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Engineering the biosynthesis of artemisinin

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ABSTRACT

Terpenoids represent the largest class of natural products with a diverse array of structures and functions. Many terpenoids have reported therapeutic properties such as antimicrobial, anti-inflammatory, immunomodulatory and Chemotherapeutic properties making them of great interest in the medical field. Terpenoids suffer from low natural yields and complicated chemical synthesis; hence there is a need for a more sustainable production method. Metabolic engineering using biosynthetic mevalonate and non-mevalonate pathways provides an excellent opportunity to construct microbial cell factories producing terpenoids. The complexity and diversity of terpenoid structures depends mainly on the action of the terpene synthases responsible for their synthesis. Amorpha- 4, 11-diene synthase (ADS) cyclizes the substrate farnesyl pyrophosphate to produce amorpha- 4, 11-diene as major product. This is considered the first committed and rate-limiting step in the biosynthesis of the antimalarial artemisinin. Here, we utilize a reported 3D model of ADS to perform mutability landscape guided enzyme engineering.

Key words: Terpenoids, Metabolic engineering, Chemotherapeutic properties, Amorpha- 4, 11-diene synthase (ADS).

INTRODUCTION

Terpenoids represent the largest class of natural products with a diverse array of structures and functions. Many terpenoids have reported therapeutic properties such as antimicrobial, anti-inflammatory, immunomodulatory and chemotherapeutic properties making them of great interest in the medical field. Terpenoids suffer from low natural yields and complicated chemical synthesis; hence there is a need for a more sustainable production method. Metabolic engineering using biosynthetic mevalonate and non-mevalonate pathways provides an excellent opportunity to construct microbial cell factories producing terpenoids. The complexity and diversity of terpenoid structures depends mainly on the action of the terpene synthases responsible for their synthesis. Amorpha- 4, 11-diene synthase (ADS) cyclizes the substrate farnesyl pyrophosphate to produce amorpha- 4, 11-diene as major product. This is considered the first committed and rate-limiting step in the biosynthesis of the antimalarial artemisinin. Here, we utilize a reported 3D model of ADS to perform mutability landscape guided enzyme engineering. A mutant library of 258 variants along sixteen active site residues was created and then screened for catalytic activity and product profile. This allowed for identification of the role of some of these residues in the mechanism.

The mutability landscape also helped to identify variants with improved catalytic activity. H448A showed ~4 fold increase in catalytic efficiency and the double mutation T399S/H448A showed that kcat has improved by ~5 times. This variant can be used to enhance amorphadiene production and in turn artemisinin biosynthesis. Our findings provide the basis for the first step in improving industrial production of artemisinin and they open up possibilities for further engineering and understanding of ADS. Artemisinin, a natural compound from Artemisia annua, is highly effective in treating drug-resistant malaria. Because chemical synthesis of this natural terpenoid is not economically feasible, its only source remains as the native plant which produces only small quantities of it, resulting in a supply that is far short of demand. Extensive efforts have been invested in metabolic engineering for the biosynthesis of artemisinin precursors in microbes.

However, the production of artemisinin itself has only been achieved in plants. Since, A. annua possesses only poorly developed genetic resources for traditional breeders, molecular breeding is the best alternative. In this review, we describe the efforts taken to enhance artemisinin production in A. annua via transgenesis and advocate metabolic engineering of the complete functional artemisinin metabolic pathway in heterologous plants. In both cases, we emphasize the need to apply state-of-the-art synthetic biology approaches to ensure successful biosynthesis of the drug. Artemisinin, a sesquiterpene lactone isolated from the Chinese medicinal plant Artemisia annua L., is an effective antimalarial agent, especially for multi-drug resistant and cerebral malaria. To date, A. annua is still the only commercial source of artemisinin. The low concentration of artemisinin in A. annua, ranging from 0.01 to 0.8% of the plant dry weight, makes artemisinin relatively expensive and difficult to meet the demand of over 100 million courses of artemisinin-based combinational therapies per year.

Since the chemical synthesis of artemisinin is not commercially feasible at present, another promising approach to reduce the price of artemisinin-based antimalarial drugs is metabolic engineering of the plant to obtain a higher content of artemisinin in transgenic plants. In the past decade, we have established an Agrobacterium-mediated transformation system of A. annua, and have successfully transferred a number of genes related to artemisinin biosynthesis into the plant. The various aspects of these efforts are discussed in this review. Artemisinin content was significantly increased in transformed material of both Artemisia species when compared to un-transformed plants. The artemisinin content within leaves of transformed lines was increased by a factor of nine, indicating that the plant is capable of synthesizing much higher amounts than has been achieved so far through traditional breeding. Expression of all artemisinin biosynthesis genes was significantly increased, although variation between the genes was observed. CYP71AV1 and ALDH1 expression levels were higher than that of ADS. Levels of the TFAR1 expression were also increased in all transgenic lines.

Trichome density was also significantly increased in the leaves of transformed plants, but no trichomes were found in control roots or transformed roots. The detection of significantly raised levels of expression of the genes involved in artemisinin biosynthesis in transformed roots correlated with the production of significant amounts of artemisinin in these tissues. This suggests that synthesis is occurring in tissues other than the trichomes, which contradicts previous theories.

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