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 complexesas 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 capacbilities. 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* biopestcides will nullify the hazards of chemical UV-blocking agents to both humans and environment. This was achieved by traditional mutantion 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 ws demonostrated 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

LIST OF ABBERIVATIONS

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