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Expression and Site-Directed Mutagenesis of Type III Polyketide Synthases

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ABSTRACT

Natural products are a well-established source of drugs, and evolution has yielded polyketides such as leinamycin and iso-migrastatin that have demonstrated anti-tumor activity. Polyketides are large metabolites with a high degree of chemical variability and are commonly produced by soil bacteria. Polyketide synthases (PKS) exist as three different archetypes, and the reaction mechanisms of ketosynthases from all archetypes is not understood. Type III PKSs exist as an independently functioning ketosynthase (KS), which primarily use coenzyme A (CoA), with some exceptions, for the biosynthesis of polyketides. We elected to focus our studies on ketosynthases, because they are responsible for forming the carbon-carbon bonds seen in polyketides. To study these Type III PKS KS, we expressed Streptomyces coelicolor germicidin synthase (Gcs) and tetrahydroxynaphthlene synthase (THNS) in E. coli and mutant versions where the catalytic active cysteine was changed to a serine or glutamine. In previous studies, serine slowed the overall progress of the reaction, and glutamine abolished carbon-carbon bond formation but promoted malonyl-CoA decarboxylation. We verified our mutations using a third party organization's fluorescent sequencing by dye termination services, as well as confirmed that an acceptable level of expression of our protein is occurring in our BL21 cell lines using SDS-PAGE and Fast Protein Liquid Chromatography (FPLC). Now that we have successfully expressed and mutated our protein, we can move forward and use substrate mimics in conjunction with our mutants to further understand the catalytic mechanism of ketosynthases.

KEYWORDS

Polyketide, Biosynthesis, Ketosynthase