Synthesis of tobacco-derived cembratriene-ol and cembratriene-diol in yeast using engineered enzymes

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Technical Notes

Keywords: Cembranoids, Tobacco, Biosynthesis, Yeast, Engineered enzymes, MVA pathway

Posted Date: November 4th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-100836/v1

Version of Record: A version of this preprint was published on February 2nd, 2021. See the published version at https://doi.org/10.1186/s12934-021-01523-4.

Abstract

Background: Cembranoids are one kind of diterpenoids with multiple biological activities, and the tobacco cembatriene-ol (CBT-ol) and cembatriene-diol (CBT-diol) have high anti-insect and anti-fungal activities, which is attracting great attention for their potential usage in sustainable agriculture. Cembranoids have been supposed to be formed through the

2-C-methyl-D-erythritol-4- phosphate (MEP) pathway, yet the involvement of mevalonate (MVA) pathway in their synthesis remains unclear. Exploring the roles of MVA pathway in cembranoid synthesis could contribute not only to the technical approach but also to the molecular mechanismfor cembranoid biosynthesis.

Results: We constructed a vector to express an engineered protein fusion of cembranol synthase (CBTS1) and the GAL4 AD domain as a N-terminal translation leader. Eventually, the engineered enzyme AD-CTBS1 was successfully expressed, which further resulted in the production of CBT-ol in yeast with an optimized MVA pathway for geranyl-geranyl diphosphate (GGPP) production, but not in other yeast strains with low GGPP supply.

Subsequently, CBT-diol was also synthesized by co-expression of engine-ered cembranol synthase (CBTS1) and cytochrome P450 hydroxylase (CYP450) in the yeast enhanced MVA pathway.

Conclusions: We demonstrated that yeast could be applied to the production of tobacco-derived CBT-ol and CBT-diol, which are anti-fungal compounds. And, established a new way to produce the tobacco-derived CBT-ol and CBT-diol in yeast with optimized MVA pathway for GGPP production. Thus, this study established a feasibility for cembranoid production via the MVA pathway and provided an alternative bio-approach for the production of cembranoids in microbes.

Background

Cembranoids are a group of natural carbocyclic diterpenes structurally composed of a 14-carbon cembrane ring. This kind of compounds were firstly identified in conifer plants and have been found to widely present in nature [1, 2]. So far, hundreds of cembranoids have been isolated from plants, insects, alligators, and marine organisms [3, 4]. Cembranoids possess multiple bioactivities, such as anti-fungal [5–7], anti-insect [8], anti-cancer [9, 10], anti-inflammatory [11–13], neuroprotection [14, 15], etc. and have great attractions to not only pharmacology but also agrochemistry. Tobacco is the land plant most abundant in cembranoids whose abundance could significantly affect the aromatic property of tobacco [16]. Cembatriene-ol (CBT-ol) and cembatriene-diol (CBT-diol) are two major cembranoids in tobacco. And, they are synthesized and secreted by the glandular trichomes on tobacco leaves [3].

As one type of diterpenoids, tobacco CBT-ol and CBT-diol are derived from geranylgeranyl diphosphate (GGPP) under the sequential catalyzation by CBTS1 (cembranol synthase) and CYP450(cytochrome P450 hydroxylase) (Fig. 1) [17–19]. And, CBT-ol and CBT-diol each have two structural isomers (i.e., α and

β isomers) [18–20]. In plant, GGPP are synthesized through two common biological pathways, i.e., the mevalonate (MVA) pathway that occurs in the cytoplasm of eukaryotes [8, 18, 21, 22] and the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway that presents in the plastids [23]. In the MVA pathway, MVA is derived from acetoacetyl-CoA, which is formed by condensation of two molecules of acetyl-coenzyme A (Acetyl-CoA), under the catalyzation by 3-hydroxy-3-Methylglutaryl synthase (HMGS) and HMG-CoA reductase [24]. Then, MVA is converted through MVA 5-diphosphate to isopentenyl diphosphate (IPP), and the IPP is converted to dimethylallyl pyrophosphate (DMAPP). In the start of MEP pathway, the pyruvate-derived (hydroxyethyl) thiamin and the C1 aldehyde group of D-glyceraldehyde 3-phosphate (GA-3P) are condensed to generate 1-deoxy-D-xylulose-5-phosphate (DXP), which is then converted to methylerythritol 4-phosphate (MEP), then MEP is catalyzed in sequential steps to form 4-hydroxy-3-methylbut-2-enyldiphosphate (HMBPP). Eventually, HMBPP is reduced to IPP and DMAPP [25, 26], which act as general precursors for terpenoids formation and can be catalyzed to produce the general precursors GGPP for diterpenoids, such as CBT-ol [27].

Previous studies demonstrated that tobacco CBT-ol and CBT-diol have high anti-insect and anti-fungal activities [5–7], respectively, and their potential application in sustainable agriculture are expected in near future. Even though their application in agricultural is greatly anticipated [8, 28, 29], it is currently limited by the high cost of preparation from natural resources. The production of CBT-ol and CBT-diol using tobacco plants is of high cost and are far from meeting the commercial demands [30, 31]. The chemical approaches for cembranoid synthesis has not been established, thus their industrial production is unable to be realized in a short period. Furthermore, chemical synthesis always yields high environmental pollution, which goes against the concept of sustainable agriculture. On the other hand, metabolic engineering of microbes for synthesizing natural plant products has recently made a great progress [32–36], which provides a way to produce natural compound via fermentation method [27]. And, a number of terpenoid compounds have been successfully synthesized in metabolic engineered bacteria or fungi, such as artemisinic acid [37], tanshinones [38, 39], resveratrol [40], ginsenoside [41]. Efforts for large-scale and inexpensive production of cembranoids are of virtual value. Therefore, the biosynthetic approach is practicable way to produce cembranoids under mild condition with lower cost.

In previous studies, tobacco cembanoids were hypothesized to be formed through the MEP pathway, and the bioengineered synthesis of CBT-ol was achieved in Escherichia coli via the metabolic engineered MEP pathway [8, 29]. Whether there is a possibility for synthesizing tobacco cembranoid via the MVA pathway remains unknown. Exploring the roles of MVA pathway in cembranoid synthesis could contribute not only to the technical approach but also to the molecular mechanism for cembranoid biosynthesis.

Materials And Methods

Used strains and expression plasmids

Three kinds of yeasts are served as the host strains for the production of tobacco CBT-ol and CBT-diol. The strains are contained with BY4742 *(MATα, his3Δ1, leu2Δ0, lys2Δ0, MET15, ura3Δ0)* [42], BY-T1

(MATa, trp1Δ, his3Δ1, leu2Δ0, lys2Δ0, MET15, ura3Δ0, dDNA::PPGK1-tHMG1-TADH1-PTEF1-LYS2-TCYC1) [43], and BY-T20 *(MATα, trp1Δ0, leu2Δ0, ura3Δ0, trp1::HIS3-PPGK1-BTS1/ERG20-TADH1-PTDH3-SaGGPS-TTPI1-PTEF1-tHMG1-TCYC1)* [44]. *Botrytis cinerea* is served as the control strain for the assay for the effect of tobacco cembranoids [28]. The vector pGADT7 (Clontech, USA), pGBKT7 (Clontech, USA) is used for the expression of Engineered enzymes.

Determination of the growth effects of tobacco cembranoids on *Saccharomyces cerevisiae* and *Botrytis cinerea*

To determine the growth effects of tobacco cembranoids on *Saccharomyces cerevisiae*, YPD agar medium (1% yeast extract, 2% peptones, 2% glucose, 1.5% agar) plates with indicated amount of CBT-ol or CBT-diol were prepared, and a cell suspension of *S. cerevisiae* BY4742 (OD600 = 0.2) was spread onto the plates by 0.5 mL/plate. Plates free of CBT-ol or CBT-diol were prepared as mock treatment control by adding same volume of pure solvent (i.e., 95% ethanol) as that for CBT-ol and CBT-diol plates, and inoculated with equal amount of yeast cells. After 4 days of cultivation at 30℃ incubator, the growth of yeast cells was observed and photographed.

To determine the effects of tobacco cembranoids on *B. cinerea*, PDA (Potato Dextrose Agar) medium plates with indicated amount of CBT-ol or CBT-diol were prepared, and a cube (⌀=5 mm) of *B. cinerea* mycelium was inoculated onto each plate. Plates for mock treatment control were prepared in a similar method as described above and inoculated with mycelium cube. After 4–6 days of cultivation at 20℃ incubator, the growth of *B. cinerea* was observed and photographed. CBT-ol and CBT-diol for preparing the YPD agar and PDA plates were isolated from tobacco trichomes as previously described [28].

Vector construction and transformation into yeast strains

To construct pGADT7-CBTS1-His vector for expressing tobacco cembanoid synthetic gene CBTS1 (GenBank: AAS46038.1), the corresponding gene sequence was fully synthesized after codon-optimization for expression in yeast and then cloned by In-Fusion (Takara, Japan) cloning method into a vector modified from pGADT7 (Clontech, USA) by deleting the AD (GAL4 activation domain) region, and a fragment encoding 6 × His tag was placed downstream of *CBTS1* for protein detection by Western Blot. And, the gene fusion encoding CBTS1-6 × His was set under the control of ADH1 promoter in the modified vector. To construct pGADT7-CBTS1 vector, the synthesized *CBTS1* gene was cloned into the original pGADT7 vector (Clontech, USA) to express a fusion protein of AD-CBTS1, in which AD serves as an expression leader for enhancing the protein expression level in yeast. In a similar method, the gene sequence of *CYP450* (GenBank: AF166332) was synthesized after codon-optimization and cloned into pGBKT7 vector to express a fusion protein of BD-CYP450, in which BD (GAL4 DNA binding domain) serves as an expression leader for expression of CYP450 in yeast. The primers used for vector construction are listed in Table S1.

The derived vectors were introduced into the indicated yeast strains, including BY4742 [42], BY-T1 [43] and BY-T20 [44] for required protein expression assays as well as CBT-ol and CBT-diol synthesis. And, the

yeast transformants were selected by cultivation on SD (synthetic defined) medium plates with desired dropout (DO) supplements (Takara, Japan).

Western Blot assay of the protein CBTS1 expressed in yeast

Western Blot assay was applied to determine the expression of 6 × His-tagged and AD-tagged fusion proteins of CBTS1 in yeast cells, using SDS-PAGE for protein isolation ECL (enhanced chemiluminescence) method for protein detection. CBTS1-His protein was blotted with HRP-labeled mouse anti-6 × His antibody, and AD-CBTS1 protein was blotted with a mouse anti-AD-Tag primary antibody and a goat anti-mouse IgG secondary antibody.

Yeast cultivation and cembranoids extraction

The positive colonies of yeast transformant were inoculated into 5 mL liquid SD/-Leu medium (for yeast expressing CBTS1) or SD/-Leu/-Trp medium (for yeast expressing both CBTS1 and CYP450), and cultured at 30℃, 220 r/min for 48 h as seed culture. The seed culture was used to inoculate YPD liquid medium at a ratio of 5%, and cultured at 30 °C and 220 r/min for indicated time period [45]. The yeast growth was monitored at a serial of time points by measuring the optical density of cell culture at 600 nm (OD600)by microplate reader. For each sample, 1 L cell culture of a 50 h and 60 h cultivation period were collected for CBT-ol or CBT-diol extraction. Then yeast cells and the cultivation broth were separated by centrifugation and extracted individually.

The yeast cells were grounded into powder in liquid nitrogen, dissolved in 20 mL ddH_2O, and further lysed for 20 min in an ultrasonic cell disruptor. The cell lysate was extracted three times with an equal volume of ethyl acetate for 30 minutes at 30℃ with agitation. After centrifugation, the upper organic phase of each extraction was collected and combined for further concentration. The cultivation broth was directly extracted with ethyl acetate in the same method. The extract was dried in a rotary evaporator at 40℃, and dissolved in 5 mL of ethyl acetate for further analyses.

Determination of CBT-ol and CBT-diol by GC-MS

The cembranoids extract obtained by above method was dried in nitrogen flow, and then dissolved in 1 mL ethyl acetate for GC-MS assay. In GC-MS assay, the sample was loaded onto the HP-5 ms column (30 m × 250 μm × 0.25 μm) of a HP7890B Gas Chromatograph coupled with HP5977A Mass Spectrometer (Agilent, USA). The column temperature was initially set at 80℃ and maintained for 1 min, and increased to 200℃ at a temperature gradient of 15℃/min to maintain for another 1 min. Then, the column was temperature was increased to 240℃ by 4℃/min and kept for additional 2 min. The mass spectra were acquired in the m/z 50 – 650 range at 70 eV (EI) using negative ionization mode. CBT-ol and CBT-diol were identified by comparing their retention times and mass spectra with those of the standards and the mass spectra data at NIST Database.

Determination of CBT-ol and CBT-diol by UPLC

The cembranoids extract obtained by above method was dried in nitrogen flow, and then dissolved in 1 mL 70% acetonitrile for UPLC assay. In the UPLC assay, 5 μL sample was injected into ultra-performance liquid chromatography (UPLC; Waters, USA) under following optimized conditions: BEH C18 column (1.7 μm, 2.1 mm × 100 mm) with the column temperature of 40 °C, a gradient mobile phase as indicated in Table S2 at the flow rate of 0.3 mL/min, and a UV detector for detection of CBT-ol and CBT-diol at 208 nm. Authentic standards CBT-ol and CBT-diol, which were isolated and purified from tobacco trichomes [28], were used to distinguish the corresponding peaks in the UPLC chromatograms of the samples.

Results And Discussion

Feasibility of *Saccharomyces cerevisiae* as the host strain

As mentioned above, tobacco cembranoids possess highly active anti-fungal activities against mold or mildew fungus1 [26, 28], and may limit the construction of fungal system for cembranoid synthesis. Whereas, their effects on yeast (a type of fungus) is still unknown. To explore the possibility of synthesizing tobacco cembranoids in yeast, we examined the growth inhibitory effects of tobacco CBT-ol and CBT-diol on *S. cerevisiae* BY4742 (genotype *MATα, his3Δ1, leu2Δ0, lys2Δ0, MET15, ura3Δ0*) [42] with mold fungus *B. cinerea* as control. The results showed that the growth of *B. cinerea* on PDA (Potato Dextrose Agar) plate was extremely suppressed by tobacco CBT-diol at 200 μM comparing to the control on plate without CBT-diol, and tobacco CBT-ol displayed a much weaker suppression on the growth of *B. cinerea* than CBT-diol (Fig. 2). However, neither CBT-diol nor CBT-ol exhibited an observable suppressive effect on the growth of yeast (Fig. 2). These findings suggest that tobacco CBT-diol may function with different patterns in yeast compared to their action in the mold fungus *B. cinerea*, and that yeast could be adopted as a fungal host for synthesizing tobacco CBT-ol and CBT-diol.

Construction of the yeast expression vector and expression for the cembranoids synthetic gene CBTS1

To construct vector for expressing tobacco cembanoid synthetic gene CBTS1 (GenBank accession: AAS46038.1), the corresponding gene sequence was fully synthesized after codon-optimization for yeast expression, which was firstly cloned under the control of ADH1 promoter in a vector modified from pGADT7 (Clontech, USA) by deletion of the GAL4 AD domain fragment. And, a 6 × His-tag was placed at the C-terminal of CBTS1 for protein detection (Figure S1). However, no protein expression could be detected by Western Blot when the obtained vector pGADT7-CBTS1-His was introduced into yeast BY-T20 (Fig. 3A). To conquer the protein expression problem, we then cloned CBTS1 into the original pGADT7 vector to express a CBTS1 fusion protein with AD domain at N-terminus as an expression leader (Figure S1). Western Blot showed that the fusion protein AD-CBTS1 was successfully expressed in yeast BY-T20 strain with the obtained vector pGADT7-CBTS1 (Fig. 3B). Thus, this vector was employed for production of CBT-ol or CBT-diol in yeast. Using a similar method, the codon-optimization CYP450 (GenBank accession: AF166332) gene was synthesized and cloned into pGBKT7 vector (Clontech, USA) to express

BD-CYP450 fusion protein in yeast for CBT-diol production. The constructed vector was designated as pGBKT7-CYP450.

Selecting the suitable yeast strain for producing cembranoids

Tobacco cembanoids are derived from the terpenoid precursor GGPP which may be produced via MEP pathway or MVA pathway in plants (Fig. 1). To develop engineered microbes for tobacco cembanoid synthesis through the MVA pathway, we selected three strains that the yeast strain BY4742 and its engineered strains BY-T1 and BT-T20 were utilized to develop cembanoids synthetic systems. BY-T1 is a yeast strain expressing a truncated HMG-CoA reductase gene (tHMG1) to increase the upstream carbon flux to MVA pathway [43], and BY-T20 is a yeast strain with high efficiency GGPP production by expressing an engineered gene module composed of tHMG1, BTS1-ERG20 gene fusion and SaGGPS[44]. Initially, pGADT7-CBTS1 vector was introduced into yeast strain BY4742, BY-T1 and BY-T20 respectively for tobacco CBT-ol synthesis. And, 1L of 50 h shake-flask culture for each yeast strain was subjected to CBT-ol extraction and chromotography assays using GC-MS (Gas Chromatography-Mass Spectroscopy). The results showed that yeast strain BY-T20 harboring the *CBTS1*-expressing vector could produced CBT-ol (Fig. 3C, 3D), while the production of CBT-ol was undectable in strain BY4742 or BY-T1 harboring the same vector (Fig. 3C). In accordance, a strong peak for GGPP was detected in the sample from BY-T20 harboring the *CBTS1*-expressing vector by GC-MS assay, while no corresponding peak could be detected in sample from BY4742 or BY-T1 harboring the same vector (Fig. 3C, 3E). These evidences suggest that tobacco CBT-ol could be synthesized through the MVA pathway in yeast BY-T20 by expressing CBTS1, and the abundance of GGPP in yeast is a key factor manipulating the formation of CBT-ol.

Production of cembatriene-ol in yeast BY-T20 with the optimized fermentation time

To measure the CBT-ol production in yeast, a standard curve was plotted based on the UPLC (Ultra-Performance Liquid Chromatography). detection data from a serial dilutions of CBT-ol standard, which was isolated and purified from tobacco trichomes [28], and the CBT-ol content of the samples was determined according this standard curve. Figure 4A shows the UPLC spectra CBT-ol standard and that for the extract from CBTS1-expressing yeast. The cultivation time was optimized by monitoring the yeast density through a 72 h shake-flask cultivation period. And, the yeast growth could reach a maximal density after 60 h of cultivation (Fig. 4B). The production of CBT-ol was determined by measuring its content in both yeast cells and the cultivation broth from 1L yeast culture. The results turned out that the yield of CBT-ol in CBTS1-expressing BY-T20 was 692.73 μg/L in cells and 863.95 μg/L in cultivation broth, accounting for a total production of about 1.56 mg/L (Fig. 4C). Since considerable amount of GGPP still presents in the AD-CBTS1 expressing yeast BY-T20 (Fig. 3C, 3E), higher CBT-ol yield is expected by further optimization of CBTS1 enzyme.

Production of cembatriene-diol in yeast BY-T20 with the optimized fermentation time

Subsequently, the production of CBT-diol in yeast was investigated by introducing both AD-CBTS1- and BD-CYP450-expression vectors into yeast BY-T20. GC-MS analysis showed a successful synthesis of CBT-diol (Fig. 5A, 5B). The production of CBT-diol was also determined by measuring its content in both

cells and cultivation broth of 1L yeast culture. Cultivation time optimization during a 72 h period showed that the growth of AD-CBTS1 and BD-CYP450 co-expressing yeast BY-T20 could reach the maximal density after 50 h of cultivation (Fig. 5C). In the UPLC analysis, two peaks consistent with the retention time of α-CBT-diol and β-CBT-diol standards were detected in the sample from yeast co-expressing AD-CBTS1 and BD-CYP450 (Fig. 5D), showing the production of both α-CBT-diol and β-CBT-diol. To measure the production of CBT-diol, a standard curve was constructed based on the UPLC detection data from a serial dilutions of CBT-diol isolated and purified from tobacco trichomes [28]. In addition, this standard curve was applied to the determination of CBT-diol content in the samples. The results showed that the production of α-CBT-diol was 13.72 μg/L in cells and 50.10 μg/L in cultivation broth, and that the production of β-CBT-diol was 13.05 μg/L in cells and 72.76 μg/L in cultivation broth. Thus, the total production of CBT-diol was about 0.15 mg/L (Fig. 5E).

Conclusion

Here we established a new method that tobacco CBT-ol could be synthesized via the MVA pathway in yeast, which is limited by the abundance of GGPP. Even though tobacco cembranoids are anti-fungal compounds, this study showed that they have no observable suppressive effect on yeast growth, implying their differential function manners in yeast and other fungi. And, yeast could be used for further synthesizing CBT-diol, whose anti-fungal activity is greatly higher that CBT-ol. Currently, the production of CBT-ol and CBT-diol in yeast under shake-flask cultivation is 1.56 mg/L and 0.15 mg/L, respectively, and greater yield is expected by improving enzyme expression level and using bioreactor cultivation. The biosynthesized CBT-diol showed different distribution patterns in yeast cells and the cultivation broth, which may be resulted from their different solubility or cell transportation. In conclusion, this work has established an alternative bio-approach for synthesizing tobacco cembranoids, which can lay the foundation for biosynthesis of diterpenoids. Moreover, The bio-approach for production of cembranoids may promote their application in sustainable agriculture and other aspects.

Abbreviations

CBT-ol: cembatriene-ol; CBT-diol: cembatriene-diol; MEP: 2-C-methyl-D-erythritol-4- phosphate; MVA: mevalonate; GGPP: geranylgeranyl diphosphate; CBTS1: cembranol synthase; CYP450:cytochrome P450 hydroxylase; Acetyl-CoA: acetyl-coenzyme A; HMGS: 3-hydroxy-3-Methylglutaryl synthase; IPP: isopentenyl diphosphate; DMAPP: dimethylallyl pyrophosphate; GA-3P: D-glyceraldehyde 3-phosphate; DXP: 1-deoxy-D-xylulose-5-phosphate; HMBPP: 4-hydroxy-3-methylbut-2-enyldiphosphate; GC-MS: Gas Chromatography-Mass Spectroscopy; UPLC: Ultra-Performance Liquid Chromatography.

Declarations

Acknowledgements

This work was supported by the Science and Technology Innovation Program of Chinese Academy of Agricultural Sciences (Elite youth program to H.Z., ASTIP-TRIC05), Yunnan Tobacco Company (2018530000241001), and Sichuan Tobacco Company (SCY201916).

Authors' contributions

Y.Z., and H.Z. conceived and designed the study. Y.Z., B.S., X.L., F.N., C.W., and Y.L. performed the experiments. Y.Z., H.Z., Y.D, M.P. and Z.Z. drafted the manuscript.

Funding

This work was supported by the Science and Technology Innovation Program of Chinese Academy of Agricultural Sciences (Elite youth program to H.Z., ASTIP-TRIC05), Yunnan Tobacco Company (2018530000241001), and Sichuan Tobacco Company (SCY201916).

Availability of data and materials

All material listed in the manuscript is available from the corresponding author.

Ethics approval and consent to participate

Not applicable

Competing interests

The authors declare no competing financial interest.

Consent for publication

All authors have read and approved the current version of the manuscript.

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Supplementary information

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Figures

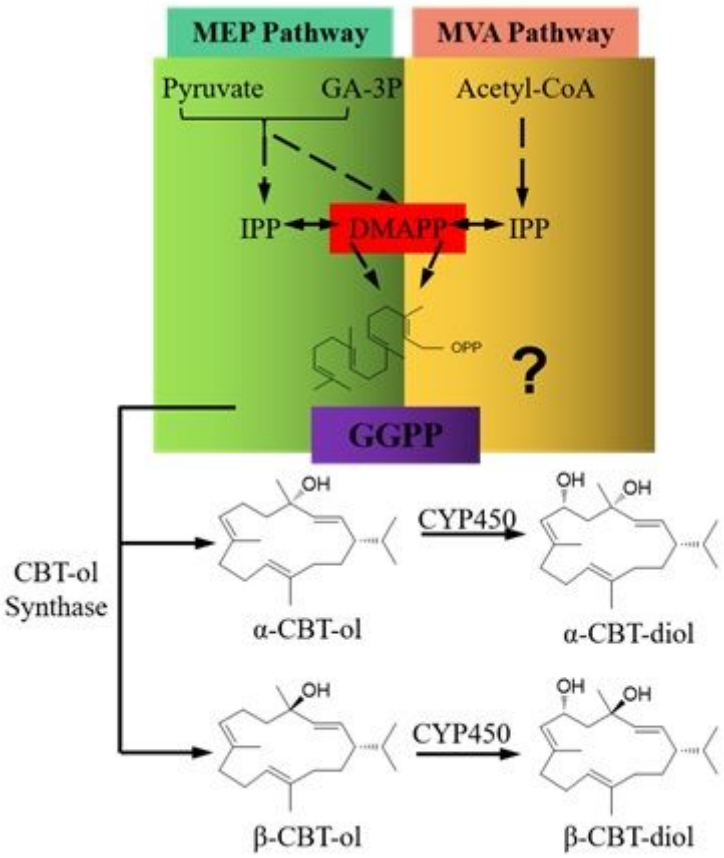

Figure 1

A schematic diagram of CBT-ol/CBT-diol synthesis in tobacco. Tobacco GGPP could be formed via MEP or MVA pathway. The MEP pathway derived GGPP was shown to be the precursor of CBT-ol/ CBT-diol, but it is unknown whether GGPP from the MVA pathway could act as their precursor. Formation of CBT-ol

from GGPP is catalyzed by CBTS1, and further synthesis of CBT-diol is catalyzed by CYP450. Both CBT-ol and CBT-diol have two structural isomers.

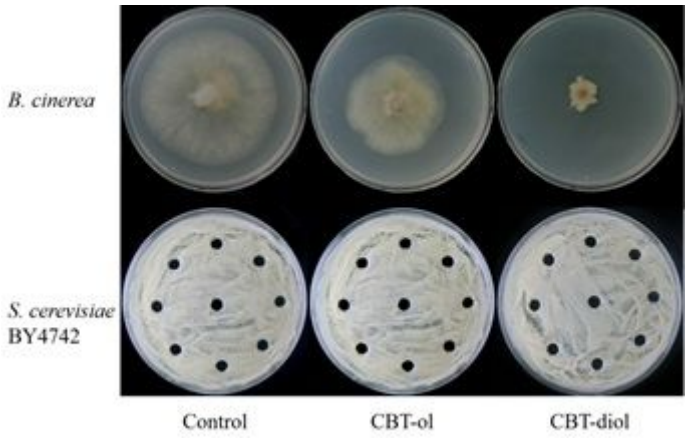

Figure 2

Growth of B. cinerea and S. cerevisiae on the medium plates with mock treatment CBT-ol, or CBT-diol.

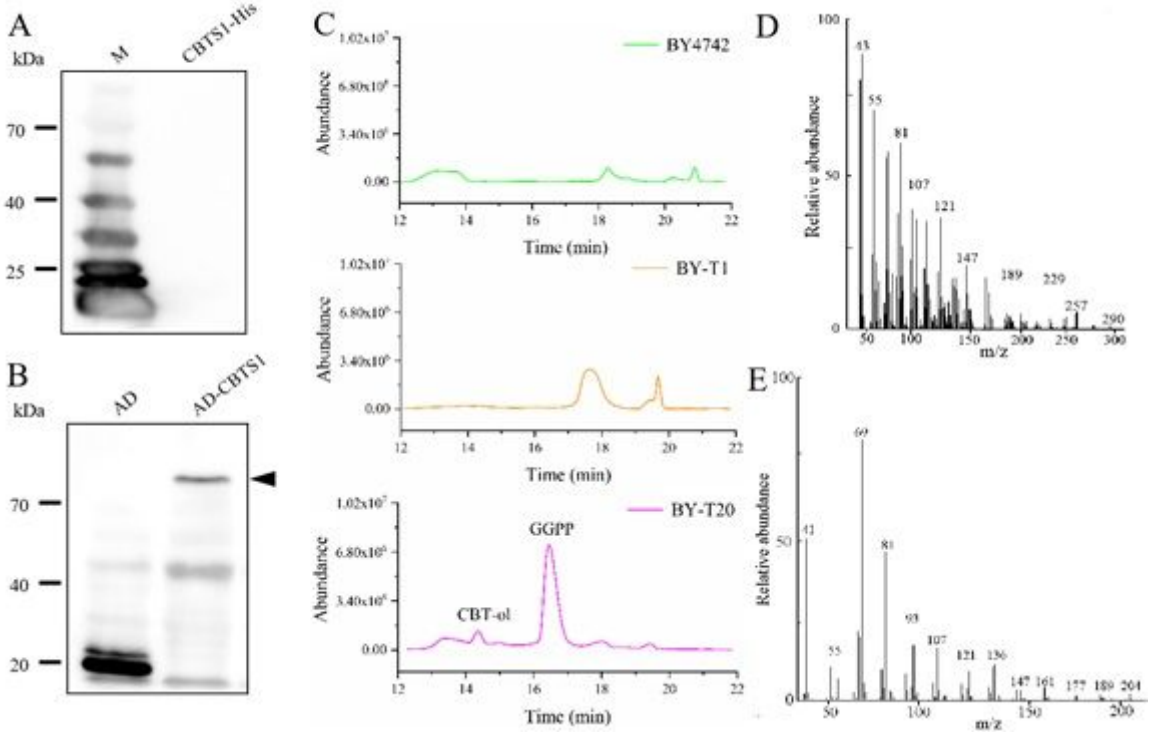

Figure 3

Production of CBT-ol in yeast. (A) Detection of CBTS1-His protein by Western Blot with anti-6×His antibody. M indicates the lane of protein Marker, as a positive control for 6×His-tagged protein. Labels at left indicate molecular weights. (B) Detection of AD-CBTS1 protein by Western Blot with anti-AD antibody. Labels at left indicate molecular weights, and the arrow at right shows the AD-CBTS1 protein. (C) GC-MS spectrums of extracts from AD-CBTS1-expressiong yeast strains BY4742, BY-T1 and BY-T20, respectively.

The GC-MS peaks for CBT-ol and GGPP are indicated. (D) The associated mass peaks of the yeast produced CBT-ol. (E). The associated mass peaks of the yeast produced GGPP.

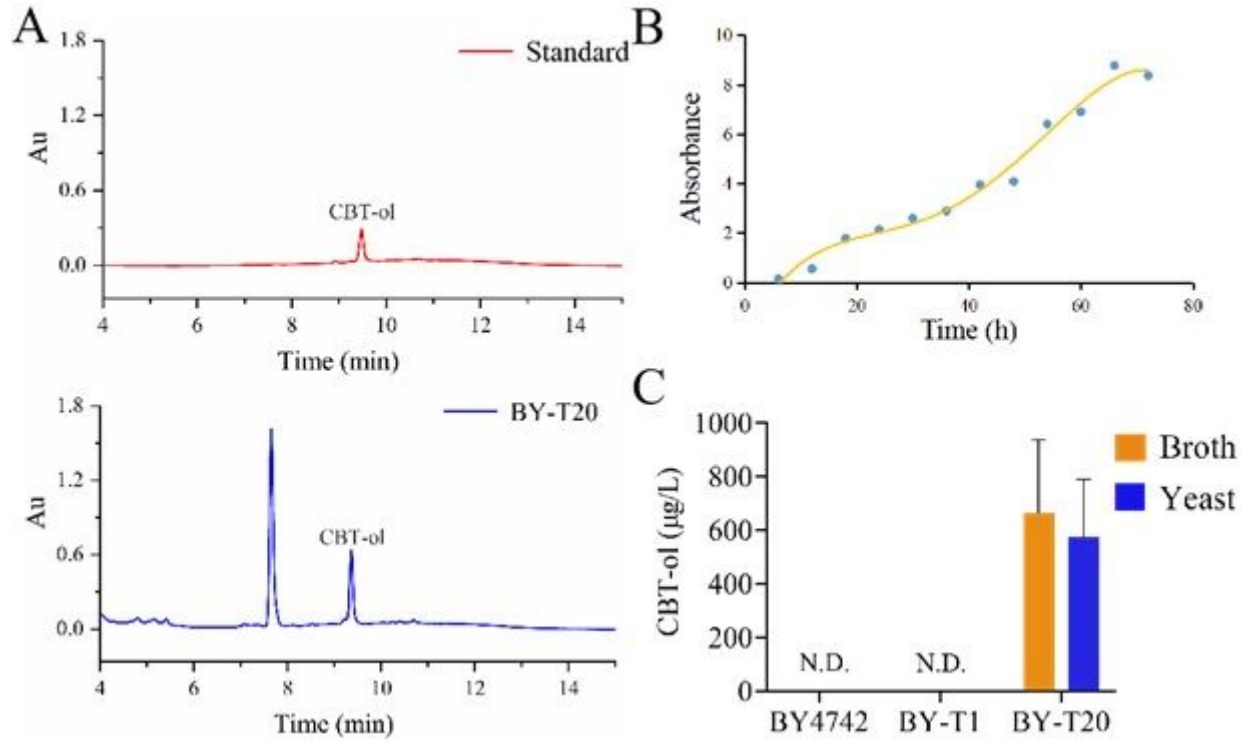

Figure 4

Production of CBT-ol in AD-CBTS1-expressiong yeast strains BY-T20. (A) Growth curve of the yeast culture.(B) UPLC detection of CBT-ol in the extract of yeast culture. The peaks for CBT-ol are indicated. (C) Content of CBT-ol in the indicated yeas cells or cultivation broth. Each values is the average of triplicates. N.D. indicates not detected, and bars indicate mean + SD.

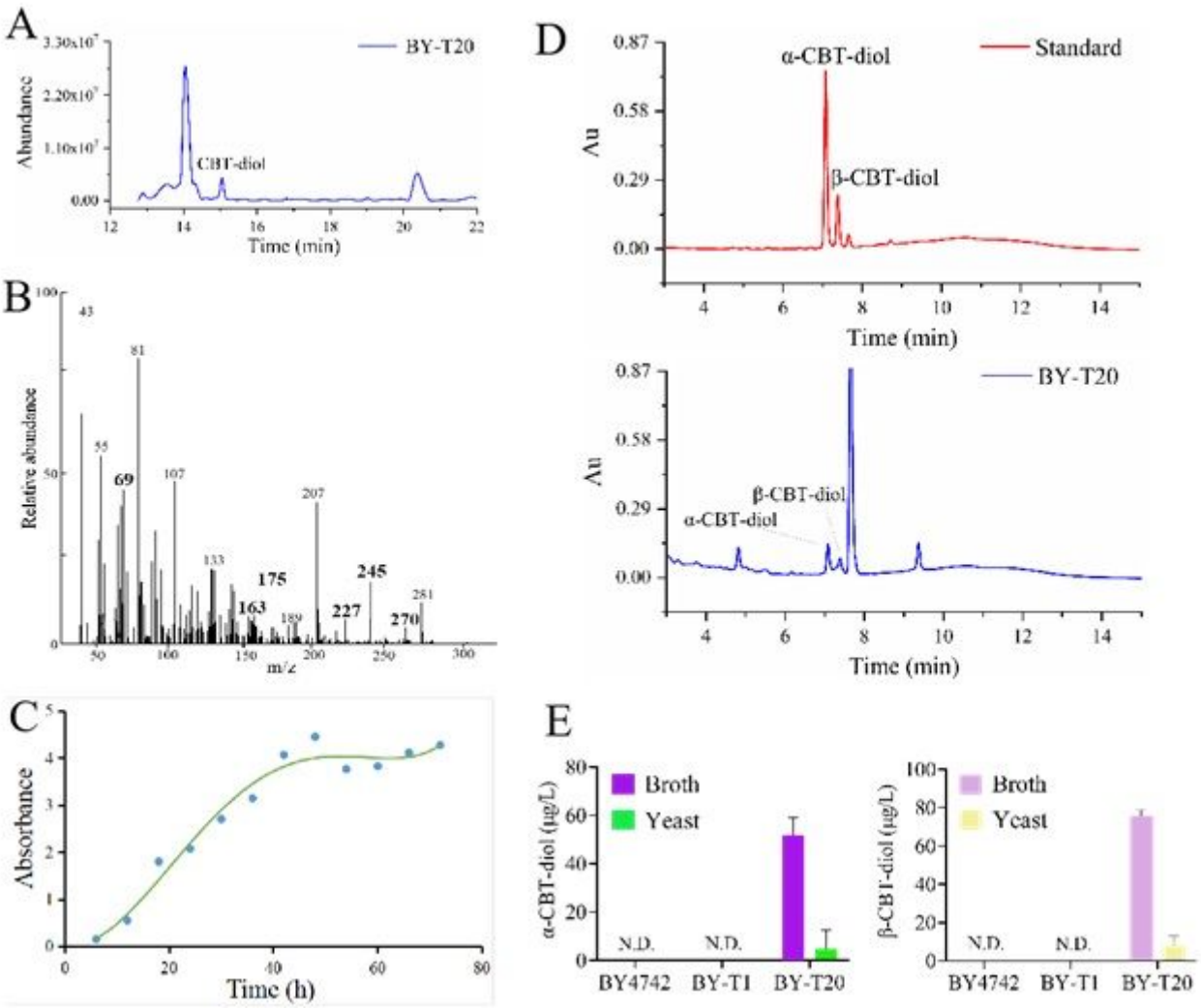

Figure 5

Production of CBT-diols in yeast strain BY-T20 expressing AD-CBTS1 and BD-CYP450. (A) GC-MS spectrums of yeast extract. The GC-MS peak for CBT-diol is indicated. (B) The associated mass peaks of the yeast produced CBT-diol. Bold numbers indicate the specific mass peaks discriminating CBT-diol from CBT-ol. (C) Growth curve of the yeast culture. (D) UPLC detection of CBT-diols in the extract of yeast culture. Peaks for α-CBT-diol and β-CBT-diol are indicated. (E) Content of CBT-diols in the indicated yeast culture or cultivation broth. Each values is the average of triplicates. N.D. indicates not detected, and bars indicate mean + SD..

Supplementary Files

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- SupplementCBTsSynthesis.pdf