Production of guaianolide sesquiterpene lactones in engineered yeast

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Research aims

Sesquiterpenoids are a group of terpenoids consisting of three isoprene units. Plants produce a vast array of useful sesquiterpenoids from a common precursor, farnesyl diphosphate (FPP). Guaianolide sesquiterpene lactones (GSLs), which are mainly found in plants, are a group of sesquiterpenoids characterized by a 5,7,5-ring structure containing a lactone ring. GSLs exhibit a wide range of pharmaceutical properties including selective inhibition of acute myelogenous leukemia stem cells[1)]. However, production of these compounds depends on their extraction from plants and suffers from low yields. Alternatively, GSLs can be produced in microbial hosts using biosynthetic genes. In this study, we aimed to identify enzymatic genes that can be used to reconstruct the GSL biosynthetic pathway to synthesize the simplest form of GSL in the yeast, *Saccharomyces cerevisiae*.

Methods

Using a combinatorial biosynthesis approach, we successfully synthesized 13-δ-guaianoic acid, a potential biosynthetic intermediate of the GSL pathway, by transforming *S. cerevisiae* with genes encoding four enzymes from different plant sources: *Aquilaria crassna* δ-guaiene synthase AcC2 (a sesquiterpene synthase catalyzing the cyclization of FPP into a sesquiterpene scaffold, δ-guaiene), *Lactuca sativa* CYP71AV3 (a cytochrome P450 monooxygenase catalyzing the hydroxylation of δ-guaiene to produce 13-hydroxy-δ-guaiene), and two dehydrogenases from *Artemisia annua*, ADH1 and ALDH1, which convert 13-hydroxy-δ-guaiene into 13-δ-guaianoic acid (Fig. 1). Therefore, to synthesize the simplest form of GSL from δ-guaianoic acid, we aimed to identify the P450(s) that catalyze the 6- or 8-hydroxylation of 13-δ-guaianoic acid, which may promote subsequent spontaneous lactone ring formation (Fig. 1). As a resource for candidate gene mining, we selected the GSL-producing species *Aquilaria sinensis* because of its available transcriptome information. To test for potential δ-guaiene oxidizing activity of the obtained candidate P450s, transgenic yeast co-expressing *AcC2* and each of the five candidate P450s

were generated, and the yeast culture extracts were analyzed by gas chromatography-mass spectrometry (GC-MS).

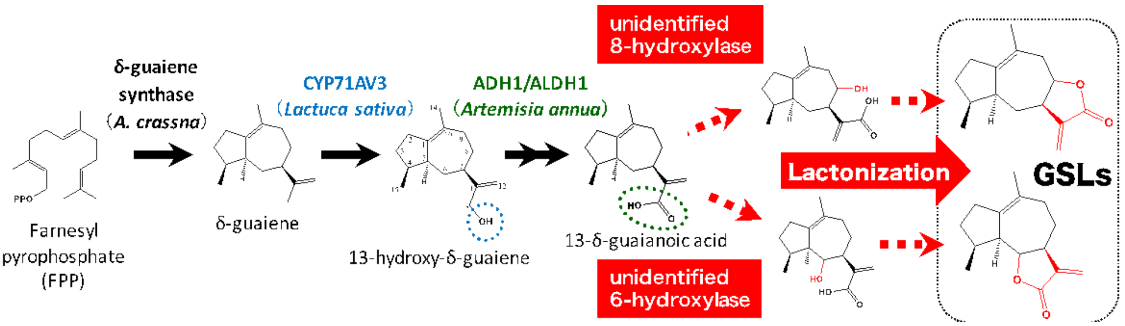

Fig. 1. Engineered pathway for biosynthesis of the simplest form of guaianolide sesquiterpene lactone (GSL) in transgenic yeast

Results

We mined candidate P450s from *A. sinensis* due to the availability of its transcriptome sequencing data. Raw reads were downloaded from the Sequence Read Archive of three data sets containing transcriptome data of differentially expressed genes under stress conditions: 1) different parts of a 5-year-old *A. sinensis* treated with formic acid[2)], 2) a callus under salt stress[3)], and 3) healthy and wounded stems[4)]. All raw reads were mixed and assembled *de novo* using Trinity software. Five candidate P450s having greater than 45% amino acid sequence identity with known sesquiterpene oxidases and showing extremely high expression in the formic acid-induced agarwood part (with a 300- to 1,600-fold increase in transcript levels) were selected. To test their potential δ-guaiene oxidizing activities, each of the five candidate P450s was co-expressed with the δ-guaiene synthase gene in an FPP high-producing yeast strain (EPY300), and the ethyl acetate extracts were analyzed by GC-MS. Among the five candidate P450s, two exhibited activity against δ-guaiene: AsCYP-1 produced 13-hydroxy-δ-guaiene (compound 1 in Fig. 2), and AsCYP-2 produced an unknown δ-guaiene oxide (compound 2 in Fig. 2). Based on its molecular mass, compound 2 was speculated to be a derivative of δ-guaiene oxidized at two different positions. We also tested the activity of AsCYP-2 against 13-δ-guaianoic acid but no activity was detected (data not shown).

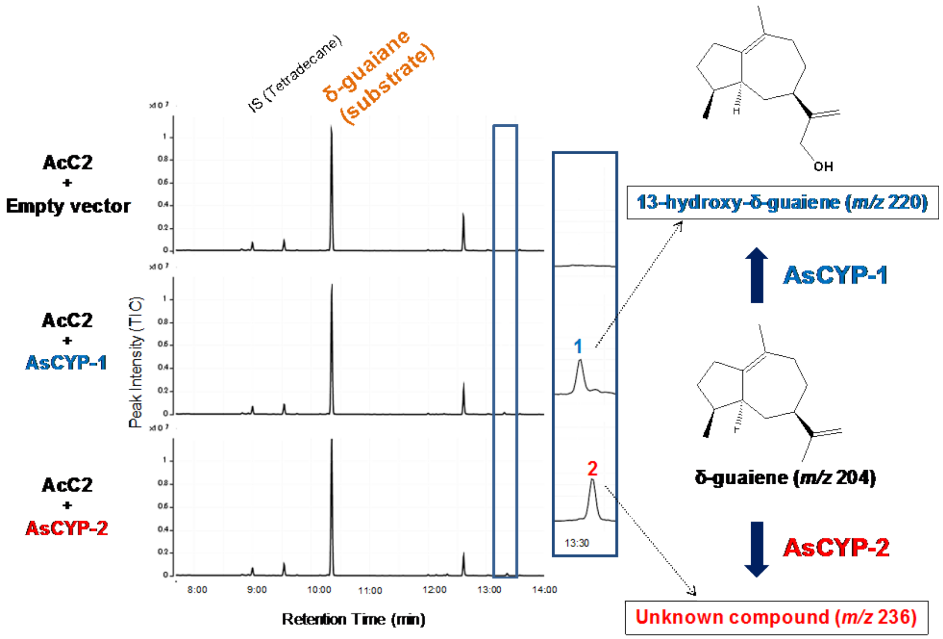

Fig. 2. Gas chromatography-mass spectrometry (GC-MS) analysis of the products of *AcC2*/candidate P450-expressing yeast

Ethyl acetate extracts of yeast culture were analyzed by GC-MS. *AcC2*/*AsCYP-1*-expressing yeast produced 13-hydroxy-δ-guaiene (compound 1). *AcC2*/*AsCYP-2*-expressing yeast produced an unknown δ-guaiene oxide (compound 2).

Conclusion

In this study, we identified five candidate P450s from *A. sinensis* transcriptome data and analyzed their potential δ-guaiene and 13-δ-guaianoic acid oxidizing activities. However, none of the candidate P450s showed desirable activities (i.e., the ability to catalyze the 6- or 8-hydroxylation of 13-δ-guaianoic acid that may promote subsequent spontaneous lactone ring formation). Although reconstruction of GSL pathways in yeast was not successful in this study, we identified two P450s active against δ-guaiene. Since both P450s (AsCYP-1 and -2) belong to the CYP71 family and sharing 48% amino acid sequence identity with each other, mining of additional candidates highly homologous to these P450s from GSL-producing plants would be a promising approach to identify desirable enzymes for reconstructing the GSL pathway in yeast.

References

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