

## ABSTRACT

The era of genomics (the study of genes and their function) began a scant dozen years ago has given biologists strong tools to study the genome of any organism such as *B. thuringiensis*. The use of biopesticides based on Bt to Spray plants, inevitably exposes them to the damaging ultraviolet light, including the wave length range of 280-320 nm (ultraviolet-B, UV-B). UV-B light inactivates the Bt insecticidal crystal protein (ICP)-spore complexes as well as the spore DNA and hence render them worthless.

Usually, a variety of chemically-synthesized sun screeners, that absorb UV-B, are added to *B. thuringiensis*-based biopesticides to ensure photoprotection and keep their insecticidal capabilities. These chemicals are either carcinogenic or mutagenic.

Therefore, the development of a biological source for production of UV-blocking agent, such as melanin, to be used in formulation of *B. thuringiensis*-based biopesticides will nullify the hazards of chemical UV-blocking agents to both humans and environment. This was achieved by traditional mutation of wild type *B. thuringiensis* and three selected mutants were capable of producing their own UV-blocking agent, the melanin pigment.

The three selected mutants (M2, M3 and M5) were characterized at both bacteriological and molecular levels. Moreover the gene encodes for the melanin production was cloned and sequenced. In batched fermentation, an optimization of the ICP and melanin production was achieved at the nutritional and physical levels. The C/N ration, the pH, temperature, inoculum size and antifoam reagent were optimized to ensure the best expression environment for melanin production. The capabilities of the produced melanin to protect the insecticidal activity of the wild type Bt strains was demonstrated either by simple addition of mixing with Wt.

More extensive are needed to maximize melanine production at large scale fermentation.

Key words:

*B. thuringiensis*, Mutation, Melanin, PCR, SDS-PAGE.

# CONTENTS

	Page
<b>Acknowledgement</b>	
<b>List of figures</b>	
<b>List of tables</b>	
<b>Abstract</b>	
<b>Abbreviations</b>	
<b>I. Introduction.....</b>	<b>1</b>
<b>II. Review of literature .....</b>	<b>6</b>
1. <i>History of Bacillus thuriengiensis</i> .....	6
2. Economics and advantages of <i>Bacillus thuringiensis</i> -based biopesticides.....	7
3. Commercial use of <i>B. thuringiensis</i> products.....	8
4. Survey of bacilli-producing insecticidal toxins.....	9
5. Taxonomy and Classification of Bt Toxins.....	10
6. Mode of action of Bt .....	11
7. Strain development.....	12
8. Commercial production of <i>B. thuringinsis</i> biopesticide and technical problems.....	13
9. Why melanin is a target .....	22
10. Phage and commercial production of <i>B. thuringinsis</i> biopesticide.....	27
11. Field application problems of <i>B. thuringiensis</i> products.....	27
<b>III. Materials and methods.....</b>	<b>33</b>
<b>I. bacterial strains .....</b>	<b>33</b>
<b>II. Methods .....</b>	<b>45</b>
Preservation of Bt stain by freeze drying .....	45
2. Batch culture condition for Bt production.....	45
3. Treatment of fodder yeast.....	45
3. The fermentor .....	46
4. Insecticidal bioassay .....	48

5. Analysis of total cellular proteins .....	49
6. Polyacrylamide gel electrophoresis of cellular proteins.....	50
7. Solubilization of cellular proteins .....	51
8. Visualization of cellular proteins.....	53
9. PCR.....	53
9. Melanin pigment characterization.....	54
10. Cloning of melanin encoding genes.....	55
<b>IV. Results.....</b>	<b>56</b>
1. <b>Bacteriological characterization of mutants through endspore staining.....</b>	<b>56</b>
2. <b>Molecular characterization of the melanin-producing mutants.....</b>	<b>58</b>
➤ protein banding pattern of vegetative cells.....	59
➤ Protein banding pattern of sporulated cells.....	60
➤ Two dimensional gel of cellular proteins.....	61
➤ PCR .....	63
3. <b>Chemical characterization of melanin pigment.....</b>	<b>64</b>
4. <b>Bioassays.....</b>	<b>66</b>
5. <b>Cloning of melanin encoding gene.....</b>	<b>69</b>
6. <b>Melanin gene sequencing .....</b>	<b>73</b>
7. <b>Optimization of fermentation process .....</b>	<b>74</b>
8. <b>Co-fermentation .....</b>	<b>84</b>
9. <b>Evaluation and estimation of melanin.....</b>	<b>85</b>
<b>V. Discussion .....</b>	<b>86</b>
<b>VI. English summary.....</b>	<b>94</b>
<b>VI. References.....</b>	<b>96</b>
<b>VII. Arabic summary</b>	

## LIST OF ABBERRIVATIONS

<b>Bt</b>	: Bacillus thuringiensis
<b>CDNA</b>	: Complementary DNA
<b>EDTA</b>	: Ethylene diamine tetraacetic acid
<b>IPTG</b>	: Isopropyl $\beta$ -D- thiogalactopyranoside
<b>LB medium</b>	: Luria-Bertani medium
<b>MCS</b>	: Multiple cloning site
<b>NaOAC</b>	: Sodium acetate
<b>NCBI</b>	: National Center for Biotechnology Information
<b>PCR</b>	: Polymerase chain reaction
<b>SDS</b>	: sodium dedocyl sulphate
<b>Rpm</b>	: round per minute
<b>RT</b>	: Reverse transcription
<b>TEMED</b>	: N, N, N, N, tetra methyl ethylene diamine
<b>X-Gal</b>	: 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galctoside
<b>D.O</b>	: Dissolved oxygen
<b>PPM</b>	: part per million
<b>FTIR</b>	: Fourier transform infrared spectroscopy
<b>PVDF</b>	: Polyvinylidene fluoride
<b>PABA</b>	: Para amino bezoic acid
<b>DOPA</b>	: Dihydroxyphenylalanine
<b>ICP</b>	: Insecticidal crystal protein
<b>Wt</b>	: wild type