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Review

Shaking up ancient scents: Insights into santalol synthesis in engineered *Escherichia coli*

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ABSTRACT

Sandalwood oil is an essential oil that is derived from sandalwood and has important uses in cosmetics and medicine. Sandalwood oil is mainly composed of α-, β- and *epi*-β-santalols, which are responsible for the pleasant woody aroma. The syntheses of santalols begin with the condensation of the universal C_5 precursors dimethylallyl diphosphate and isopentenyl diphosphate via farnesyl diphosphate (FPP) synthase. The resulting FPP undergoes multiple rearrangements and cyclization via santalene synthase to generate α-, β- and/or *epi*-β-santalenes, which are finally hydroxylated at the C_{12} position by cytochrome P450 monooxygenase in cooperation with an NADPH-dependent cytochrome P450 reductase (CPR) to form santalols. Advances in metabolic engineering and protein engineering can increase the use of enzymes to synthesize valuable chemicals. In this review, we summarize the metabolic pathway for santalol synthesis and associated enzymes. We also review the advances in metabolic engineering and biotechnology that can be utilized to manipulate *Escherichia coli* for santalol synthesis. We expect that the insights brought out by this review will shed light on metabolic engineering processes for santalol production in *E. coli*.

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1. Introduction

Sandalwood oil is a precious essential oil with characteristic soft, warm, woody and milky-nutty scents [1]. It is obtained from the heartwood and roots of mature (>25 years), oil-producing Santalaceae (*Santalum* genus) via steam distillation (Fig. 1A). Four *Santalum* species contribute to the sandalwood oil market, including *Santalum album*, which is native to South Asia; *S. spicatum*, which is grown in Western Australia; *S. austrocaledonicum*, which is grown in Vanuatu and New Caledonia; and *S. yasi*, which is grown in Fiji and Tonga [2]. Sandalwood oil is a mixture of sesquiterpenoid olefins and alcohols (>90%) and mainly consists of α- and β-santalols (50–70%) [3], which are mainly responsible for the pleasant woody aroma. The oil compositions are variable, depending on extraction methods and biological sources [2,4]. The well-known East Indian sandalwood oil contains approximately 80% santalols (α-, β- and *epi*-β-) with minor amounts of α-bergamotol, α- and β-santalenes, α-bergamotene, etc. (Fig. 1B and C) [5], which is standardized in ISO (3518:2002) for quality control [6]. Sandalwood and its oil have been used in religious

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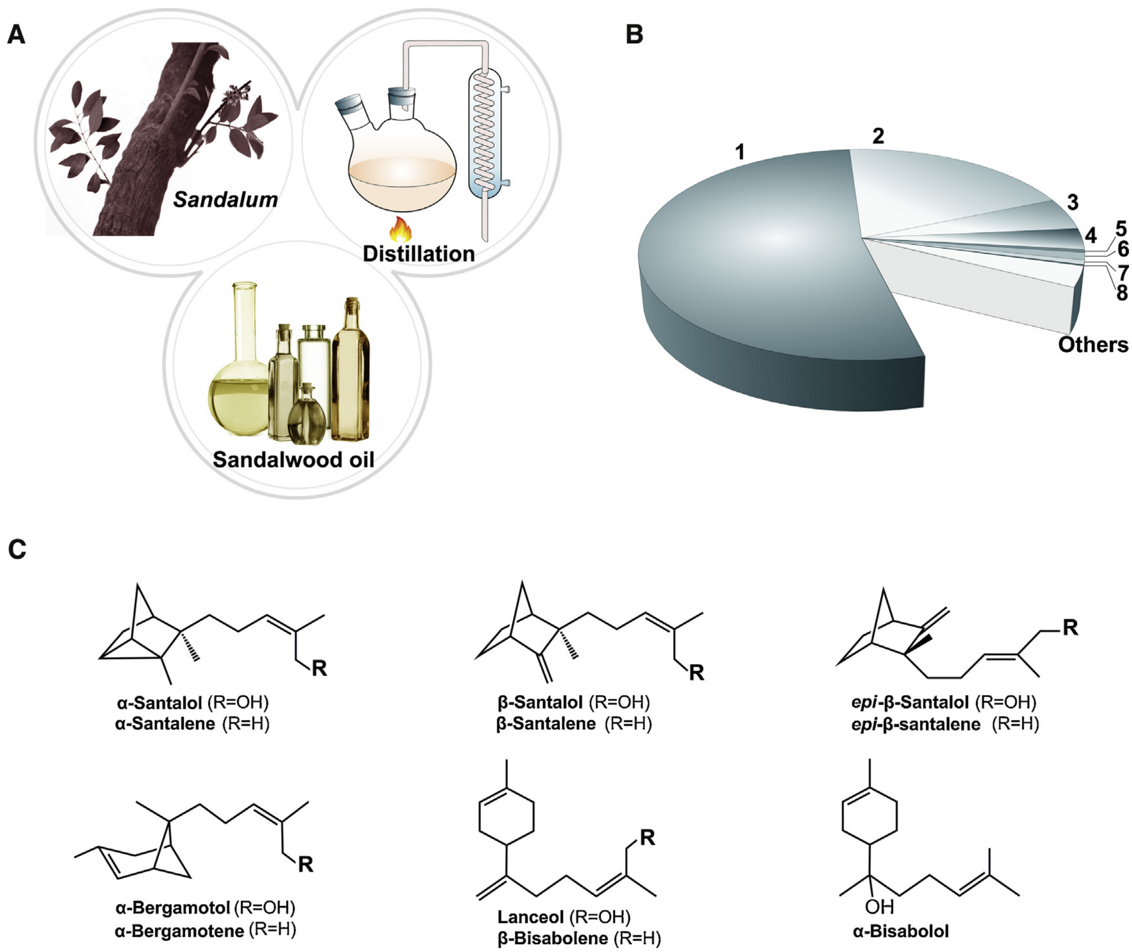

Fig. 1. Overview of natural occurring sandalwood oil. (A) Preparation of sandalwood oil. Sandalwood oil is extracted from matured trees of the genus *Santalum* via steam distillation. (B) Composition of sandalwood oil from *Santalum album*. 1, α-santalol (54.2%); 2, β-santalol (19.2%); 3, *epi*-β-santalol (5%); 4, α-bergamotol (3.3%); 5, α-santalene (0.5%); 6, β-santalene (1.1%); 7, *epi*-β-santalene (0.7%); 8, α-bergamotene (0.2%); and others, farnesol, α-bisabolol, *etc.* (C) Chemical structures of its main components.

rituals and Ayurvedic medicine for millennia [1]. The current interests in sandalwood oil are growing in the aromatherapy, cosmetics and food industries due to its sedative action and fragrance [2]. The Flavor and Extract Manufacturers Association (FEMA) has approved sandalwood oil as a generally recognized as safe (GRAS) flavoring ingredient for use in food [7]. In addition, the main α-santalol component is found to prevent the development of skin tumors in mice and reduce the likelihood of actinic keratosis and skin cancer [8]. However, the increasing demand for this essential oil is not likely to be met due to limited global resources and poor sustainable plantation of sandalwood, arising from the slow growth and hemiparasitic nature of Santalaceae, habitat destruction and government restriction of sandalwood harvest [1]. Oil production by *in vitro* micropropagation of Santalaceae is still in its infancy [9], which usually requires great efforts on genomics, transcriptomics and metabolomics in this precious tree. Chemists have designed several approaches to produce santalols and their derivatives, while the complex architecture of santalols (bi-/tri-cyclic) results in very low overall yields and product purity from chemical synthesis [10–12]. Currently, there are only a small selection of commercially available synthetic substitutes that mimic natural sandalwood scents, such as Sandranol (Symrise), Sandalore (Givaudan) and Polysantol (Firmenich), which have structures similar to santalol [13]. However, metabolic engineering of living microorganisms to synthesize and refine santalols via regio- and stereo-selective properties of biological enzymes can afford a sustainable and environmentally friendly alternative. *Escherichia coli* possesses superior engineering capacity to other heterologous hosts such as *Saccharomyces cerevisiae*, owing to its fast growth rate and massive genetic tools [14]. In this review, we will summarize the synthetic pathways of santalols and provide insights into the production of sandalwood oil in *E. coli* with recent advances in metabolic engineering and its enabling technologies.

2. Pathway engineering for santalol synthesis

The pathways and enzymes associated with santalol biosynthesis have been elucidated. Metabolic engineering of *E. coli* can be adopted to produce santalols, the main components of sandalwood oil. The recently developed strategies for the production of terpenoids could offer insight into the engineering process to improve santalol yields.

2.1. Santalol synthesis pathway and its constituent enzymes

Santalols (α-, β- and *epi*-β-) in sandalwood oil are sesquiterpenoids derived from the universal C_5 precursors dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP). Carbon substrates can be assimilated to DMAPP and IPP via either the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway in most bacteria and plastids or the mevalonic acid (MVA) pathway in eukaryotes and some bacteria. There are many excellent reviews for more comprehensive information on the both pathways [15]. Farnesyl diphosphate (FPP) synthase assembles these two