Tryptophanase inhibition by NusB elimination in SVS1144 E.Coli: a potential biofilm synthesis control point

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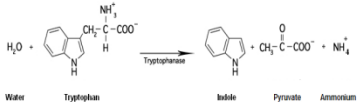
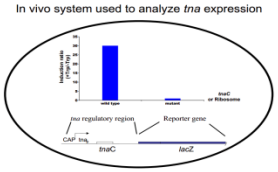
Introduction

The purpose of my research this summer was to determine what the NusB gene's role is in Tryptophanase expression in the SVS1144 strain of *Escherichia Coli.* Tryptophanase is an enzyme that catalyzes the conversion of L-tryptophan to indole and other metabolites . Indole acts a a chemical messenger that signals biofilm synthesis in bacteria. A biofilm is a phenomena when bacterial cells growing in large groups stick to each other on a living or non-living surface. The synthesis of biofilms is seen commonly in several human diseases such as: UTI's after urinary catheterization, middle ear infections, and gingivitis.





Methods

Production of SVS1144 electrocompetent cells

Electrocompetent cells were produce by following a protocol that allowed for introduction of an electrical current to allow permeability in the SVS1144 cell membranes, allowing for entrance of the plasmid.

Electrotransformation of SVS1144 electrocompetent cells + pkd46 plasmid

A transformation was performed by use of electroporation the SVS1144 cells in the presence of the plasmid, allowing for entry. Cells were electroporated at 1.7 kV., then plated on Kanamycin plates and grown at 30°C

PCR amplification of NusB::Km

Polymerase Chain Reaction was used to amplify the kanamycin resistant gene in this step, to be used as a replacement for NusB and allow for comparison.

Transformation of SVS1144+pkd46 with NusB::Km

The final transformation was done on X-gal plates to compare the activity of NusB presence and absence in SVS1144 and as well as W3310 E. Coli cells.

Blue White Screen

Presence of the NusB gene in a blue white screen will not show a precipitate blue color. However, absence of the gene allows for precipitation of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside causing the blue coloration.

Results

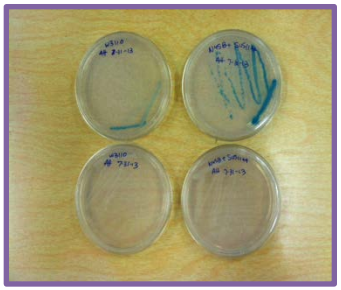
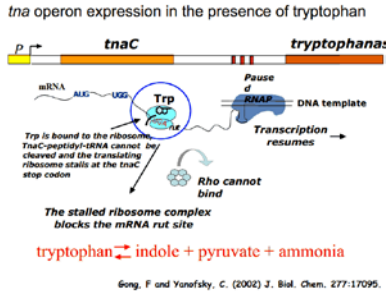


Figure B: Blue White Screen Results



Conclusion and Future Works

After completion of my research it was found that when the NusB gene is eliminated, it inhibits Tryptophanase action.

Further work can be done to develop or determine a mechanism in which manipulation of the NusB gene to inhibit TNA production of indole, therefore inhibiting the startup signaling for biofilm synthesis in manifestations involving pathogenic E.Coli.

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