Biosynthesis of Bromocoumaric Acid in Bromoalterochromide A.

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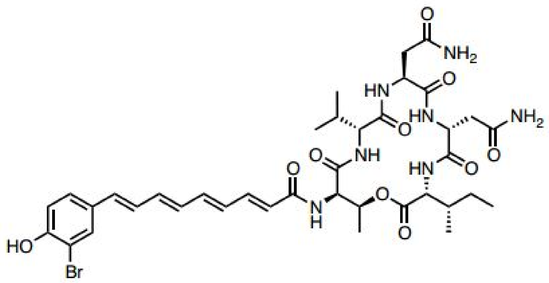
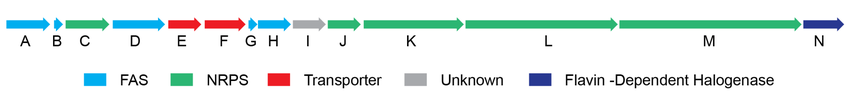
Overview and Background

The main point of our project is to study the biosynthesis of bromoalterochromide by looking into the four enzymes that are responsible for the production of the bromocoumaric acid portion of the molecule. Bromoalterochromide is a compound naturally produced by the marine bacteria *Pseudoalteromonas Piscicida*. The compound was captured and the gene cluster was isolated last year. We then studied the compound further by looking at the four enzymes, AltA, AltB, AltC, and AltN (Halogenase).

Bromoalterochromide Compound

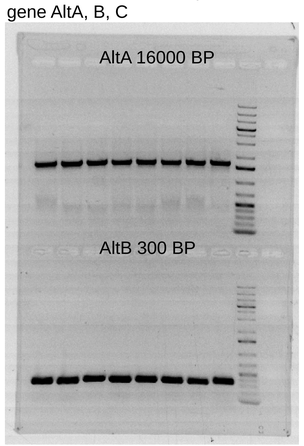


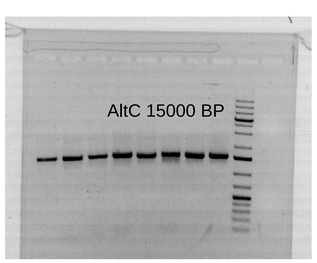
Biosynthetic Gene Cluster for Bromoalterochromide A.



The compound starts from Tyrosine. AltA removes the hydrogen and amino group and generates coumaric acid with a carbon double bond. AltB (ACP) and AltC work together to attach coumaric acid to the ACP which AltN uses to create the brominated compound.

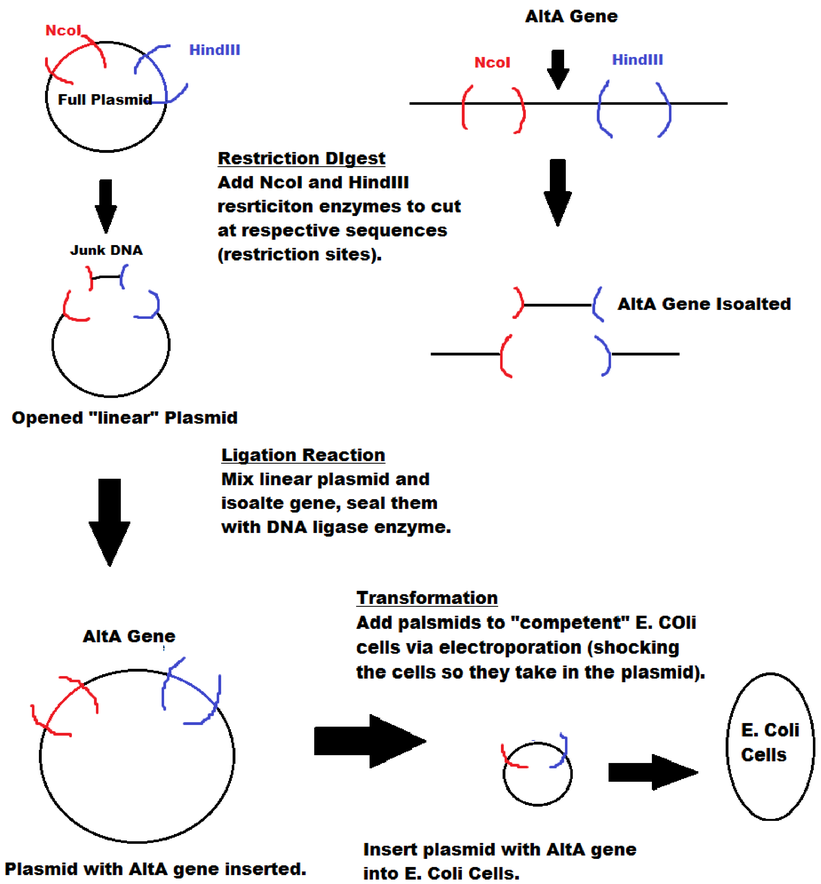
Gel results for PCR amplification of gene AltA, B, C





Alt A is 16000 base pairs, AltB is 300 BP and AltC is 15000 BP
The results for this gel were beautiful.

Generating gene expression system



Future

If I had more time in lab I would have worked to get AltB and C at the same point AltA is and transform them to BL21 Gold and run protein expressions like we did for AltN. We would do this on a larger scale and isolate the enzyme. Our ultimate goal is to test *in vitro* the purified enzymes to confirm their proposed roles in the biosynthesis of the bromocoumaric acid portion of Bromoalterochromide A.

Acknowledgements

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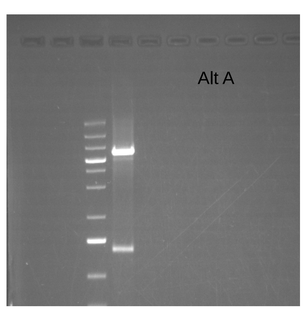
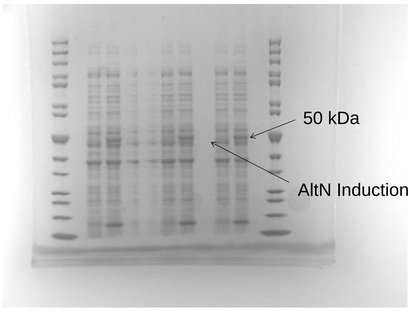


This successful ligation of Alt A shows the plasmid (big piece) and the gene (small piece).

Procedure

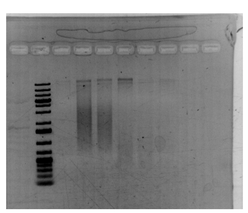
We grew *P. Piscicida* JCM 20779 which was cultured in 3mL of A1 broth and incubated overnight and then isolated gDNA. We then grew *E. Coli* containing pHis8 cultured in 5mL of LB broth with the antibiotic Kanamycin (Kan). After isolating the plasmid we ran a restriction digestion on the pHis8 and isolated the linear plasmid. We then took the gDNA from *P. piscicida* and ran PCR to obtain AltA , AltB, and AltC, and ran a gel to analyze the products (which separates the DNA by length and screens what we want out). One for A worked, all of B worked and none of C worked. Since our original PCR's didn't work we set up a second one, using only the gene cluster inserted into the pCCOI plasmid from last year. The PCR results were beautiful! We then purified the PCR product, and performed a restriction digest on the PCR product. At this point we had our isolated genes for AltA, AltB, and AltC so we set up a ligation reaction which mixes the linear plasmid, the isolated gene, and sealing them with a DNA ligase enzyme. We transferred the cells through electroporation which shocks the cells so they open up and take in our plasmid. We could confirm that the ligation of AltA was successful. We then ran a trial protein expression for pHis8 AltN.

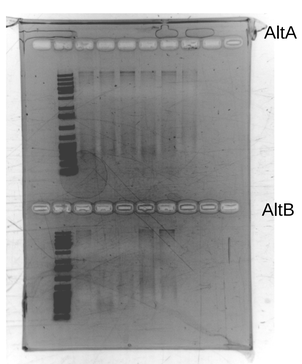


Results

The first time we ran a PCR the results were not solid so we ran a second PCR using only the gene cluster inserted into the pCCOI Plasmid from last year and the results were awesome. We then took these and performed a restriction digest on the PCR product and purified the restriction digest. When we performed a ligation reaction we mixed the linear plasmid, amplified genes and sealed the DNA with a ligase enzyme. We transformed the cells through electroporation and plated them with antibiotics. Colonies grew and we picked 3 from each (A, B, C). We ran a restriction digest on the isolated pHis8 AltA , B, and C plasmid and the gel came out smeared with no genes present. We are now looking closer to see why this may be. We tested our cultures and ran restriction digests on the results. A second attempt resulted in successful ligation for AltA.

AltC





Restriction Digest gels for AltA, B, C to see if the plasmid took up the cells. The results were blurred. This is where we began troubleshooting.