STUDIES ON PRODUCTION, STRAIN IMPROVEMENT 
AND BIOLARVICIDAL ACTIVITY OF 
BACILLUS THURINGIENSIS

SUMMARY OF THE 
THESIS SUBMITTED TO 
THE MAHARAJA SAYAJIRAO UNIVERSITY OF BARODA 
FOR THE DEGREE OF 
DOCTOR OF PHILOSOPHY 
IN 
MICROBIOLOGY

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OCTOBER 1998
SUMMARY
• *Bacillus thuringiensis* var. *kurstaki* HD-1 and *Bacillus thuringiensis* var. *israelensis* were effective against *H. armigera* and *A. aegypti* respectively, hence studied with respect to production with shake flask and 2.0 L bioreactor.

• In basal glucose yeast extract salt medium (GYS) the biomass, 2.30 g/l and insecticidal crystal protein, 0.25 mg/l were obtained within 24 h having yield 108 mg crystal protein/g biomass and productivity 0.0104 g/l/h.

• Glucose was suitable carbon source. At 0.5 g% glucose concentration sharp crystals were obtained which turned amorphous, at higher concentration (1 g%). The other preferred carbon source was glycerol.

• Among the complex organic nitrogen sources, yeast extract, beef extract, casein and peptone were equally promoting the biomass and crystal protein production.

• Ammonium sulphate was the best inorganic nitrogen source at the concentration of 0.2 g%.

• The GYS medium containing glucose 0.5 g%, yeast extract 1 g% and ammonium sulphate, 0.2 g% resulted in 4.5 g/l biomass, 0.72 mg/ml crystal protein and $2.5 \times 10^{12}$ spores/ml within 60 h. The yield and productivity obtained were 160 mg crystal protein/g biomass and 0.012 g/l/h respectively.

• At lower working volume (wv) ($k_{La}$ 19.65/h) dry weight 4.8 g/L and crystal protein 0.81 mg/ml while at 40% (wv) ($k_{La}$ 6.89/h) dry weight 3.5 g/l and crystal protein 0.52 mg/ml were obtained. At 10%wv the yield and productivity obtained were 168 mg crystal protein/g biomass and 0.0135 g/l/h respectively. While at 40% wv the yield and productivity obtained were 148 mg crystal protein/g biomass and 0.0086 g/l/h respectively.
- In a 1.5 L capacity bioreactor ($k_{\text{a}} = 63/h$) dry weight 5.5 g/l and crystal protein 0.92 mg/ml obtained. The yield and productivity obtained were 167 mg crystal protein/g biomass and 0.0153 g/l/h respectively.

- Production of the insecticidal crystal protein was detected by dot blot immunoassay after 12 h.

- Enhanced larvicidal activity of Bt var. israelensis and B. sphaericus was obtained by conjugation. The LC$_{50}$ values for transciipients TC1, TC2 and TC3 were $0.026 \pm 0.008$, $0.04 \pm 0.01$ and $0.03 \pm 0.012 \mu g/ml$ as compared to LC$_{50}$ values of donor Bti and recipient Bs, $0.035 \pm 0.006$ and $0.09 \pm 0.03 \mu g/ml$ respectively.

- Lysozyme concentration (1 mg/ml) converted 99% of the Bti and 91% of the Bs cells to protoplasts. Regeneration frequencies of protoplasts were 43.82% for Bti and 24.9% for Bs respectively.

- In case of Bt var. kurstaki, protoplast formation and regeneration frequency were 95.65% and 2.038% respectively, while in Bs 91.71% and 24.4% respectively.

- The strain Bt var. kurstaki NRRL HD-1 produced bipyramidal and cuboidal inclusions of the cry I and cry II class of insecticidal crystal proteins respectively.

- Quantitative analysis of the neutral hexoses by HPLC revealed that glucose (4%) and mannose (1%) accounted for all of the carbohydrate present in the ICPs of Btk HD-1.
Various solubilising reagents and conditions were employed to solubilize the crystal protein of Bt. The reagent C containing 100 mM Na$_2$CO$_3$ (pH 10.5), 10 mM EDTA and 1% β-mercaptoethanol was the suitable solubilizing agent at 28 ± 2°C. Laemmli's buffer 3x was also suitable solubilizing reagent for SDS-PAGE analysis of ICPs of Bt.

Proteolytic activity in the intact as well as solubilized crystals could not be detected at pH 9.0 but proteolytic activity was detected in the Bt culture broth.

A strong λ max at 280 nm was observed when the solubilized ICPs were subjected to UV scan.

Intact crystals were isolated by step gradient centrifugation (80, 72, 67 and 50% w/v sucrose). Crystals were banded at the interface of 80 and 72% and 72 and 67% sucrose (w/v).

By Sephacryl S-300 gel filtration chromatography two protein peaks were obtained having ~130 and ~67 kDa proteins.

Multiple polypeptide bands were observed when the spore crystal complex was directly solubilized in the 3x Laemmli's sample buffer. Both the 130 and 72 kDa bands were observed.

The purified crystals of Bt var kurstaki HD-1 showed a polypeptide band of 130 kDa while Bt var thuringiensis HD-2 showed a doublet at 135 and 140 kDa. The anti Btk HD-1 antibody did cross react with both HD-1 and HD-2 crystal proteins.

The crystal protein solubilized at higher alkaline pH showed multiple bands and aggregation of glycoprotein forming higher molecular weight proteins.
A broad smear at the bottom of the gel indicated the extensive degradation of subunit of the insecticidal crystal protein due to higher pH and longer incubation time.

SDS-PAGE analysis of the cryIV class crystal proteins of Bt var. israelensis and some soil isolates revealed the presence of five polypeptides ranging from 130, 128, 66, 45 ad 29 kDa.

In vitro activation of the Btk HD-1 crystal protein was achieved by trypsin and midgut juice of H. armigera. The protein (130 kDa) was partially cleaved at 2 h of treatment but after 4 h treatment proteolytically stable polypeptide (55 kDa) was generated.

Btk HD-1 antibody did cross react with 130 and 67 kDa polypeptides of Bt var. kurstaki HD-23, Bt var. thuringiensis HD-2 and Bt var. kurstaki TO3A001.

By dot blot immunoassay, production of ICPs were detected 12 h onwards.

LC$_{50}$ values of a Btk preparation on H. armigera and Spodoptera sp. larvae were 38 ± 1.3 and 285 ± 2.6 ng/well, while the LC$_{50}$ values of the solubilized crystal proteins were 20 ± 0.8 and 100 ± 1.2 ng/well respectively.

Bt var. kurstaki HD-1 and Bt var. thuringiensis ICPs were not haemolytic. In vitro assay method for the assessment of toxicity of Bti preparation was developed.

Two Btk preparations were quantified by dot blot immunoassay. The lowest concentration of insecticidal crystal protein detected by dot blot immunoassay were 310 and 156 ng for two Btk preparations.