Biochemical Analysis of Terpenes in Rice Blast Fungus

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Overview

Rice Blast disease caused by the fungal pathogen *Magnaporthe oryzae* poses a threat to global food security. The disease is known to cause approximately 10-30% of rice crop loss each year, which can feed 60 million people annually. To understand what allows the pathogen to infect the rice crops without any defence, researchers have come to study secondary metabolites very closely.

Image 1: Rice plants in India

In fungi specifically, the secondary metabolites help in environmental interactions. One such class of specialized metabolites that are produced in fungi are terpenes. Terpenes are a large group of compounds made of unsaturated hydrocarbons, sometimes they can be volatile too. They are aromatic compounds found mainly in plants. (give examples). However, not much is known about the exact function of such metabolites in the fungal organism. Also, the relationship between pathogenicity and secondary metabolites remain unclear. To understand this relationship, we are studying the genes that code for specialized metabolites in laboratory situations.

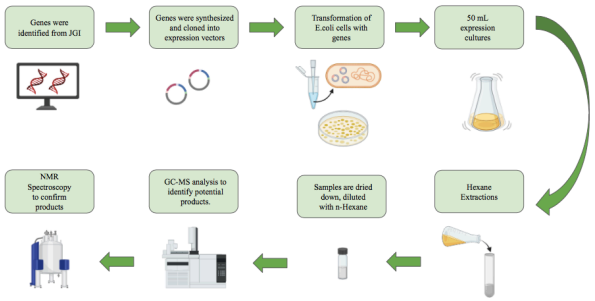
In 2007, a chimeric enzyme was found in Fungi where the Prenyltransferase (PT) and the Terpene Synthase (TPS) were fused together. PTs help the PTs determine the prenyl carbon chain length, whereas TPSs generate the structural complexity of the molecular scaffolds, forming various ring structures. The fusion of these enzymes could reflect a possible acceleration in product flux in fungi to produce terpenoids.

Our primary focus was to observe the possible terpene products that certain genes from *M.oryzae* produced to study the biosynthesis of these products and the enzymes involved in the process.

Objectives

- Biochemical Analysis of terpenes (sesterterpenes) in *M.oryzae*.
- Catalytic Promiscuity of Sesterterpene Synthases.

Method



Results

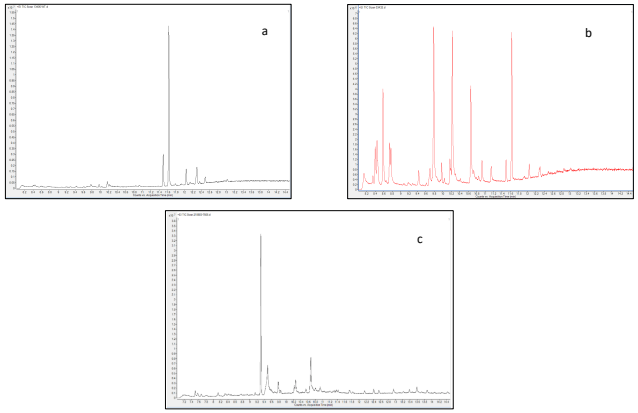


Figure 2: The GC graphs a, b and c show the products formed by Genes 1, 2 and 3. The graphs on the top show Gene 1 (left) and Gene 2 (Right) where the suspected product is present at the 11.6 peak. For Gene 3, the product can be seen at the 9.3 peak.

Structural Schematic

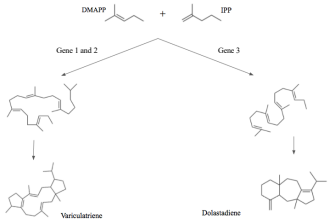


Figure 3: The terpenoid precursors DMAPP and IPP lead to the eventual formation of the suspected products.

Motifs

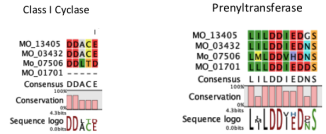


Figure 4: The two enzymes that form the chimeric enzyme are shown here with their motifs.

Promiscuity

To test the catalytic promiscuity of sesterterpene synthases in the genes of interest, the mutant version of the gene was provided a different carbon backbone of 20 Carbons – GGPP (Geranylgeranyl Diphosphate). The mutant versions did not make the expected products seen from Figure 2.

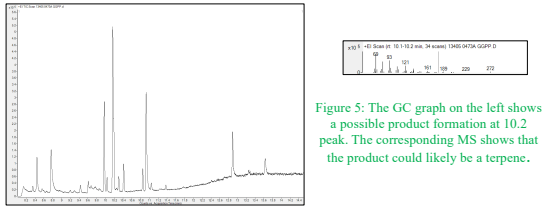


Figure 5: The GC graph on the left shows a possible product formation at 10.2 peak. The corresponding MS shows that the product could likely be a terpene.

Summary

Based on the study conducted over the summer, it has become evident that the genes can be mutated to prevent the suspected product formation. The catalytic promiscuity experiment showed us that when provided with different carbon backbones the enzymes can use it to produce terpene-like products. However, the study must be continued to exactly determine the kind of products being formed.

Future Work and Extensions

Since the genes of *M.oryzae* have been studied at the laboratory level and we can observe that the suspected products are terpenes, we can assume that they play some role in the pathogenesis of the organism.

Next step would be to use these results as a control and study the organism itself by knocking out the specific genes and observing how that affects the organism's function. If the function is found to be crucial to the pathogenesis aspect of the organism, then we can further investigate how *M.oryzae*'s interaction with rice can be modified through the understanding of the gene's functions.

References and Acknowledgements

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