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Engineering of bacterial terpene synthases for improved terpenoid production in Escherichia coli

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PURPOSE OF THE ABSTRACT
Terpenoids are a highly diverse group of natural products that are of considerable industrial interest. Monoterpenoids (C10) are, for example, used as flavour and fragrance ingredients, as antimicrobials in household products, but also as precursors for anti-cancer drugs and alternative fuels. Increasingly, engineered microbes are used for the production of terpenoids to replace natural extracts and chemical synthesis. All terpenoids are produced from the C5 building blocks isopentenyl diphosphate and dimethylallyl diphosphate, which are combined by prenyl pyrophosphate synthases resulting in substrates of varying lengths. Terpene synthases (TSs), the enzymes responsible for the wide structural diversity of terpenoids, guide the linear carbocationic intermediates along one of many cyclisation paths via exertion of subtle steric and electrostatic control, before the reaction is terminated by deprotonation or nucleophilic water attack.
In addition to plants, bacteria are a rich source for TS activity. Bioinformatics searches of available bacterial genomes revealed over 2000 genes with a class I TS domain. In addition to 2-methyl isoborneol and geosmin synthases, TSs long known to be present in soil bacteria, many classical TSs were identified in a wide variety of bacterial species [1]. Most of these enzymes consist of a single class I TS domain only and exhibit sesqui- (C15) or di-terpene (C20) synthase activity. Until recently, only two known bacterial monoterpene synthases (MTSs) had been identified, a bi-functional linalool/nerolidol synthase (bLinS) and a cineole synthase (bCinS). Interestingly, these enzymes show a high-level of product fidelity, where plant MTSs are often highly promiscuous yielding product mixtures. We used a multi-disciplinary approach involving, data-mining, bio-informatics, X-ray crystallography, site-directed mutagenesis and computational chemistry to unravel the mechanism of high-fidelity bacterial TSs.
Linalool is a valuable acyclic monoterpenoid product. bLinS from Streptomyces clavuligerus produces linalool from geranyl diphosphate (GPP, C10) and nerolidol from farnesyl diphosphate (FPP, C15). We engineered bLinS for increased linalool production in E. coli by constricting the active site, improving both linalool titre and purity [2]. bCinS from S. clavuligerus is the only known ‘true' bacterial MTS and produces bi-cyclic 1,8-cineole from GPP at high purity, unlike plant CinS from Greek sage (CinS_Sf). We identified residues involved in key steps in the bCinS cyclisation cascade, including water attack and carbocation stabilisation. We also show that bCinS produces cineole almost exclusively via S-a-terpineol, where CinS_Sf appears to use both S- and R-isomers [3,4]. 10-epi-cubebol synthase from Sorangium cellulosum (ScCubS) forms >93% products with a tri-cyclic cubebane scaffold from FPP. We identified key residues that control the distribution between several early-stage cations which determine whether the final product is derived from the mono-, bi or tri-cyclic germacrane, cadalane, or cubebane hydrocarbon scaffold respectively [5].
Our results show that each terpene product requires a tailored active site, where each residue has a unique and precise function, and unlike high-fidelity enzymes, promiscuous enzymes may supply multiple routes to the end product. However, progress is slow and knowledge often does not translate to other TSs. Due to the absence of strong protein interactions with the carbocation intermediates, there is a remarkable lack of sequence-function relationship within the class I TS family, making product-outcome predictions from sequences alone highly challenging. Future work is therefore directed towards combining sequence diversity data, with product profiles and structural information in data-driven approaches for the predictive engineering of TS activity [6].

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

Enzyme engineering | Synthetic biology | Terpene synthase | Terpenoids

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