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Biosynthesis of 4-acetylantroquinonol B in *Antrodia cinnamomea* via a pathway related to coenzyme Q synthesis

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ABSTRACT

The biosynthesis pathway for production of 4-acetylantroquinonol B (4-AAQB) by *Antrodia cinnamomea* was investigated by adding various precursors to the culture medium. Adding 4-hydroxybenzoic acid (4-HBA) significantly increased the production of 4-AAQB. Since 4-HBA is an intermediate of the shikimate pathway and 4-AAQB and coenzyme Q (CoQ) are similar in structure, we suspected that the pathway for producing 4-AAQB was closely related to the biosynthesis of CoQ. Since the isoprenoid chain of CoQ is synthesized via the mevalonate pathway, we added oleic acid to the culture medium and confirmed that the addition significantly increased the production of 4-AAQB. Furthermore, adding coenzyme Q_0 into the fermentation broth was found to be the most effective way to increase the production of 4-AAQB. We suspect that coenzyme Q_0 forms CoQ, after which CoQ is converted to 4-AAQB via unknown steps. The increase in 4-AAQB production due to the addition of CoQ_{10} further demonstrated that the biosynthesis pathway of 4-AAQB from *A. cinnamomea* is closely related to CoQ.

1. Introduction

Antrodia cinnamomea is a well-known medicinal fungus often produced commercially by submerged fermentation in Taiwan. This fungus is known for its potent anti-hepatocellular carcinoma function. In a previous study, the key anti-hepatic compound from the mycelium of *A. cinnamomea*, 4-acetylantroquinonol B (4-AAQB), was isolated and identified. This compound can inhibit proliferation of hepatocellular carcinoma cells HepG2 with an IC_{50} of 0.1 μg/mL [1]. When the HepG2 cells were treated with 4-acetylantroquinonol B, CDK2 and CDK4 decreased and p27 increased in a dose-dependent manner [2]. The treatment also increased the levels of p53 and p21 proteins. A previous study found that in the biosynthesis of 4-acetylantroquinonol B [3], based on the biosynthesis pathway of coenzyme Q, the benzoquinone ring might be produced from shikimic acid via the shikimate pathway and that the polyisoprenoid side chain may be produced from farnesyl diphosphate via the mevalonate pathway. Furthermore, the structure of 4-AAQB is very similar to that of coenzyme Q, so we suspected that the biosynthesis pathway of 4-AAQB was closely related to Coenzyme Q.

Previous reports have discussed the biosynthesis of coenzyme Q. For *Saccharomyces cerevisiae*, the benzoquinone ring is formed from 4-hydroxybenzoic acid (4-HBA), which can be synthesized from chorismate via the shikimate pathway [4]. In *E. coli*, the aromatic ring also comes from 4-HBA, and nine enzymes involved in modification of the ring [5]. Recent research on the overproduction of coenzyme Q_{10} in microorganisms has been extensive [6]. Throughout the pathway, the exogenous addition of correct precursors to the medium, and overexpression of various enzyme genes are strategies for improving the yield of coenzyme Q_{10}. We proposed that in *A. cinnamomea*, 4-AAQB was synthesized via the biosynthesis pathway of CoQ, after which CoQ would be converted to 4-AAQB. Various precursors of CoQ were tested in this study in order to construct the possible biosynthesis pathway of 4AAQB in *A. cinnamomea* (Fig. 1).

2. Materials and methods

2.1. Microorganism and reagents

Antrodia cinnamomea BCRC35716 was obtained from the Bioresoures Collection and Research Center (BCRC) of the Food Industry Research and Development Institute (Hsinchu, Taiwan). Potato dextrose agar (PDA), malt extract, and peptone were obtained from Difico (Sparks, MD, USA). 4-hydroxybenzoic acid (4-HBA), 2, 3-dimethoxy-5-methyl-*p*-benzoquinone (coenzyme Q_0), coenzyme Q_{10}, L-phenylalanine (Phe), tyrosine (Tyr), vanillic acid (VA), 2, 4-dihydroxybenzoic acid (2,4-DHBA), 4-aminobenzoic acid (4-ABA)

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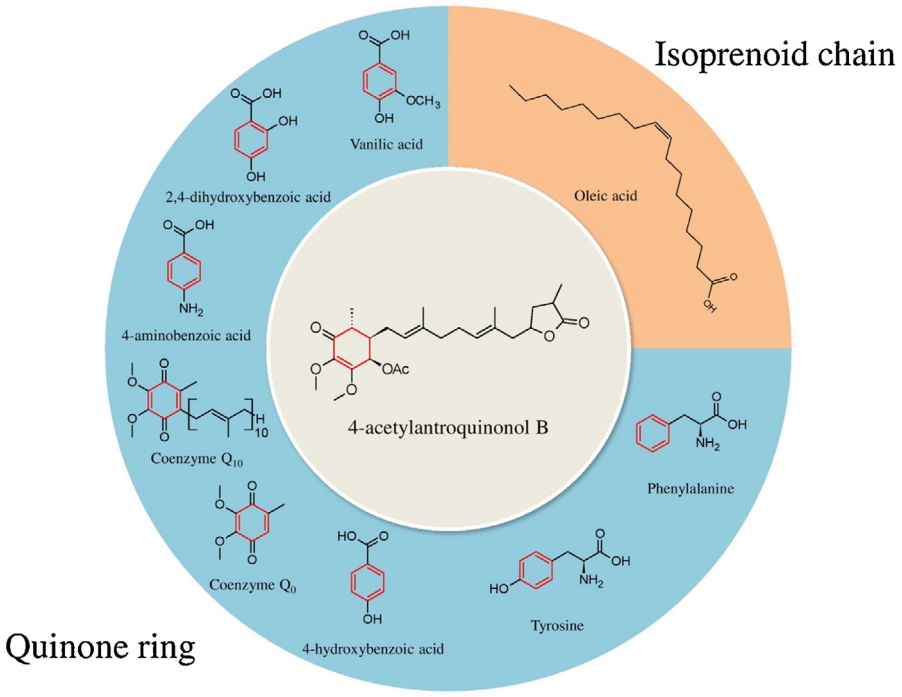

Fig. 1. Precursors for 4-acetylantroquinonol B.

and oleic acid were obtained from Sigma-Aldrich (St. Louis, Mo, USA).

2.2. *Shake-flask fermentation of* Antrodia cinnamomea

The seed culture was maintained on 39 g L^{-1} potato dextrose agar at 25 °C and transferred to a fresh agar plate every 4 weeks. To prepare the inoculum, the mycelium of *A. cinnamomea* was transferred from the Petri dish to a 500 mL flask containing 200 mL of medium (components: glucose 2.0%, malt extract 2.0%, and peptone 0.1%) and incubated at 25 °C for 7 days for mycelium growth. Then, 20 mL of the flask culture was transferred to a 500 mL flask containing 200 mL medium. The mixture was fermented at 25 °C for 4 weeks in a rotary shaker at 100 rpm. The initial pH of the medium was 5 adjusted by 0.1 N NaOH or 0.1 N HCl.

The *A. cinnamomea* was cultivated in the medium described above with 0.01% phenylalanine, tyrosine, coenzyme Q_0, coenzyme Q_{10}, vanillic acid, 2, 4-dihydroxybenzoic acid, 4-aminobenzoic acid and 0.01, 0.02, 0.05 or 0.1% 4-HBA. Precursors were combined in the same medium with both 0.01% 4-HBA and coenzyme Q_0 or 0.1% oleic acid added. The pH value of the medium was adjusted to 5 by adding 0.1 N NaOH or 0.1 N HCl, and then the mixture was sterilized at 121 °C for 20 min.

2.3. *Determination of biomass and 4-acetylantroquinonol B content*

To recover the mycelium, the fermentation broth was through Whatman No. 1 filter paper and the filtrate was washed twice with distilled water. Biomass was then determined after freeze-drying. The ethanol extract of the mycelium of *A. cinnamomea* was obtained by extracting freeze-dried mycelium (0.1 g) with 95% ethanol (2 mL) by sonication at 25 °C for 1 h. The extract was centrifuged at 25 °C for 1 h at 10,000 rpm. The supernatant was filtered through 0.45 μm membrane and then the concentration of 4-AAQB was analyzed by Agilent 1100 HPLC system (Agilent, USA) equipped with an UV detector. A COSMOSIL $5C_{18}$-MS-II column (4.6mm × 250 mm, 5 μm) was used for separation. The mobile phase was composed of H_2O (A), 0.1% phosphate buffer:methanol = 7:13 (B) and acetonitrile (C). The elution profile was as follows: 0–45 min, B 100%; 45–60 min, A:C = 90:10; 60–75 min, A:C = 10:90; 75–90 min, B 100%; the flow rate was 1 mL/min and the detection wavelength was set at 254 nm. Pure 4-AAQB isolated from the mycelium of *A. cinnamomea* in a previous study (Lin et al., 2011) [2] was diluted to 31.25, 62.5, 125, 250, 500 and 1000 μg/mL for constructing the standard curve (R^2 = 0.9993).

2.4. *Statistical analysis*

Significant differences among means ($p < 0.05$) were determined by one-way analysis of variance and Duncan's multiple-range test (SAS Institute Inc., Cary, NC, USA).

3. Results and discussion

The biosynthesis pathway of CoQ is different in different species (Fig. 2). Yeast and fungi can make the benzoic ring from 4-HBA, and yeast is also able to derive the ring of CoQ from the folate precursor *para*-aminobenzoic acid (*p*ABA) [7]. In vertebrates, mammals can incorporate phenylalanine and tyrosine into the benzenoid ring of CoQ, and 4-hydroxybenzoic acid is a possible intermediate [4]. Since the structure of 4-acetylantroquinonol B is very similar to that of CoQ, in this study, we used some of the important intermediates in CoQ synthesis as precursors to determine whether these intermediates also play significant roles during the synthesis of 4-AAQB, so that we could try to construct the biosynthesis pathway of 4-AAQB.

3.1. *Effect of aromatic amino acid on the biosynthesis of 4-acetylantroquinonol B*

In the biosynthesis of CoQ in *E. coli*, 4-HBA is formed from shikimate via chorismate. Research shows that *S. cerivisiae* forms 4-HBA via two different pathways [8]. In yeast cells, 4-HBA can

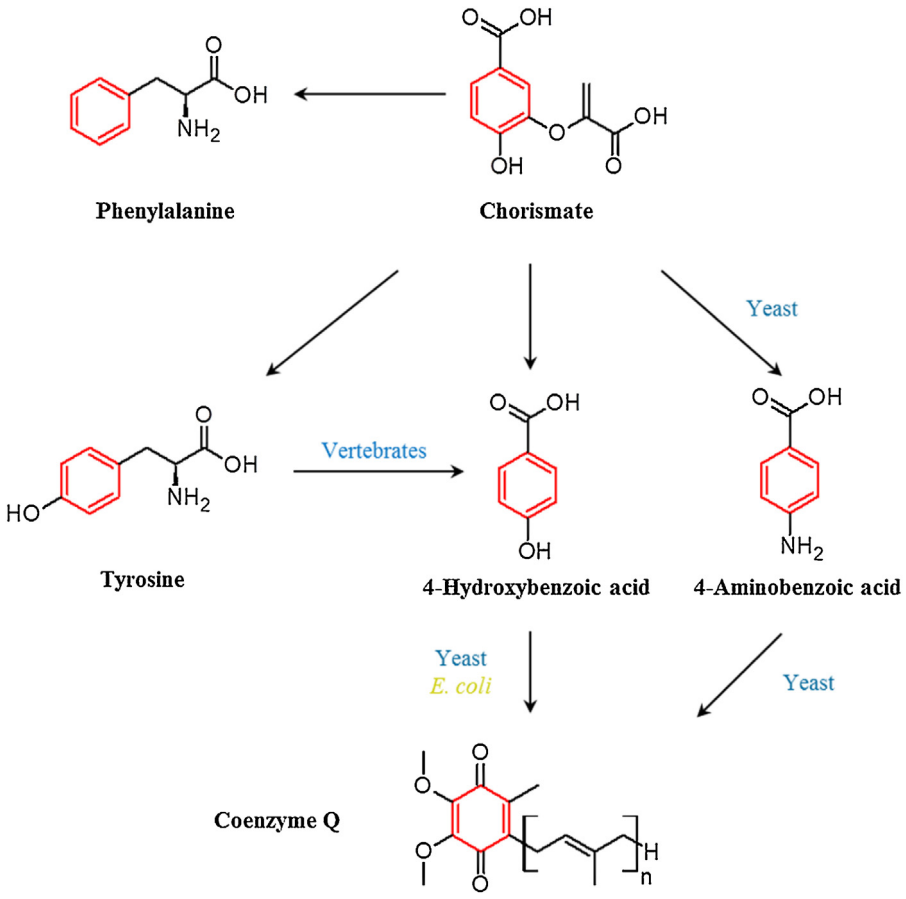

Fig. 2. The biosynthesis pathway of coenzyme Q in *E. coli*, yeast and vertebrates.

be produced directly from chorismate or alternatively from tyrosine. Furthermore, when mutations do not allow the use of tyrosine for CoQ biosynthesis via the shikimate pathway, the pathway can be completed by providing 4-HBA [9]. In animal cells, 4-HBA is formed from the essential amino acid tyrosine [10]. In rat livers, both tyrosine and phenylalanine serve as precursors for 4-HBA, and the association of phenylalanine is thought to proceed by its conversion to tyrosine [9]. Since both tyrosine and phenylalanine may be the possible precursors of 4-AAQB, these two amino acids were added into the fermentation medium to increase the production of 4-AAQB.

The effects of tyrosine and phenylalanine (0.01%) on the production of biomass and 4-AAQB are shown in Fig. 3. Although supplementation of 0.01% tyrosine and phenylalanine had no significant effect on the biomass ($p > 0.05$), it significantly decreased the production of 4-AAQB ($p < 0.05$). The amount of 4-AAQB was about half the amount of control when 0.01% tyrosine was added, and was 10 times less than the amount of control when 0.01% phenylalanine was presented. Therefore, neither tyrosine nor phenylalanine is a main precursor of 4-AAQB in *A. cinnamomea*. The fact that addition of tyrosine or phenylalanine decreases the production of 4-AAQB may suggest that the whole ubiquinone and other terpenoid-quinone biosynthesis pathway was directed to produce plastoquinol/tocopherol, instead of ubiquinone. A study on antroquinonol, which is a ubiquinone derivative and structure similar to 4-AAQB, showed that the addition of tyrosine and phenylalanine not only had no effect on antroquinonol production but also decreased biomass production [11]. Furthermore, both tyrosine and phenylalanine could be used as nitrogen sources during submerged fermentation of *A. cinnamomea*, leading the metabolic pathway to primary metabolism but not to secondary metabolism.

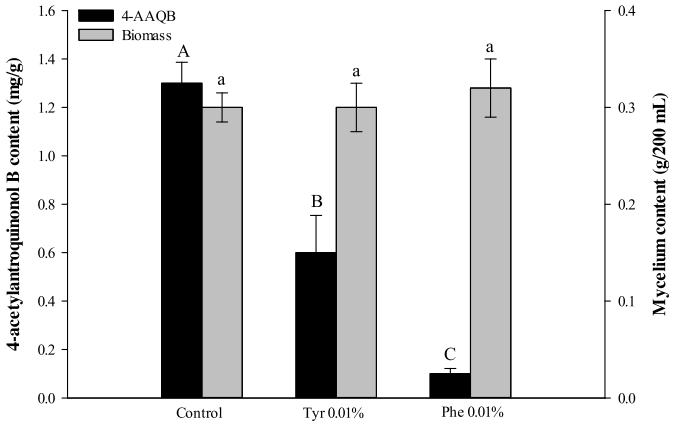

Fig. 3. Effects of tyrosine and phenylalanine supplementation on the 4-acetylantroquinonol B and biomass production of *A. cinnamomea* in 500 mL shake flask cultures. Each value is the mean of three determinations, and the error bars indicate the standard deviations from three independent samples. The bars labelled with different capital letters (A, B, and C) indicate the amount of 4-AAQB in mycelium are significantly different ($p < 0.05$); and the bars labelled with different lowercase letters (a, b, and c) indicate the amount of biomass are significantly different ($p < 0.05$).

These may be the reasons why the amount of 4-AAQB significantly decreased as compared with the control.

3.2. *Effect of 4-HBA on the biosynthesis of 4-acetylantroquinonol B*

For both prokaryotes and eukaryotes, 4-HBA is an essential intermediate to form CoQ. For instance, *S. cerevisiae* can form 4-

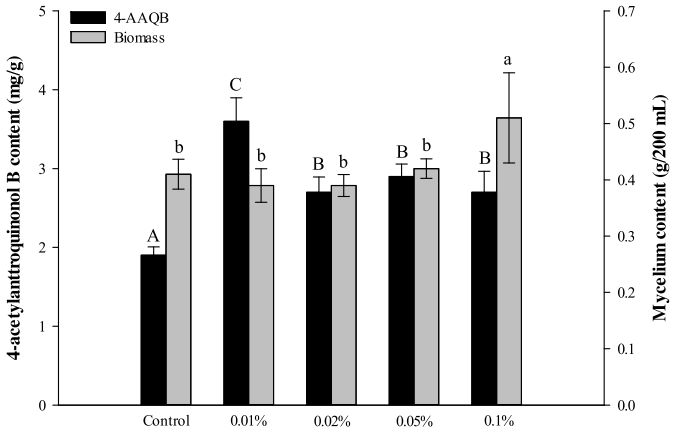

Fig. 4. Effects of 4-hydroxybenzoic acid (4-HBA) supplementation on the 4-acetylantroquinonol B and biomass production of *A. cinnamomea* in 500 mL shake flask cultures. Each value is the mean of three determinations, and the error bars indicate the standard deviations from three independent samples. The bars labelled with different capital letters (A, B, and C) indicate the amount of 4-AAQB in mycelium are significantly different ($p < 0.05$); and the bars labelled with different lowercase letters (a, b, and c) indicate the amount of biomass are significantly different ($p < 0.05$).

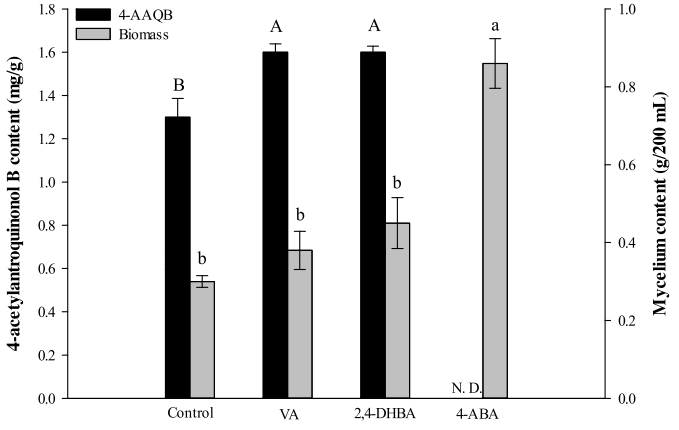

Fig. 5. Effects of vanillic acid (VA), 2,4-dihydroxybenzoic acid (2,4-DHBA) and 4-aminobenzoic acid (4-ABA) supplementation on the 4-acetylantroquinonol B and biomass production of *A. cinnamomea* in 500 mL shake flask cultures. Each value is the mean of three determinations, and the error bars indicate the standard deviations from three independent samples. The bars labelled with different capital letters (A, and B) indicate the amount of 4-AAQB in mycelium are significantly different ($p<0.05$); and the bars labelled with different lowercase letters (a, and b) indicate the amount of biomass are significantly different ($p < 0.05$).

HBA from both shikimate and tyrosine and then synthesize CoQ. In *E. coli*, 4-HBA can be formed only from shikimate [8]. Since we propose that 4-AAQB may have a biosynthesis pathway similar to that of CoQ, 4-HBA might be the most important intermediate for the biosynthesis of 4-AAQB. The effects of 4-HBA supplementation (0.01, 0.02, 0.05 and 0.1%) on the biomass and 4-AAQB production during fermentation are given in Fig. 4. Supplementation of 0.01%, 0.02% and 0.05% of 4-HBA had no effect on biomass, but adding 0.1% 4-HBA to the medium significantly increased biomass compare to control ($p < 0.05$). As expected, supplementation of 4-HBA significantly increased the production of 4-AAQB, and the addition of 0.01% 4-HBA increased 4-AAQB production approximately 2-fold of the control. This result shows that 4-HBA is indeed an important intermediate in the biosynthesis pathway of 4-AAQB. Hu also found that supplementation of 0.01% of 4-HBA during fermentation of *A. camphorate* could significantly increase the production of antroquinonol [11]. However, our results also showed that the amount of 4-AAQB decreased at higher concentration of 4-HBA.

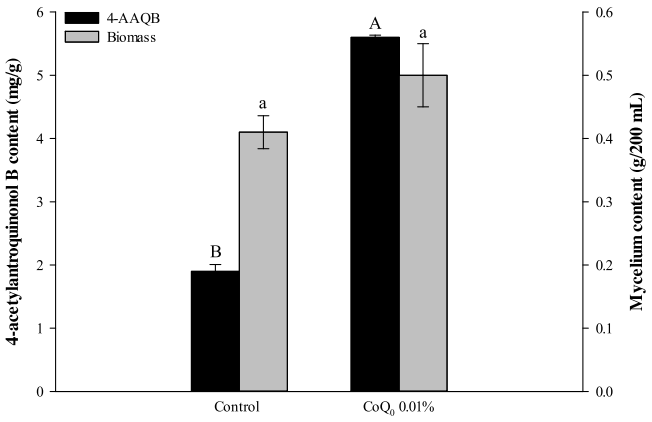

Fig. 6. Effects of coenzyme Q_0 supplementation on the 4-acetylantroquinonol B and biomass production of *A. cinnamomea* in 500 mL shake flask cultures. Each value is the mean of three determinations, and the error bars indicate the standard deviations from three independent samples. The bars labelled with different capital letters (A, and B) indicate the amount of 4-AAQB in mycelium are significantly different ($p < 0.05$); and the bars labelled with different lowercase letters (a, and b) indicate the amount of biomass are significantly different ($p < 0.05$).

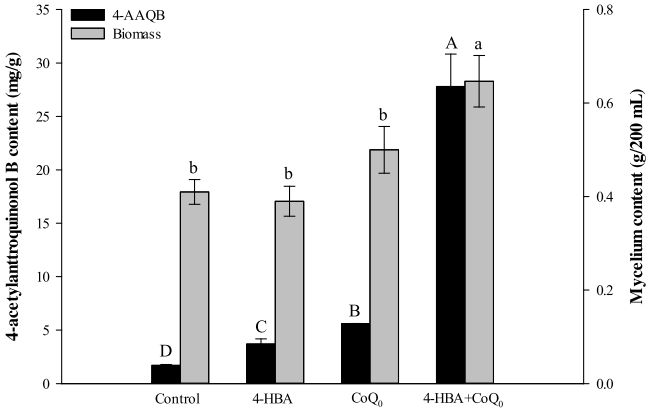

Fig. 7. Effects of 4-hydroxybenzoic acid and coenzyme Q_0 supplementation on the 4-acetylantroquinonol B and biomass production of *A. cinnamomea* in 500 mL shake flask cultures. Each value is the mean of three determinations, and the error bars indicate the standard deviations from three independent samples. The bars labelled with different capital letters (A, B, C and D) indicate the amount of 4-AAQB in mycelium are significantly different ($p < 0.05$); and the bars labelled with different lowercase letters (a, b, c and d) indicate the amount of biomass are significantly different ($p < 0.05$).

Since the enzyme that converts chorismate to 4-HBA has a strong product inhibition [12], it is reasonable that a higher amount of 4-HBA would decrease the production of 4-AAQB.

3.3. *Effect of 4-HBA analogs on the biosynthesis of 4-acetylantroquinonol B*

Wessjohann et al. [13] found that the enzyme that links the polyisoprenoid chain to 4-HBA is non-specific; compounds with a particular structure can also be the substrates of the enzyme. Furthermore, Marbois et al. [14] and Pierrel et al. [7] found that 4-aminobenzoic acid, which is a precursor of folate, can also be the aromatic ring precursor of CoQ. Therefore, we tested vanillic acid, 2,4-dihydroxybenzoic acid, and 4-aminobenzoic acid to see if they also had effects similar to those of 4-HBA.

The results of supplementation of these compounds are given in Fig. 5. Vanillic acid and 2,4-dihydroxybenzoic acid did not increase biomass significantly, but 4-aminobenzoic acid, which is a precursor of folate in some microorganisms [15], did significantly increase the biomass, which was about 2.7-fold of the control ($p < 0.05$). On

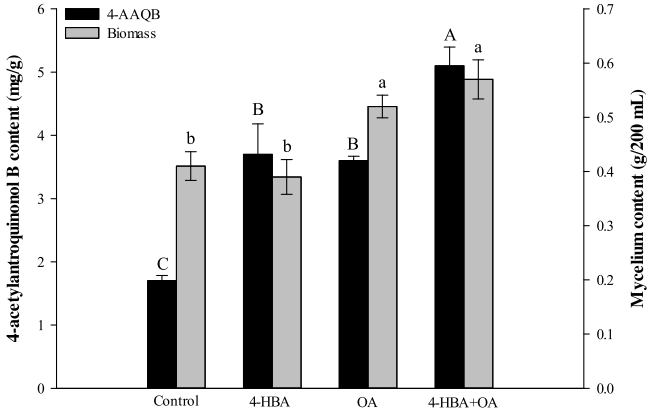

Fig. 8. Effects of 4-hydroxybenzoic acid and oleic acid (OA) supplementation on the 4-acetylantroquinonol B and biomass production of *A. cinnamomea* in 500 mL shake flask cultures. Each value is the mean of three determinations, and the error bars indicate the standard deviations from three independent samples. The bars labelled with different capital letters (A, B, and C) indicate the amount of 4-AAQB in mycelium are significantly different ($p < 0.05$); and the bars labelled with different lowercase letters (a, b, and c) indicate the amount of biomass are significantly different ($p < 0.05$).

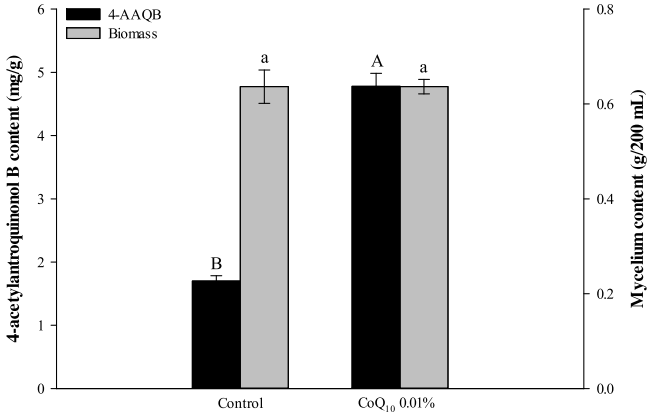

Fig. 9. Effects of coenzyme Q_{10} supplementation on the 4-acetylantroquinonol B and biomass production of *A. cinnamomea* in 500 mL shake flask cultures. Each value is the mean of three determinations, and the error bars indicate the standard deviations from three independent samples. The bars labelled with different capital letters (A, and B) indicate the amount of 4-AAQB in mycelium are significantly different ($p < 0.05$); and the bars labelled with different lowercase letters (a, and b) indicate the amount of biomass are significantly different ($p < 0.05$).

the other hand, vanillic acid and 2,4-dihydorxybenzoic acid significantly increased 1.2-fold of 4-AAQB production ($p < 0.05$). Ozeir et al. [16] and Xie et al. [17] reported that vanillic acid can bypass the C5-hydroxylation step of coenzyme Q_6 biosynthesis in coq6 yeast mutants and that 2,4-dihydroxybenzoic can restore the production of coenzyme Q_6 in coq7 yeast mutants. These results showed that both vanillic acid and 2,4-dihydroxybenzoic acid can be the precursors of 4-acetylantroquinonol B. However, 4-aminobenzoic acid completely inhibited the production of 4-AAQB. In the study of Ozeir et al. [18], the protein Coq6, which catalyzes the C5-hydroxylation reaction of coenzyme Q, is able to carry out the C4-deamination reaction when the yeast is grown with exogenous 4-aminobenzoic acid. However, for *A. cinnamomea*, it is quite possible that the enzyme does not have the ability to remove the C4-amino group, with resultant inhibition of 4-AAQB production. Furthermore, since 4-aminobenzoic acid is the precursor of folate, adding this compound may lead the biosynthesis pathway to the formation of folate and not to the synthesis of 4-AAQB.

3.4. Effect of coenzyme Q_0 on the biosynthesis of 4-acetylantroquinonol B

Coenzyme Q_0 is rarely found in nature, but Wu et al. [19] isolated it from submerged fermentation of the mycelium of *A. cinnamomea* BCRC 36799, and Shen et al. [20] also isolated coenzyme Q_0 from *Antrodia salmonea*, which is in the same genus as *A. cinnamomea*. Chung et al. [21] identified and isolated coenzyme Q_0 from the fermentation filtrate of *A. cinnamomea* 35716, the strain used in this study. According to Chung et al., the concentration of coenzyme Q_0 began to increase when the growth of mycelium reached the stationary phase after 2 weeks of fermentation, and the amount of coenzyme Q_0 continued to increase until week 4. This trend indicated that the coenzyme Q_0, like other quinone metabolites in *A. cinnamomea*, is a secondary metabolite. According to the research of Yu et al. [22] on biosynthesis of benzenoids in *A. cinnamomea*, the deletion of polyketide synthase gene pks63787 would result in the loss of ability to synthesize several benzenoids, including coenzyme Q_0, indicating that coenzyme Q_0 is synthesized by polyketide pathway. The effects of coenzyme Q_0 (0.01%) on the production of biomass and 4-AAQB during fermentation are given in Fig. 6. Supplementation of 0.01% coenzyme Q_0 had no significant effect on biomass ($p > 0.05$), but higher concentration (0.05% and 0.1%) of coenzyme Q_0 would completely inhibit the mycelia growth of *A. cinnamomea*. However, supplementation of 0.01% of coenzyme Q_0 increased about 3-fold of 4-AAQB production as compared to control, and this was the most effective compound tested in this study for increasing 4-AAQB production (5.60 ± 0.04 mg/g dried mycelium). Based on this result, we propose that coenzyme Q_0 is also an intermediate during the synthesis of 4-AAQB. Yu et al. [22] found that the coenzyme Q_0 is a product of polyketide pathway. We suspect coenzyme Q_0 may be converted to 4-AAQB through still unidentified steps. It has been suggested that modification of the benzoquinone ring comprises the rate limit steps of the synthesis of CoQ [23]. The coenzyme Q_0 does not need further modification on the functional groups but 4-HBA does. It should form CoQ more easily than 4-HBA, which is why it could increase the production of 4-AAQB better than 4-HBA could. From the results mentioned above we can conclude that the biosynthesis of 4-AAQB involves both of 4-HBA and coenzyme Q_0.

3.5. Effect of combination of coenzyme Q_0 and 4-HBA

From our results, we can deduce that coenzyme Q_0 and 4-HBA might both be materials used in forming the benzoquinone ring, and these two compounds were demonstrated to be the most effective precursors for enhancing 4-AAQB production. In order to elucidate whether these two compounds synthesize 4-AAQB through the same or different the biosynthesis pathway, we added these two compounds (0.01% each) together to the fermentation medium, and the results are given in Fig. 7. We found that these two compounds increased 4-AAQB production (27.80 ± 3.04 mg/g) approximately 16 times of the control. As mentioned before, Yu et al. [22] found that the production of coenzyme Q_0 is via polyketide pathway. It is also known that 4-HBA is produced through shikimate pathway. Therefore, these two compounds enter the synthesis of 4-AAQB via different pathways, which can explain why addition of these two compounds together in the culture medium would further increase the 4-AAQB production. Moreover, other research also found that addition of these two compounds together also enhanced the production of antroquinonol [11].

3.6. Effect of combination of 4-HBA and oleic acid

We have demonstrated that 4-HBA is a precursor of 4-AAQB and we suspected that it might contribute to the formation of the ben-

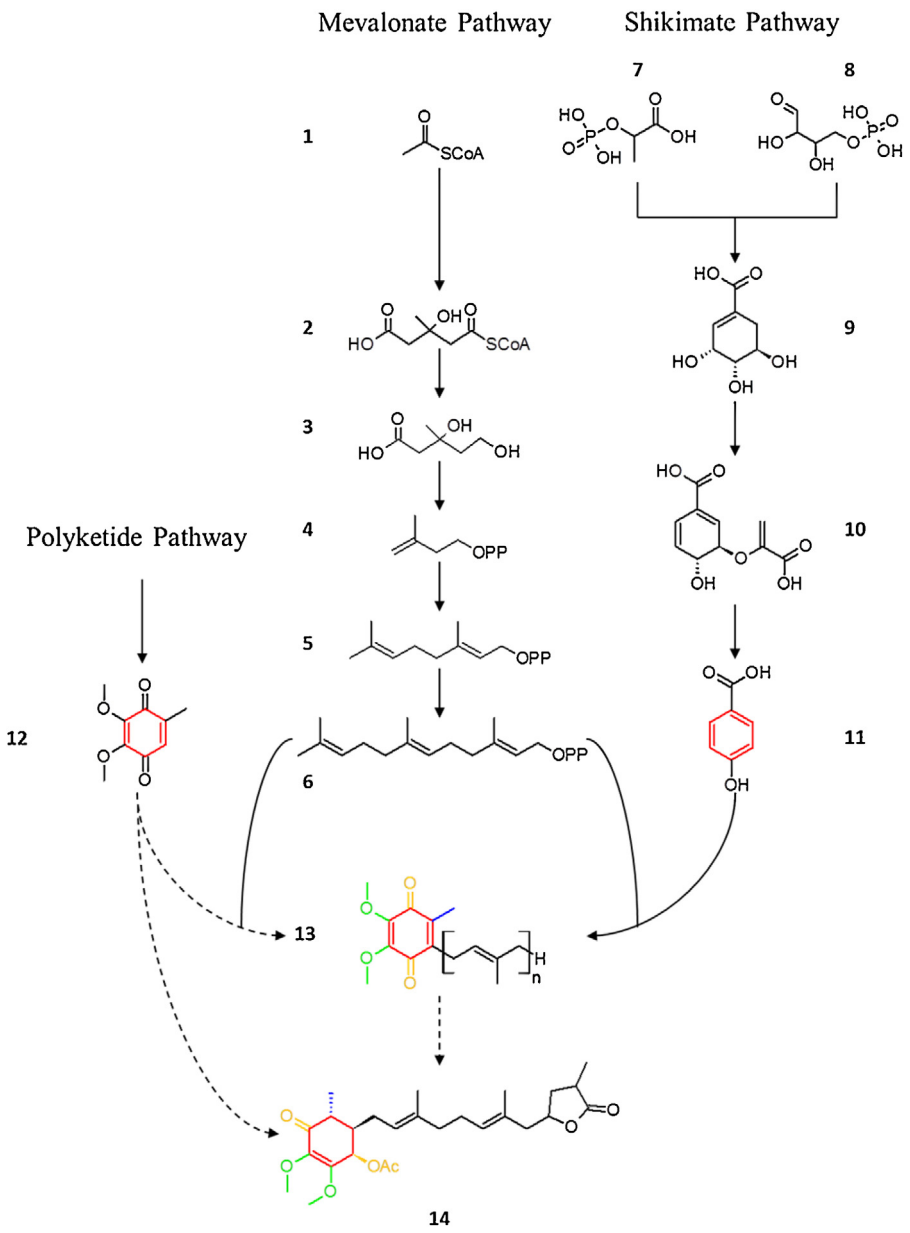

Fig. 10. The proposed biosynthesis pathway of 4-acetylantroquinonl B.
1. Acetyl-CoA, 2. Hydroxymethylglutaryl-CoA, 3. Mevalonic acid, 4. Isopentenylpyrophosphate, 5. Geranyl pyrophosphate, 6. Farnesyl pyrophosphate, 7. Phosphoenolpyruvate, 8. D-erythrose 4-P, 9. Shikimic acid, 10. Chorismic acid, 11. 4-Hydroxybenzoic acid, 12. Coenzyme Q_0, 13. Coenzyme Q_{10}, 14. 4-Acetylantroquinonol B. The dotted line represents an unknown pathway for synthesizing 4-AAQB from coenzyme Q_0, and the solid line represents the pathways already known.

zoquinone ring of 4-AAQB. For the isoprenoid chain of 4-AAQB, we suspected that the synthesis of this part might be similar to that of CoQ, which is synthesized via the mevalonate pathway. Therefore, we added both 4-HBA and oleic acid to the fermentation medium to investigate if whether this combination, one as the precursor for the benzoquinone ring and the other for the isoprenoid chain, could effectively increase the production of 4-AAQB. The results are given in Fig. 8. We found that the combination of these two precursors indeed increased the amount of 4-acetylantroquinonol B in mycelium, and it was about 1.5 times higher than the addition 4-HBA or oleic acid alone ($p < 0.05$). This result further demonstrates that the synthesis of 4-AAQB is similar to the synthesis of CoQ. Although the addition of the two precursors significantly increased the 4-AAQB content in mycelium as compared to the result of addition either 4-HBA or oleic acid alone, the increment of 4-AAQB was still modest. The possible explanation might be that oleic acid is not the precursor only for isoprenoid side chain. For instance, the oleic acid may be metabolized to acetyl-CoA, and acetyl-CoA may enter either mevalonate pathway or polyketide pathway. Therefore, the combined addition of 4-HBA and oleic acid failed to achieve the result that is corresponding to the amount we added.

3.7. *Effect of coenzyme Q_{10} on the biosynthesis of 4-acetylantroquinonol B*

Coenzyme Q is a redox active lipid that functions in the electron transport chains of mitochondria and plasma membranes, and it plays an important role as an antioxidant [24]. The membrane-bound isoprenoid ubiquinone is found in nearly all living organisms, and the chain length of CoQ depends on species [25]. Research has found that the expression levels of COQ genes in *A. cinnamomea* are noticeable in both the mycelium and the fruiting

body, but much higher in the mycelium [26]. This result gives a clue that CoQ biosynthesis may play an important role in the biosynthesis of antroquinonol derivatives. We assumed that the biosynthesis pathway of CoQ and 4-AAQB were closely related, or that they might be synthesized on the same pathway and that 4-AAQB was a metabolic product of CoQ. To confirm this hypothesis, we added 0.01% of coenzyme Q_{10} to the fermentation medium and the result are given in Fig. 9. It is shown that coenzyme Q_{10} had no significant effect on the amount of biomass production ($p > 0.05$), but increased approximately 3-fold of 4-AAQB production ($p < 0.05$). This result may be evidence that 4-AAQB is a metabolic product of coenzyme Q_{10}, Gille et al. [27] investigated the metabolic degradation of CoQ and found a common metabolic pathway through ω-oxidation or β-oxidation of the side chain leading to the carboxylate product Q-acid. We speculate that the formation of a carboxyl group during the metabolic pathway may be closely connected to the γ-lactone structure on the side chain of 4-AAQB. Only a few benzoic compounds have γ-lactone; in *A. cinnamomea*, only two compounds, antroquinonol and 4-acetylantroquinonol B, have this structure. To date, no reports have focused on the biosynthesis of compounds like this, but for γ-lactone related compounds, such as natural flavors, fungi can transform some fatty acids into lactones through the biosynthesis steps of hydroxylation, β-oxidation and lactonisation [28]. For unsaturated fatty acids, when the hydroxyl group is positioned near the hydroxyl group of the carboxylic end, lactonisation occurs. Since CoQ can be metabolized to Q-acid, it is very possible that the carboxyl group of Q-acid can undergo the same steps and form the lactone ring, subsequently becoming 4-AAQB, but more study is needed to confirm this possibility.

4. Conclusion

This study compared the structures of 4-AAQB and CoQ and proposed that the biosynthesis pathway of 4-AAQB is similar and closely related to the biosynthesis of CoQ. The proposed biosynthesis pathway of 4-AAQB is given in Fig. 10. We have demonstrated that 4-HBA is a precursor of 4-AAQB and that it contributes to the formation of the benzoquinone ring of 4-AAQB. Since adding of both 4-HBA and oleic acid significantly increased the production of 4-AAQB, we suspect that the synthesis of the isoprenoid chain might also occur via the mevalonate pathway, as in the case of CoQ, and that oleic acid is not only a material used in the isoprenoid chain. It may also be a precursor of the gamma-lactone ring of 4-AAQB. Furthermore, the adding of coenzyme Q_0 to the fermentation broth was found to be the most effective way to increase the production of 4-AAQB. (Although the biosynthesis pathway of coenzyme Q_0 is still unclear, we suspect that coenzyme Q_0 forms CoQ, after which CoQ is converted to 4-AAQB via unknown steps.) The biosynthesis of coenzyme Q_0 is from polyketide pathway, and it can be converted to 4-AAQB via unknown steps.

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