Biological Synthesis of Chiral *p*-Coumaroyl Glycerol

Min Kyung Song,[†] GeunYoung Sim,[†] Su Jin Lee,[†] Bong-Gyu Kim,[‡] Mihyang Kim,[†] Taegum Lee,[†] Youhoon Chong,[†] and Joong-Hoon Ahn[†,*]

[†]*Department of Bioscience & Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul 05029, South Korea. *E-mail: jhahn@konkuk.ac.kr*
[‡]*Gyeongnam National University of Science and Technology, Department of Forest Resources, Jinju-si 52725, South Korea*
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Biological synthesis has advantages over chemical synthesis owing to regioselectivity and enantioselectivity. Enantioselective synthesis is a key pharmaceutical process because different enantiomers display different biological activity.[1] Three approaches can be used to synthesize a specific form of an enantiomer.[2] Among these approaches, using an enantiomerically pure starting material (enantiomerically pure synthon) is currently the most popular approach.[2] However, development of enantiomerically pure materials is still challenging. Glycerol itself does not contain a chiral center; however, chiral glycerol derivatives have been used to synthesize enantiomerically pure biological molecules such as platelet aggregation factor, phospholipids, and β-blockers.[3,4] To synthesize enantiomer-specific glycerol derivatives, suberin biosynthesis in some plants[4] was harnessed to generate hydroxycinnamoyl glycerol. We have maximally harnessed natural mimics to synthesize an enantiomerically pure glycerol derivative *p*-coumaroyl glycerol.

Enzymatic esterification is a well-known reaction in biological synthesis, catalyzed majorly by lipases.[5–7] Some members of the plant BAHD (benzylalcohol *O*-acetyltransferase [BEAT], deacetylvindoline 4-*O*-acetyltransferase [DAT], anthranilate *N*-hydroxycinnamoyl/benzoyltransferase [HCBT], anthocyanin *O*-hydroxycinnamoyltransferase [AHCT]) family also catalyze esterification reactions, while others catalyze amidification.[8] One of the BAHD enzymes, hydroxycinnamoyl transferases (HCTs), uses hydroxycinnamoyl-CoA as an acyl donor and diverse acyl acceptors including shikimate or quinate, malate, tartrate, and *D*-(hydroxy)phenyllactate.[9–12]

Hydroxycinnamic acid (HC) is a secondary metabolite of phenylalanine or tyrosine through a deamination reaction. Phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL) have been characterized from plants and microorganisms.[13,14] Introduction of these genes into heterologous systems such as *Escherichia coli* and *Saccharomyces cerevisiae* helped synthesize hydroxycinnamic acids from simple sugars such as glucose and glycerol in these systems.[15] The carboxyl group of HCs conjugates with alcoholic and amine functional groups. In biological systems, these conjugation reactions are carried out by HCTs. In the present study, we used OsHCT4 (hydroxycinnamate transferase from *Oryza sativa*) to synthesize chiral *p*-coumaroyl glycerol by conjugating glycerol with an asymmetrical HC, and its absolute configuration and enantiomeric purity were determined.

Os4CL and *OsHCT4* were harnessed for *p*-coumaroyl glycerol synthesis. HCs including *p*-coumaric acid were activated through CoA attachment by Os4CL to generate HC-CoA. OsHCT4 uses HC-CoA as an HC group donor and glycerol as an acceptor to generate an HC-glycerol conjugate.[16] Two constructs were generated: the first construct (pC-p4CL-pHCT4) contained *Os4CL* and *OsHCT4* under the control of T7 promoters; the second construct (pC-p4CL-HCT4) contained both genes under the control of one T7 promoter. To select the optimal construct to generate the *p*-coumaroyl glycerol conjugate, we assessed each construct by feeding *p*-coumaric acid in M9 broth supplemented with 2% glucose. *E. coli* transformed with the operon-type construct generated approximately 21.9 mg/L of *p*-coumaroyl glycerol, while *E. coli* transformed with the pseudo operon-type construct generated approximately 8.3 mg/L. Therefore, the operon-type construct was used for further analysis. On using glycerol instead of glucose as a carbon source, the *p*-coumaroyl glycerol yield was 66 mg/L, which was 3-fold that with glucose as the carbon source.

Thereafter, we synthesized *p*-coumaroyl glycerol from glucose and glycerol. *p*-Coumaric acid could be directly synthesized from tyrosine by tyrosine ammonia lyase (TAL). In addition, $aroG^f$ and $tyrA^f$, which are feedback inhibition resistant versions of *aroG* and *tyrA*, respectively, and involved in tyrosine biosynthesis,[17] along with *TAL* were overexpressed. *TAL*, $aroG^f$, and $tyrA^f$ were subcloned into one *E. coli* expression vector, pA-aroG^f-TAL-tyrA^f. Constructs pA-aroG^f-TAL-tyrA^f and pC-p4CL-HCT4 were transformed into *E. coli*, and *p*-coumaroyl glycerol synthesis was examined via high-performance liquid chromatography (HPLC), using this strain (B-PG3). Several peaks were

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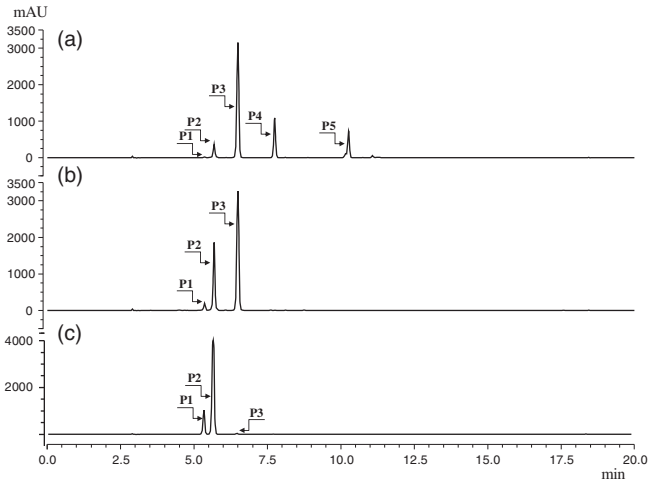


Figure 1. High-performance liquid chromatographic analysis of reaction products from strain B-PG1 cultured with glucose (a), from strain B-PG2 cultured with glucose (b), or from strain B-PG3 cultured with glycerol (c). P1, 2-*O*-*p*-coumaroyl glycerol; P2, 1-*O*-*p*-coumaroyl glycerol; P3, *p*-coumaric acid; P4, 3-acetyl-1-*O*-*p*-coumaroyl glycerol; P5, 2,3-diacetyl-1-*O*-*p*-coumaroyl glycerol.

observed (Figure 1(a)); a peak at 4 min (P3) had the same retention time as that of *p*-coumaric acid, suggesting that it is a *p*-coumaric acid synthesized from tyrosine by TAL. Molecular masses of P1, P2, P4, and P5 were 238.0, 238.0, 280.1, and 322.1 Da, respectively. The molecular mass of P1 and P2 corresponded with the predicted molecular mass of *p*-coumaroyl glycerol. Nuclear magnetic resonance (NMR) analysis of each product revealed that P1 is 2-*O*-*p*-coumaroyl glycerol and P2 is 1-*O*-*p*-coumaroyl glycerol. 1-*O*-*p*-Coumaroyl glycerol was a major product, and 2-*O*-*p*-coumaroyl glycerol was a minor product. We examined the reaction products corresponding to P4 and P5. Based on the molecular mass, P4 seemed to donate one acetyl group to *p*-coumaroyl glycerol, and P5 seemed to contain two acetyl groups conjugated with *p*-coumaroyl glycerol. P4 and P5 were 3-acetyl-1-*O*-*p*-coumaroyl glycerol and 2,3-diacetyl-1-*O*-*p*-coumaroyl glycerol, respectively, both being unexpected products.

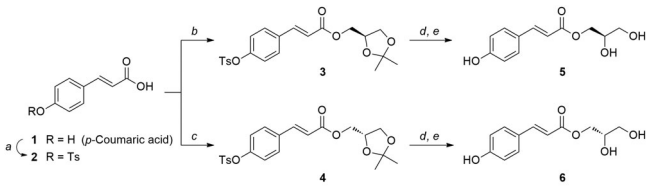
The protein encoded by chloramphenicol resistance gene *CmR* in pACYC-duet1 used herein transfers an acetyl group from acetyl-CoA to chloramphenicol. This protein transferred an acetyl group to glycerol. To test this, we replaced *CmR* with the ampicillin resistance gene *AmpR* in pA-aroG^{f}-tyrA^{f}-SeTAL, and the resulting construct was named pA(AmpR)-aroG^{f}-tyrA^{f}-SeTAL. We investigated whether the new strain transformed with pA(AmpR)-aroG^{f}-tyrA^{f}-SeTAL produced acetylated products. As expected, the acetylated *p*-coumaroyl glycerols were not synthesized in the strain harboring pA(AmpR)-aroG^{f}-tyrA^{f}-SeTAL (Figure 1(c)), indicating that CmR is involved in glycerol acetylation (Figure 1(c)). To simplify the reaction product, we then used constructs containing *AmpR*.

Strain B-PG4 still contained *p*-coumaric acid (Figure 1 (b)), which was not converted to *p*-coumaroyl glycerol. There are two possible explanations for the aforementioned observation: first, conjugation of glycerol and *p*-coumaroyl-CoA is slower than the conversion of tyrosine to *p*-coumaric acid; second, conversion of *p*-coumaric acid to *p*-coumaroyl-CoA is slower than the conversion of tyrosine to *p*-coumaric acid. The second explanation is more probable owing to a limited glycerol supply. To assess the first possibility, we subcloned *OsHCT* and *Os4CL* into a higher-copy-number plasmid and examined whether the resulting strain produced *p*-coumaroyl glycerol at greater levels. However, the strain transformed with the higher-copy-number construct did not produce more *p*-coumaroyl glycerol and still contained unreacted *p*-coumaric acid, suggesting that the first possibility was not likely. On using glycerol instead of glucose as a carbon source and substrate for *p*-coumaroyl glycerol, no *p*-coumaric acid was detected in the strain, and this strain produced *p*-coumaric acid at greater levels (Figure 1(c)), thus suggesting that glycerol was the limiting factor. Using this strain, we synthesized approximately 799.9 mg/L of *p*-coumaroyl glycerol after 63 h of incubation in M9 broth supplemented with 2% glycerol.

For unequivocal determination of the absolute configuration and enantiomeric purity of biologically synthesized *p*-coumaroyl glycerol, chemical syntheses of enantiomerically pure (*R*)- and (*S*)-isomers were attempted. Briefly, commercially available *p*-coumaric acid (**1**, Scheme 1) was treated with *p*-TsCl in the presence of NaOH to generate coumaric acid tosylate **2**. Coupling of **2** with (*S*)- and (*R*)-2,-2-dimethyl-1,3-dioxolane-4-methanol in the presence of EDC yielded the corresponding coumaric acid esters **3** and **4**, which were easily converted to enantiomerically pure (*S*)- and (*R*)-*p*-coumaroyl glycerols **5** and **6** after sequential deprotection of the tosyl and isopropylidene moieties.

Optical rotation of the enzymatically synthesized *p*-coumaroyl glycerol was measured and compared with that of chemically synthesized (*S*)- and (*R*)-isomers (Table 1). Specific rotation of the enzymatic product was concurrent with that of (*S*)-*p*-coumaroyl glycerol, and its enantiomeric purity was as high as 98.6%.

In summary, glycerol is an inexpensive material. In plants, glycerol is a component of an insoluble lipophilic polymer, suberin,[18] comprising a polyaromatic and polyaliphatic domain,[19] and glycerol potentially serves as the



Reagents and Conditions: (a) *p*-TsCl, NaOH, H_2O, 0 °C; (b) (*S*)-(+)-2,2-Dimethyl-1,3-dioxolane-4-methanol, EDC, DMAP, DCM, rt; (c) (*R*)-(-)-2,2-Dimethyl-1,3-dioxolane-4-methanol, EDC, DMAP, DCM, rt; (d) TBAF, THF, rt; (e) THF-2N HCl (1:1), rt.

Scheme 1. Synthesis of enantiomerically pure (*S*)- and (*R*)-isomers of *p*-coumaroyl glycerols (**5** and **6**).

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Table 1. Specific rotations of the enzymatic product and two chemically synthesized enantiomers of *p*-coumaroyl glycerol.

Compounds	Specific rotation
Enzymatic product	$[\alpha]_D^{25}$–13.9 ± 0.2° (c = 1.02, CH_3OH)
5 (*S*)-*p*-coumaroyl glycerol	$[\alpha]_D^{27}$–14.1 ± 0.2° (c = 0.33, CH_3OH)
6 (*R*)-*p*-coumaroyl glycerol	$[\alpha]_D^{26}$ 14.3 ± 0.1° (c = 0.46, CH_3OH)

interlink aliphatic monomer. Caffeoyl acid, an HC, fatty acids, and glycerol, constitute an ester present in cotton,[20] and caffeoyl glycerol ester is reportedly present in oat.[21,22] This prompted us to identify the gene encoding an esterification enzyme between HCs and other molecules, including glycerol, quinic acid, and shikimic acid. This study reports an enzyme that converts prochiral glycerol into chiral *p*-coumaroyl glycerol. Using this enzyme, (*S*)-*p*-coumaroyl glycerol, with an optical purity of 98.6%, was synthesized.

Experimental

Construction of the *E. coli* expression vector: *aroG*, *tyrA*, and *SeTAL* were cloned as previously reported.[23] The selection marker was replaced through homologous recombination using the Quick and Easy Conditional Knockout Kit (Gene Bridges, Heidelberg, Germany). A chloramphenicol resistance gene of pACYCDuet-1 vector (Merck, Darmstadt, Germany) was replaced by the ampicillin resistance gene through homologous recombination. Two primers, 5′-TCAGGCGTAGCACCAGGCGTTTAAGGGCACCAATA ACTGCCTTAAAAAAATTACCAATGCTTAATCAGTG-3′ as the forward primer and 5′-CGGGCGTATTTTTTGA GTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAAT AAATACATTCAAATATGTA-3′ as the reverse primer (Underlined sequences are obtained from the pGEMT-easy vector encompassing the ampicillin resistance gene; other sequences are obtained from the pACYC-duet vector flanking the chloramphenicol resistance gene) were used to amplify the ampicillin resistance gene of pGEMT-easy vector (Promega, Madison, WI USA) as a template. The PCR product was used to replace the chloramphenicol resistance gene of pACYCDuet-1, located at position 697–1438.

Two constructs containing both *Os4C*L and *OsHCT4* were generated. In the first construct, pC-p4CL-pHCT4, both genes were regulated by independent T7 promoters. *Os4CL* (GenBank BAD27987) was first subcloned into the EcoRI/NotI site of pCDFDuet-1 vector and then *OsHCT*4 (GenBank 115 466 807) was subcloned into the NdeI/EcoRV site of the resulting construct containing *Os4CL*. The second construct, pC-4CL-HCT4 contained two genes regulated by one T7 promoter. *OsHCT4* was amplified using 5′-ATGAATTCGATGGCGACGGTG GACGTGCTG-3′ (OsHCT4-F; EcoRI site is underlined) as the forward primer and CATCTAGATCATGCCAA TCGCGCCACCTCG (XbaI site is underlined) as the reverse primer, and *Os4CL* was amplified with 5′-ATGGTTACAAATTAATCTAGAAGGAGGATTACAA-AATGGATCCGATGGGG-3′ (XbaI site is underlined, and the ribosomal binding site (RBS) is in low case) and 5′-ATGATATCTCATGCCAATCGCGCCACCTCG-3′ (Os4CL-R). The resulting PCR products were digested with XbaI, and both genes were ligated and re-amplified with primers OsHCT4-F (operon) and Os4CL-R (operon). The resulting PCR product was digested with EcoRI/NotI and subcloned into the corresponding sites of the pCDF-1 Duet vector.

Metabolite analysis. *p*-Coumaroyl glycerol was synthesized in *E. coli* as previously described,[16] with a minor modification: glycerol was added as a carbon source instead of glucose. To purify the reaction product, the culture was extracted with ethyl acetate, followed by evaporation to dryness on a rotary vacuum evaporator. The metabolites were then dissolved in methanol. The semipreparative C18 reversed-phase column (Spherisorb 5 μm ODS2, 10 mm × 250 mm) was used with the Agilent 1260 infinity HPLC system (Palo Alto, CA, USA). The mobile phase comprised 72% water (A) and 28% acetonitrile (B). Isocratic elution was carried out at a flow rate of 3 mL/min.

^{1}H NMR spectra of the compounds in DMSO-d_6 were recorded at 400 and 100 MHz, on a (Bruker, Billerica, MA, USA) at 296 K. 3-Acetyl-1-*O*-*p*-coumaroylglycerol: ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.58 (d, *J* = 15.2 Hz, 1H), 7.55 (d, *J* = 8.4 Hz, 2H), 6.80 (d, *J* = 8.4 Hz, 2H), 6.39 (d, *J* = 15.2 Hz, 1H), 4.10 (d, *J* = 5.3 Hz, 2H), 4.02 (d, *J* = 11.0 Hz, 2H), 3.94 (dd, *J* = 11.0, 5.3 Hz, 1H), 2.02 (s, 3H). 2,3-Diacetyl-1-*O*-*p*-coumaroylglycerol: ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.59 (d, *J* = 15.8 Hz, 1H),7.57 (d, *J* = 8.2 Hz, 2H), 6.79 (d, *J* = 8.2 Hz, 2H), 6.39 (d, *J* = 15.8 Hz, 1H), 4.25 (m, 1H), 4.05 (m, 2H), 4.01 (m, 2H), 1.98 (s, 6H). 2-*O*-*p*-coumaroylglycerol: ^{1}H NMR δ(ppm) in DMSO-d_6: 7.56 (d, *J* = 15.9 Hz, 1H), 7.54 (d, *J* = 8.6 Hz, 2H), 6.79 (d, *J* = 8.6 Hz, 2H), 6.37 (d, *J* = 15.9 Hz, 1H), 4.8 (m, 1H), 3.48–3.58 (m, 4H); 1-*O*-*p*-coumaroylglycerol: ^{1}H NMR δ(ppm) in DMSO-d_6: 7.57 (d, *J* = 15.9 Hz, 1H), 7.55 (d, *J* = 8.6 Hz, 2H), 6.79 (d, *J* = 8.6 Hz, 2H), 6.39 (d, *J* = 16.0 Hz, 1H), 4.15 (dd, *J* = 11.2, 4.1 Hz, 1H), 4.01 (dd, *J* = 11.2, 6.5 Hz, 1H), 3.73–3.68 (m, 1H), 3.41 (dd, *J* = 11.1, 5.5 Hz, 1H), 3.37 (dd, *J* = 11.3, 6.0 Hz, 1H).

Authentic samples of (*R*)-1-*O*-*p*-coumaroyl glycerol (**5**) and (*S*)-1-*O*-*p*-coumaroyl glycerol (**6**) were prepared as follows (Scheme 1):

Synthesis of *p*-coumaric acid *p*-toluenesulfonate (**2**). To a solution of *p*-coumaric acid (0.30 g, 1.83 mmol) in H_2O (20 mL), NaOH (0.18 g, 4.57 mmol) was added followed by *p*-toluenesulfonyl chloride (*p*-TsCl, 0.45 g, 2.38 mmol) at 0 °C. After stirring at room temperature for 12 h, the reaction mixture was acidified and filter-washed with H_2O. The obtained white solid was air-dried to produce **2**: ^{1}H NMR (acetone-d_6) δ 7.76 (d, *J* = 8.4 Hz, 2H), 7.70 (d, *J* = 8.6 Hz, 2H), 7.63 (d, *J* = 16.1 Hz, 1H), 7.49 (d,

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J = 8.0 Hz, 2H), 7.10 (d, J = 8.7 Hz, 2H), 6.51 (d, J = 16.0 Hz, 1H), 2.47 (s, 3H).

Synthesis of (*R*)-1,2-isopropylidene glyceryl *p*-coumaric acid *p*-toluenesulfonate (**3**) and (*R*)-1,2-isopropylidene glyceryl *p*-coumaric acid *p*-toluenesulfonate (**4**). To a suspension of **2** (0.10 g, 3.14 mmol) in dichloromethane (20 mL), (*R*)-(−)-2,2-dimethyl-1,3-dioxolane-4-methanol (0.058 mL, 4.71 mmol) or (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (0.058 mL, 4.71 mmol) was added, followed by addition of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.21 g, 1.10 mmol) and *N*,*N*-dimethylpyridine (134 mg, 1.10 mmol). After stirring at room temperature for 4 h, and the reaction mixture was concentrated *in vacuo*. The crude product was purified via column chromatography on a silica gel (dichloromethane: ethyl acetate = 20:1) to obtain **3** or **4** as a pale yellow solid; ^{1}H NMR (acetone-d_6) δ 7.76 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 8.6 Hz, 2H), 7.67 (d, J = 16.1 Hz, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.11 (d, J = 8.7 Hz, 2H), 6.56 (d, J = 16.0 Hz, 1H), 4.37 (ddd, J = 12.1, 10.8, 4.8 Hz, 1H), 4.25 (dd, J = 11.5, 4.6 Hz, 1H), 4.19 (dd, J = 11.5, 5.8 Hz, 1H), 4.11 (dd, J = 8.4, 6.5 Hz, 1H), 3.80 (dd, J = 8.4, 6.0 Hz, 1H), 2.46 (s, 3H), 1.37 (s, 3H), 1.31 (s, 3H).

Synthesis of (*R*)-1-*O*-*p*-coumaroyl glycerol (**5**) and (*S*)-1-*O*-*p*-coumaroyl glycerol (**6**). To a solution of 3 or 4 (0.15 g, 0.35 mmol) in tetrahydrofuran (THF, 10 mL), tetrabutylammonium fluoride (1 M in THF) (0.80 mL, 2.77 mmol) was added. After stirring at room temperature for 2 h, the reaction mixture was concentrated *in vacuo* and used for the subsequent step without further purification.

The *p*-coumaric acid ester obtained above (**3** or **4**) (0.10 g, 0.72 mmol) in THF (10 mL) was treated with 2 M HCl (10 mL). After stirring at room temperature for 1 h, the reaction mixture was concentrated to eliminate volatiles. The aqueous layer was extracted thrice with ethyl acetate, and the combined organic layers were washed with brine, dried over MgSO4, filtered, and concentrated *in vacuo*. The crude product was purified via column chromatography on a silica gel (hexane: acetone = 3:2) to give **5** or **6** as a white solid: ^{1}H NMR (acetone-d_6) δ 7.57 (d, J = 15.9 Hz, 1H), 7.55 (d, J = 8.6 Hz, 2H), 6.79 (d, J = 8.6 Hz, 2H), 6.39 (d, J = 16.0 Hz, 1H), 4.15 (dd, J = 11.2, 4.1 Hz, 1H), 4.01 (dd, J = 11.2, 6.5 Hz, 1H), 3.73–3.68 (m, 1H), 3.41 (dd, J = 11.1, 5.5 Hz, 1H), 3.37 (dd, J = 11.3, 6.0 Hz, 1H).

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References

1. A. S. de Miranda, L. S. M. Miranda, R. O. M. A. de Souza, *Biotechnol. Adv.* **2015**, *33*, 372.
2. A. Rouf, S. C. Taneja, *Chirality* **2014**, *26*, 63.
3. D. Breitgoff, K. Laumen, M. P. Schneider, *J. Chem. Soc. Chem. Commun.* **1986**, *20*, 1523.
4. T. Harada, H. Nakajima, T. Ohnishi, M. Takeuchi, A. Oku, *J. Org. Chem.* **1992**, *57*, 720.
5. S. K. Karmee, *Appl. Microbiol. Biotechnol.* **2009**, *81*, 1013.
6. M. E. de Araújo, Y. E. Franco, M. C. Messias, G. B. Longato, J. A. Pamphile, P. O. Carvalho, *Planta Med.* **2017**, *83*, 7.
7. P. Y. Stergiou, A. Foukis, M. Filippou, M. Koukouritaki, M. Parapouli, L. G. Theodorou, E. Hatziloukas, A. Afendra, A. Pandey, E. M. Papamichael, *Biotechnol. Adv.* **2013**, *31*, 1846.
8. J. C. D.'. Auria, *Curr. Opin. Plant Biol.* **2006**, *9*, 331.
9. L. Hoffmann, S. Maury, F. Martz, P. Geoffroy, M. Legrand, *J. Biol. Chem.* **2003**, *278*, 95.
10. M. L. Sullivan, *Plant Physiol.* **2009**, *150*, 1866.
11. M. L. Sullivan, *Planta* **2014**, *239*, 1091.
12. M. Sander, M. Petersen, *Planta* **2011**, *233*, 1157.
13. J. Rösler, F. Krekel, N. Amrhein, J. Schmid, *Plant Physiol.* **1997**, *113*, 175.
14. C. B. Jendresen, S. G. Stahlhut, M. Li, P. Gaspar, S. Siedler, J. Förster, J. Maury, I. Borodina, A. T. Nielsen, *Appl. Environ. Microbiol.* **2015**, *81*, 4458.
15. D. G. An, M. N. Cha, S. P. Nadarajan, B. G. Kim, J.-H. Ahn, *Appl. Biol. Chem.* **2016**, *59*, 173.
16. I. A. Kim, B.-G. Kim, M. Kim, J.-H. Ahn, *Phytochemistry* **2012**, *76*, 25.
17. T. Lütke-Eversloh, G. Stephanopoulos, *Appl. Microbiol. Biotechnol.* **2007**, *75*, 103.
18. L. Moire, A. Schmutz, A. Buchala, B. Yan, R. E. Stark, U. Ryser, *Plant Physiol.* **1999**, *119*, 1137.
19. P. E. Kolattukudy, *Science* **1980**, *208*, 990.
20. A. Schmutz, T. Jenny, N. Amrhein, U. Ryser, *Planta* **1993**, *189*, 453.
21. D. G. H. Daniels, H. F. Marin, *J. Sci. Fd. Agric.* **1968**, *19*, 710.
22. D. Fomergue, D. K. Kosma, *Plants (Basel)* **2017**, *6*, 25.
23. M. J. Kim, B.-G. Kim, J.-H. Ahn, *Appl. Microbiol. Biotechnol.* **2013**, *97*, 7195.

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