Investigating Catalytic Residues in Class I Active Site of a Fungal Diterpene Synthase

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Background:

Diterpene synthases create secondary metabolites known as diterpenes. Diterpenes are recognized in plants for their antimicrobial and antifungal properties. They allow plants to communicate with their environments and play roles in plant defense and plant reproduction. Diterpene synthases synthesize these secondary metabolites by performing numerous cyclization reactions driving promiscuity of diterpene product outcome. Novel studies have detected the presence of diterpene synthases genes in fungi. We seek to understand the biochemical mechanisms behind terpene formation. This study investigates the catalytic residues in class I active site of a fungal diterpene synthase through site directed mutagenesis and combinatorial expression.

Purpose:

Magnaporthe, is the causative agent of rice blast disease and accounts for devastating damage to cereal crop yield worldwide annually. This plant pathogenic fungi infects numerous cereal and grass species at an alarming rate, causing lesions on all parts of the plant in all the respective hosts. We seek to better understand plant - pathogen interaction.

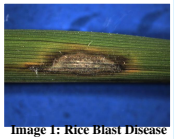


Image 1: Rice Blast Disease

Schematics:

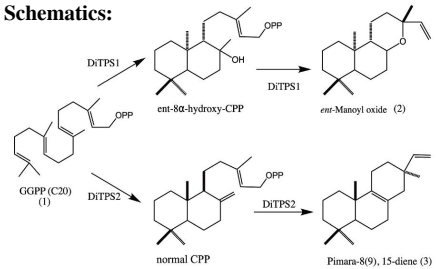


Figure 4: Biosynthesis of labdane related diterpenes by *M.oryzae*

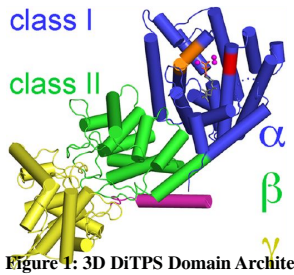


Figure 1: 3D DiTPS Domain Architecture

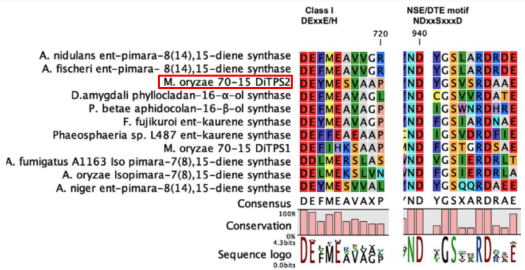
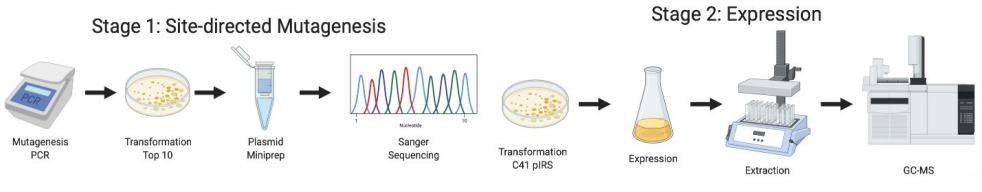


Figure 2 : Motifs and alignments of class I motifs of *M. Oryzae* diterpene synthases with charactered diterpene synthases

Methods:



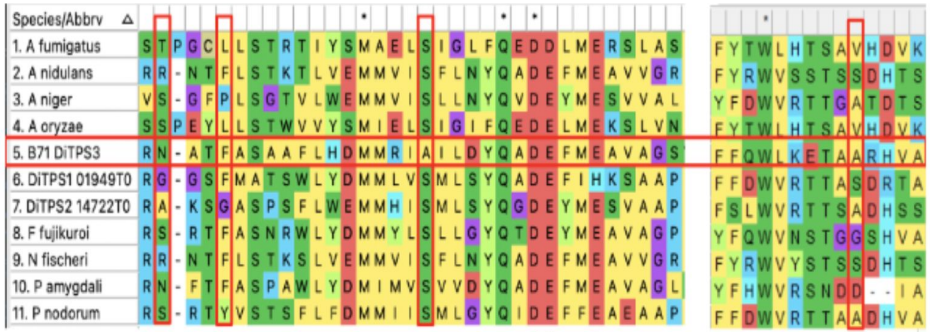


Figure 3: Phylogenetically guided mutagenesis of mutants N632G (1), F635G (2), A648S (3) and A791S (4).

Future directions:

- Determine other residues of catalytic importance through mutagenesis
-Determine structural and functional significance of product outcomes
-Contribution to ongoing projects
-Agricultural use against multiple pathogens and environmental factors
-Investigate other fungi and their diterpene metabolism

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