optimal growth, a variety of macro and microelements which function as co-factors of numerous enzyme reactions (Sarrafzadeh *et al.* 2005). In minimal medium, various ingredients are too expensive for large-scale production of *B. thuringiensis*. Therefore, agricultural products and by-products were evaluated as sources of nutrients for production of *B. thuringiensis*. Cotton seed meal, defatted soya flour and corn gluten meal were found to be the most efficient substrate for the production of *B. thuringiensis* biomass (Morris *et al.* 1997). Bengal gram (Poopathi and Kumar 2003), defatted groundnut cake, gram flour and soy bean (Desai and Shethna 1991) have been used as different nitrogen sources for production of *B. thuringiensis*.

## Conclusion

In the present study, six different cost effective nitrogen sources such as cotton seed meal, soya meal, sunflower meal, urea, ammonium sulfate and potassium nitrate were evaluated for biomass production. The results in this study indicated that cotton seed meal supported the highest biomass production of Bt followed by soya meal containing medium (Figure 1) and that 1.0% concentration of cotton seed meal produced the optimum biomass after 48h of incubation i.e.  $1.3 \times 10^{10}$  cfu/ml which was comparable to growth in LB medium ( $1.1 \times 10^{10}$  cfu/ml) (Table 1). Therefore, LB medium could be easily replaced for industrial scale production of *B. thuringiensis* because of its high cost in comparison to medium containing potato extract (as carbon source) and cotton seed meal (as nitrogen source).

## References

- Alves L.F.A., Alves S.B., Pereira R.M., Capalbo D.M.F. (1997): Production of *Bacillus thuringiensis* Berliner var. *kurstaki* grown in alternative media. *Biocontrol Sc. Tech.*, **7** (3): 377-383.
- Desai S.Y., Shethna Y.I. (1991): Production and formulation of *B. thuringiensis* var. *israelensis* and *B. sphaericus* 1593. *Indian J. Med. Res.*, **93**: 318-323.
- Harish J. (2006): Development and evaluation of different formulations of *Bacillus thuringiensis* for management of *Helicoverpa armigera*. Ph.D. Thesis, Department of Biotechnology and Molecular Biology, CCS HAU Hisar.
- Johnson V., Shah R.N., Shah D. N., Patel K.A., Mehta M.H.: (1994): Production of *Bacillus thuringiensis* based bioinsecticide: Influence of various nitrogen sources on process economics. In : *Microbes for better living*. 161-167 pp.
- Morris O.N., Kanagaratnam P., Converse V. (1997): Suitability of 30 agricultural products and by-products as nutrient sources for laboratory production of *Bacillus thuringiensis* subsp. *aizawai*. *J. Invert Pathol.*, **70**: 113-120.
- Obeidat M., Hassawi D., Ghabeish I. (2004): Characterization of *B. thuringiensis* strains from Jordan and their toxicity to the lepidoptera, *Ephestia kuehniella*. *African J. Biotech.*, **3** (11): 622-626.
- Poopathi S., Kumar K.A. (2003): Noval fermentation media for production of *B. thuringiensis israelensis*. *J. Econ. Entomol.*, **96** (4): 1039-1044.
- Sarrafzadeh M.H., Guiraud J.P., Lagneau C., Gaven B., Carron A., Navarro J.M. (2005): Growth, sporulation, delta-endotoxins synthesis, and toxicity during culture of *B. thuringiensis* H 14. *Curr. Microbiol.*, **51** (2): 75-81.
- Schisler D.A., Slininger P.J., Behle R.W., Jackson M.A. (2004): Formulation of *Bacillus* spp. for biological control of plant diseases. *Phytopathol.*, **94** (11): 1267-1271.

## **PRODUKCIJA BIOMASE *Bacillus thuringiensis* S<sub>6</sub> POMOĆU RAZLIČITIH JEFTINIHZVORA AZOTA**

- originalni naučni rad -

**Harish Dhingra**

*Katedra za prirodne nauke, Fakultet za umetnost, prirodne nauke i trgovinu,  
Modijev institut za tehnologiju i nauku, Radžastan, Indija*

**Kamla Chaudhary**

*Fakultet za biotehnologiju i molekularnu biologiju, Harjanski poljoprivredni  
univerzitet "CCS", Hisar, Indija*

### **Rezime**

Poslednjih godina, *Bacillus thuringiensis* privlači sve veću pažnju zbog primene u programima integralne zaštite od insekatskih štetočina poljoprivrednih i šumskih kultura i insekata vektora bolesti ljudi i ostalih sisara. Pošto su prekomerna upotreba i negativni uticaj hemijskih pesticida na zemljište, vodu, ljudsko zdravlje, divlji svet i ekološku ravnotežu doveli do primene mikrobioloških insekticida u suzbijanju insekatskih štetočina iz redova Lepidoptera, Coleoptera, Diptera i Hemiptera, učinjen je pokušaj da se razviju jeftine Bt formulacije za kontrolu *Helicoverpa armigera*. Agroindustrijski ostaci i njihovi sporedni proizvodi mogu se koristiti u industrijskog proizvodnji biomase *B. thuringiensis*. Ispitana je primena različitih jeftinih agroindustrijskih izvora azota, kao što su pamukova sačma, sojina sačma, sunco-kretova sačma, urea, amonijum sulfat i kalijum nitrat, u proizvodnji biomase Bt.

Od svih ispitivanih izvora azota, maksimalna biomasa izolata *B. thuringiensis* ostvarena je u podlozi sa 1.0% pamukove sačme u osnovnoj podlozi. Produkcija biomase u ovoj podlozi istovetna je produkciji biomase ostvarenoj u LB podlozi nakon 48-časovnog rasta.