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Enhancing Lovastatin Biosynthesis in Oyster Mushrooms (*Pleurotus ostreatus*) using Phytohormones

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Abstract

The biosynthesis of lovastatin, the anti-cholesterol compound, in oyster mushrooms *(Pleurotus ostreatus)*, has the potential to be enhanced by utilizing phytohormones, which activate the expression of certain genes. This research aimed to determine the best type and concentration of phytohormone among auxin, gibberellin, and cytokinin, as well as the best mycelial colonization percentage in F2 medium to realize the greatest increase in lovastatin biosynthesis in oyster mushrooms. Lovastatin was extracted from the fruiting bodies and mycelia and analyzed by spectrophotometry. The analysis of *CYP450* linked to lovastatin biosynthesis was conducted by quantitative polymerase chain reaction (qPCR) using samples containing the highest concentration of lovastatin. The results showed that adding phytohormones increased the lovastatin concentration in the fruiting bodies and mycelia of *P. ostreatus*. The highest lovastatin content was observed in the 10 ppm gibberellin treatment by applying 75% mycelial colonization in the F2 medium. Furthermore, this gibberellin treatment also demonstrated increased expression of the *CYP450* gene in the fruiting bodies and mycelia. In conclusion, phytohormone treatments with the right timing and appropriate concentration increased lovastatin biosynthesis in *P. ostreatus* as well as related gene expression.

Keywords: CYP450 gene, lovastatin, mycelial percentage, phytohormones, Pleurotus ostreatus

Introduction

Sedentary lifestyle has increased the number of cases of hypercholesterolemia in Indonesia, particularly in people living in urban areas. Based on 2013 Indonesian public health data (Riskesdas), 35.9% of the Indonesian population <15 years reported having an abnormal cholesterol levels. Consumption of functional foods can overcome this problem. Therefore, the development of local functional foods is required [1].

Oyster mushrooms (*Pleurotus ostreatus*) are a local functional food in Indonesia, which could be developed further as a potential drug source for countering cholesterol levels as it contains the anti-cholesterol compound lovastatin as a secondary metabolite [2]. Lovastatin reduces cholesterol levels by inhibiting hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, which is the enzyme that catalyzes the reduction of HMG-CoA to mevalonate during cholesterol synthesis [3].

Lovastatin biosynthesis in *P. ostreatus* has the potential to be enhanced using phytohormones. Phytohormones act as precursors that affect cell physiology and can activate the expression of certain genes [4]. Several phytohormones that affect the growth of oyster mushrooms are auxin, gibberellin, and cytokinin [5, 6]. Hence, this study was conducted to determine how phytohormones affect lovastatin biosynthesis in oyster mushrooms. Several factors that affect lovastatin synthesis, such as type, concentration, and the correct timing of the treatment were analyzed. The correct timing for the treatment was represented by the percentage of mycelia colonized in F2 medium, which represented the age of the culture.

Materials and Methods

Mushroom strain and culture preparation. The *P. ostreatus* culture was obtained from a traditional oyster mushroom farm in Pangalengan, Bandung, Indonesia. The culture was grown in Potato Dextrose Agar (PDA) and inoculated in F1 medium, which contained sawdust, sorghum, brown rice, sugar, NPK fertilizer, and water. Sorghum replaced corn. Mycelia from the F1 medium became the seed for the solid-state fermentation F2 medium, which contained rice bran, corn, NPK, $CaCO_3$, and sawdust [7]. The fungal culture was incubated in F2 medium for 2–5 weeks before the treatments.

Phytohormone treatments. Auxin (indole acetic acid [IAA]), gibberellin (GA), and cytokinin (KIN) were used as the hormone treatments. The phytohormone solutions were sterilized through a 0.2 µm syringe filter and sprayed onto F2 medium 75% colonized with mycelia. Table 1 shows the different phytohormone treatments. Different ages of mycelial culture were used to determine the correct timing of the hormone treatments. The ages of the mycelial cultures were represented by the percentage of mycelia colonization in F2 medium. IAA (5 ppm), GA (10 ppm), and KIN (10 ppm) were used as the treatments. Table 2 shows the variations in the percentages of mycelial colonization on F2 medium. The percentages of mycelial colonization in F2 medium are illustrated in Figure 1.

Table 1. Phytohormones and their Concentrations

Variation	Phytohormone	Phytohormone concentration (ppm)
V1	IAA	1
V2		5
V3		10
V4	GA	5
V5		10
V6		15
V7	KIN	5
V8		10
V9		15
Control	None	0

Table 2. Variation in Mycelial Colonization for the Phytohormone Treatments

Variation	Phytohormone Treatment	Mycelial Colonization in Medium F2 (%)
T1	IAA 5 ppm	50
T2		75
T3		100
T4	GA 10 ppm	50
T5		75
T6		100
T7	KIN 10 ppm	50
T8		75
T9		100
Control	None	75

Figure 1. Percentage of Mycelia in F2 Medium: 50% (Left), 75% (Middle), and 100% (Right)

Lovastatin extraction and analysis. Four-day-old fruiting bodies and mycelia from solid medium were dried at 50 °C for 48 h, then powdered. Wet and dry biomass was measured in grams. Lovastatin was extracted from 1 g of powdered sample using 20 ml of acetonitrile by shaking for 60 min at 160 ppm on a rotary shaker. The mixtures were centrifuged for 10 min at 6,000 rpm [8] and filtered through no. 1 Whatman filter paper. The filtrate was used for the spectrophotometric analysis (UV-1900; Shimadzu, Tokyo, Japan) at a detection wavelength of 237 nm. The lovastatin concentration was determined based on a standard curve prepared from the commercial anti-cholesterol medicine "Lotyn" containing 20 mg of lovastatin. To prepare the standard solutions, lovastatin was powdered, mixed with 10 ml of absolute ethanol: 0.1 N NaOH (1:1, v/v), and heated at 50 °C for 20 min. HCl (1 M) was added to the mixture until pH 7.7 was reached, and then the solution was filtered through no. 2 Whatman filter paper [7].

RNA extraction, RT-qPCR, and gene expression analysis. RNA extraction and the lovastatin biosynthesis related gene expression analysis were performed on fruiting body and mycelial samples from the treatment with the highest increase in lovastatin concentration. The whole parts of 4-day-old fruiting bodies were used. The mycelial samples originated from cultures grown on PDA (Himedia) that were sprayed with phytohormones on day 5 and further incubated for another 5 days. Each sample was analyzed in duplicate.

The harvested fruiting bodies and mycelia were frozen in liquid nitrogen and ground with a sterile mortar and pestle. Total RNA was extracted from 0.1 g of the frozen sample using the Geneaid GENEzol™ Reagent according to the manufacturer's instructions. The purity of the extracted RNA was verified using the Nanodrop Lite Spectrophotometer (ThermoScientific, Waltham, MA, USA). The extracted RNA was used as a template for reverse transcription with the Toyobo ReverTra Ace™ quantitative polymerase chain reaction (qPCR) RT Master Mix and the gDNA Remover in the Veriti™ 96 Well Thermal Cycler according to the manufacturer's protocols. cDNA was diluted 1:10 (v/v) for real-time PCR using primer pairs (Table 3) and SensiFAST SYBR® No-ROX mix (2×). The qPCR conditions were pre-denaturation for 2 min at 95 °C, denaturation for 5 s

Table 3. Primer Sequences for the RT-qPCR Analysis

Gene	Type of primer	Primer sequence (5'→3')	Resource
CYP450	*forward*	GTTCTCAGGTGGCATACGGT	[10]
	reverse	AGTTATTGCCTACGACGA TGG	
act1	*forward*	AGTCGGTGCCTTGGTTAT	[11]
	reverse	ATACCGACCATCACACCT	

at 95 °C, annealing for 10 s at 60 °C, and 40 cycles of extension for 15 s at 72 °C. The threshold cycle (Ct) values were used to calculate the relative gene expression of the target gene normalized to the actin gene as the reference gene. The relative RT-qPCR quantification was performed using the $2^{-\Delta\Delta Ct}$ method [9]. The PCR product was sequenced for confirmation and analyzed further using the Web SD-search tool to identify the family of the protein that was coded.

Statistical analysis. All treatments were run in triplicate. The means and standard deviations were obtained from the test replicates. All data were analyzed by one-way analysis of variance (ANOVA) and a posthoc Tukey's test. A *p*-value <0.05 was considered significant. The statistical analysis was performed using Minitab v. 21.1.

Results and Discussion

The Effect of Phytohormone and their Concentrations on the Lovastatin Concentration and Fungal Biomass. Secondary metabolite biosynthesis is induced by nutrients in the environment [12]. The concentration of induction molecules in the environment can affect the lovastatin concentration. Adding 100 nM butyrolactone I increase lovastatin biosynthesis in *Aspergillus terreus* by 150% [12, 13]. The production of lovastatin by *A. terreus* increased 1.8-fold after adding linoleic acid [14].

Adding phytohormones to the cultures increased the lovastatin concentration produced by the fruiting bodies and mycelia of *P. ostreatus*. The top three highest lovastatin levels produced by the mycelia in response to the phytohormone treatments were realized by adding 10 ppm GA, 10 ppm KIN, or 10 ppm IAA. The increase in lovastatin in the fruiting bodies displayed a similar pattern, but the lovastatin concentrations in the fruiting bodies (Figure 2A) were higher than those in mycelia (Figure 2B). This difference was caused by the expression of different genes in the mycelia and fruiting bodies, which occurs until the maturation of the fruiting body [15]. Higher lovastatin concentrations in fruiting bodies than mycelia were also reported in a study of *Pleurotus eryngii* [16].

The increase in lovastatin concentration after adding phytohormones to the cultures was caused by changes in nitrogen metabolism. Exogenous IAA increases total nitrogen and NO_3^-, as well as nitrate reductase activity, which facilitates the reduction of nitrate to ammonium [17]. Adding KIN stimulates nitrate reductase activity [18] while adding GA increases nitrogen usage efficiency by increasing the uptake of nitrogen from the environment through nitrate reductase activity [19]. Measurement of the medium pH supported enhanced nitrate reductase activity, as the pH values of the treatments were higher than the pH of the control (data not shown). Nitrogen plays an important role in lovastatin biosynthesis [12], as changes in nitrogen metabolism affect lovastatin biosynthesis.

Adding GA increases the glucose uptake rate as well as affects nitrogen metabolism. GA promotes the conversion of glucose to pyruvate but inhibits glycolysis and the citric acid cycle. Pyruvate is converted to acetyl Co-A [20]. Acetyl Co-A is a lovastatin precursor, which is converted to dihydromonacolin L, monacolin L, and then to monacolin J, to produce lovastatin acid [12]. The increase in the precursor enhanced the synthesis of the product.

The highest lovastatin concentration was observed in the 10 ppm GA treatment. GA regulates carbon and nitrogen metabolism, and lovastatin biosynthesis in *P. ostreatus* is affected by carbon and nitrogen concentrations. Higher carbon and nitrogen concentrations increase the lovastatin concentration, although nitrogen plays a more important role [2]. GA treatment may have increased carbon and nitrogen metabolism, which would increase lovastatin biosynthesis.

The fungal biomass observations revealed a different effect of the phytohormone treatments. Data on the dry weight of the fruiting bodies indicated no significant difference from the control (Figure 3). However, the dry weights of the fruiting bodies from the 5 and 10 ppm GA treatments were the lowest compared to the others. This may have been caused by inhibited central carbon metabolism by GA [20], which is the main biomass production pathway [21].

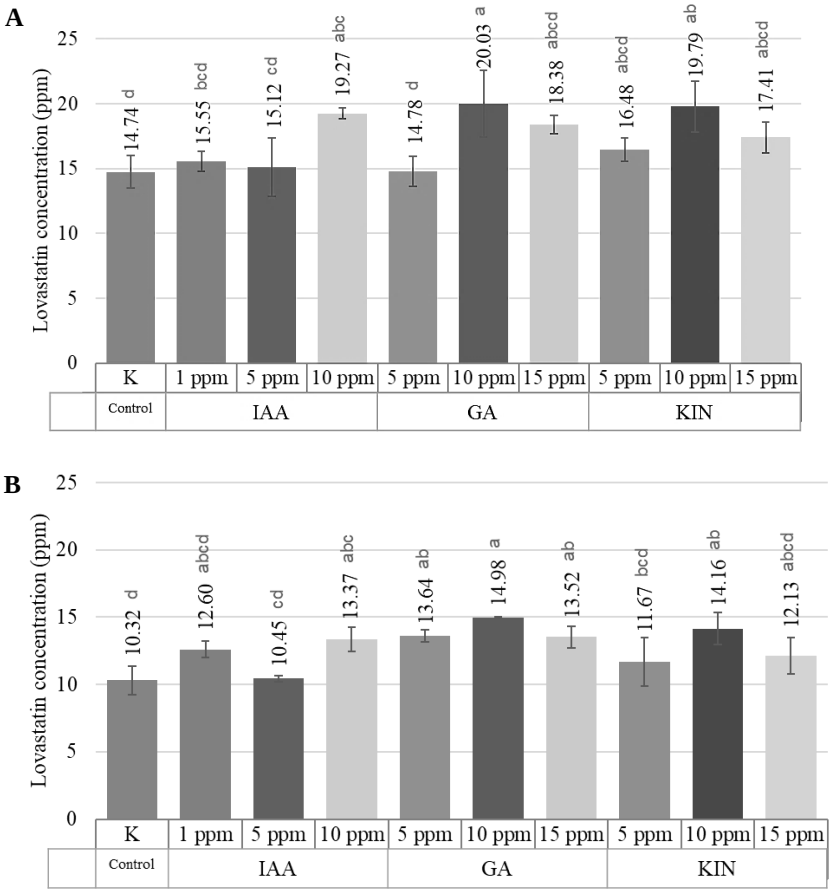

Figure 2. Effect of the Phytohormone Treatments on Lovastatin Concentrations in Fruiting Bodies (A) and Mycelia (B) of *Pleurotus ostreatus* (n = 3; t = 23.7 ± 0.8 °C, RH = 88.2 ± 3.9%). Columns with Different Letters are Significantly Different Based on One-way Analysis of Variance and Posthoc Tukey's Test ($p < 0.05$)

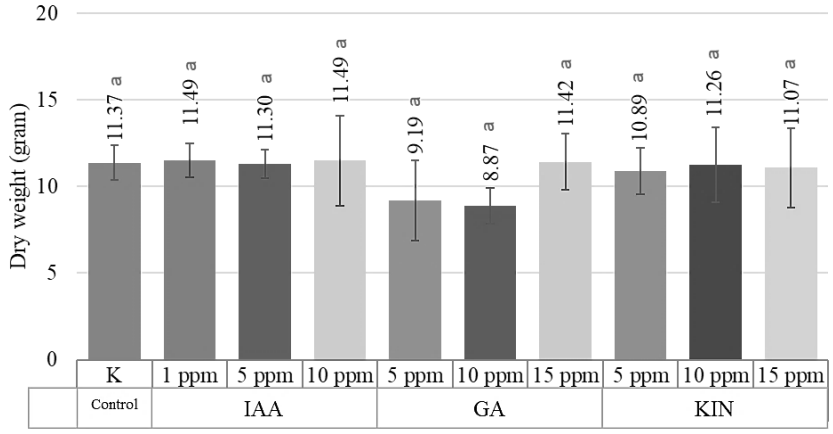

Figure 3. Effect of the Phytohormone Treatments on Dry Weight of *Pleurotus ostreatus* Fruiting Bodies (n = 3; t = 23.7 ± 0.8 °C, RH = 88.2 ± 3.9%). Columns with Different Letters are Significantly Different Based on One-way Analysis of Variance and Posthoc Tukey's Test ($p < 0.05$)

The Effect of Mycelial Percentage and Phytohormones on the Lovastatin Concentration and Fungal Biomass. Lovastatin biosynthesis occurs at the stationary phase or idiophase [22]. Adding linoleic acid to 2-day-old *Aspergillus terreus* spores decreases lovastatin yield [14], even though linoleic acid is a growth hormone precursor [23]. The age of the culture when the induction compound is added affects lovastatin

biosynthesis. In this study, the ages of the cultures were represented by the percentage of mycelia colonization in F2 medium. Cultures in the growth phase (tropophase) have 50% mycelial colonization, whereas cultures in the transition phase between the growth phase and stationary phase (idiophase) are represented by 75% mycelial colonization, and cultures in the early stationary phase have 100% mycelial colonization.

The best times to add the phytohormones varied. Adding GA and KIN (10 ppm each) induced the highest lovastatin concentration in the fruiting bodies and mycelia when added to F2 medium with 75% mycelial colonization (Figure 4A, B). The average lovastatin concentration decreased when 10 ppm GA and KIN were added to cultures with 100% mycelial colonization. The best time to add GA and KIN was during the transition from the growth phase (tropophase) to the stationary phase (idiophase). A similar pattern was found in a study of the biosynthesis of the secondary metabolites in *Scenedesmus* and *Chlorella*, in which the highest concentration of astaxanthin was obtained when linoleic acid was added at the end of the growth phase and the beginning of the stationary phase [23]. Adding 5 ppm IAA to cultures with 100% mycelial colonization resulted in the highest lovastatin concentration in the fruiting bodies. The highest lovastatin concentration in mycelia was detected when 5 ppm IAA was added to 50% mycelial colonization in F2 medium (Figure 4A, B). Such discrepancies were caused by differences in gene expression in the mycelia and fruiting bodies as a response to chemical compounds in the environment [15].

The opposite effect of adding phytohormones was seen in the dry weight of the fruiting bodies. Most of the dry weights of the fruiting bodies and mycelia in the phytohormone treatments tended to be lower than the control, although not significantly different, except for the 10 ppm GA treatment at 100% mycelial colonization (Figure 5). Higher lovastatin biosynthesis caused a lower dry weight of fungal biomass. Older spores resulted in less biomass but an increase in the lovastatin titer in *Aspergillus terreus*. This result was related to the fact that lovastatin is primarily synthesized during the stationary phase [24]. A similar result was observed in our study.

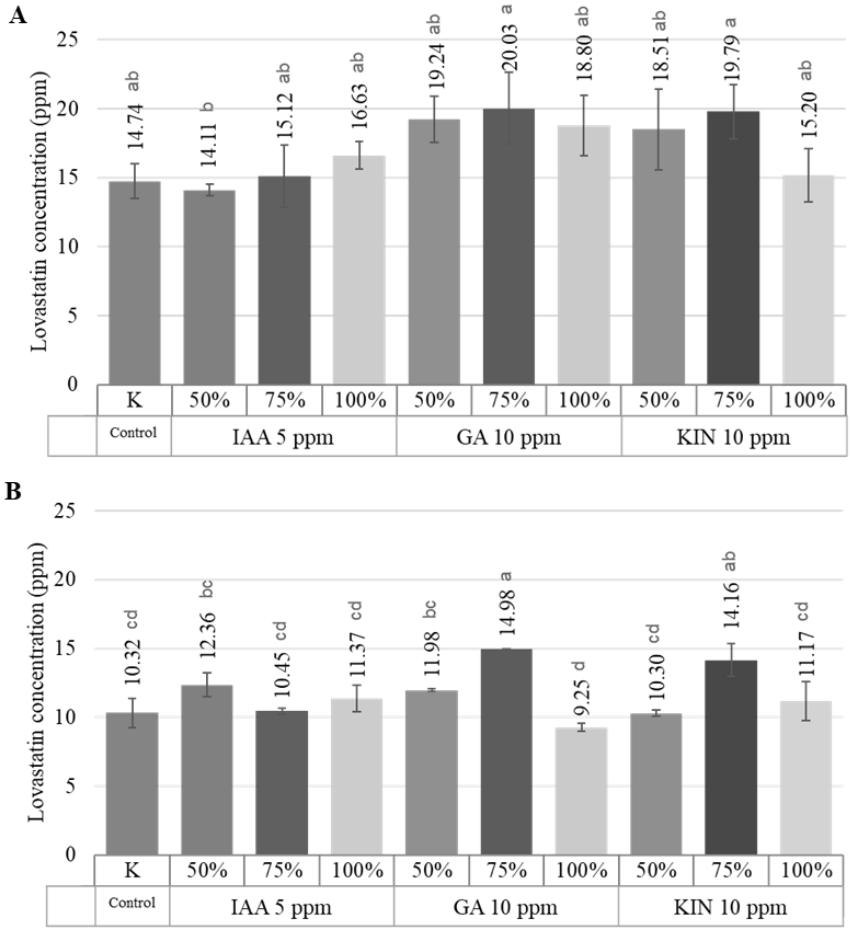

Figure 4. The Effect of the Phytohormone Treatments and Mycelia Percentage on Lovastatin Concentration in Fruiting Bodies (A) and Mycelia (B) of *Pleurotus ostreatus* (n = 3; t = 23.7 ± 0.8 °C, RH = 88.2 ± 3.9%). Columns with Different Letters are Significantly Different Based on One-way Analysis of Variance and Posthoc Tukey's Test ($p < 0.05$).

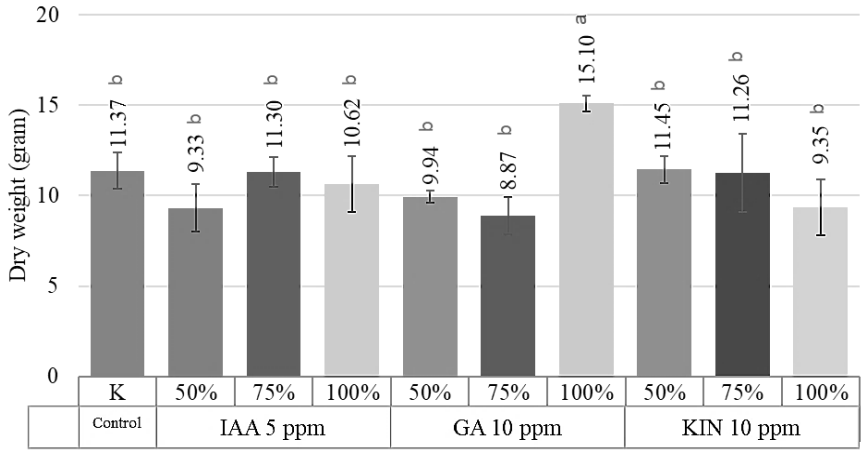

Figure 5. The Effect of the Phytohormone Treatments and Mycelia Percentage on Dry Weight of *Pleurotus ostreatus* Fruiting Bodies (n = 3; t = 23.7 ± 0.8 °C, RH = 88.2 ± 3.9%). Columns with Different Letters are Significantly Different Based on One-way Analysis of Variance and Posthoc Tukey's Test (p<0.05)

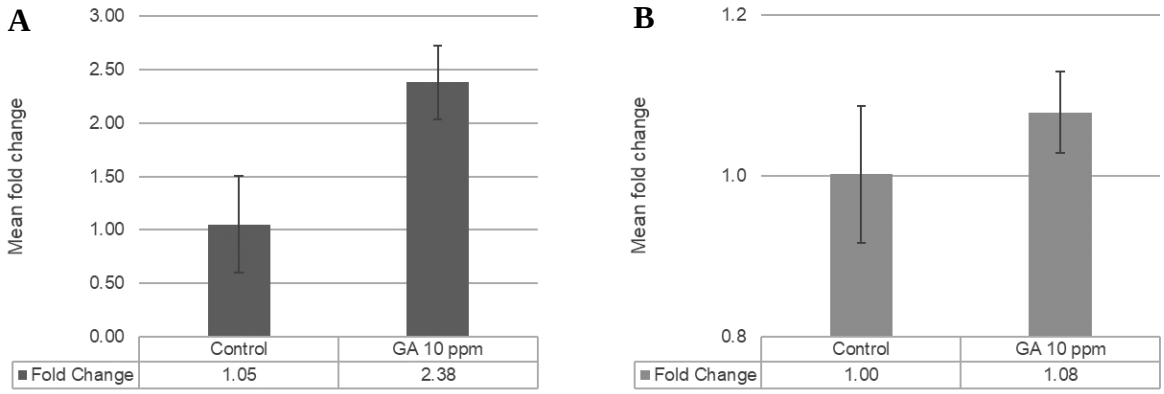

Figure 6. Expression of *CYP450* Gene in Fruiting Bodies (A) and Mycelia (B) of *Pleurotus ostreatus* after the 10 ppm GA Treatment at 75% Mycelial Percentage Compared to the Control

Effect of Adding Phytohormones and the Mycelial Percentage on *CYP450* Gene Expression. Lovastatin biosynthesis is coded by the lovastatin biosynthetic gene cluster. One of the important genes in the cluster is *lovA* (*cytochrome P450 monooxygenase* or *CYP450*), which converts monacolin L to monacolin J in *Aspergillus terreus* [12]. *CYP450* has a role during secondary metabolite biosynthesis in *P. ostreatus* [10]. This gene was analyzed in this study.

Previous data showed that the highest lovastatin concentration was obtained after adding 10 ppm GA at 75% mycelial colonization in F2 medium. The expression analysis showed that *CYP450* expression was higher in the fruiting bodies and mycelia from the treatments compared to the control (Figure 6). Consequently, GA affected the regulation of the lovastatin biosynthesis gene. GA is known to affect gene expression related to secondary metabolite biosynthesis in plants. A study in *Arabidopsis* showed that the expression of genes related to astaxanthin and sesquiterpene is regulated by GA [25]. A similar phenomenon occurs in *P. ostreatus*.

The expression of *CYP450* was higher in fruiting bodies than in mycelia. This result confirms previous data showing higher lovastatin concentrations in fruiting bodies than mycelia. In addition, this result suggests that lovastatin is mostly synthesized in *P. ostreatus* fruiting bodies.

The lovastatin gene cluster in *P. ostreatus* is not well-characterized. Sequencing result of the *CYP450* gene resulted *P. ostreatus* uncharacterized protein (PC9H_01141411), partial mRNA (Accession number XM_036780896.1). The coded protein was analyzed further to determine its family. As a result, the protein had the same superfamily as the cytochrome P450 from *P. eryngii* (Accession number KAF9497426.1) and the cytochrome P450 monooxygenase from *A. terreus* (AAD34565.1), i.e., the cytochrome P450 superfamily.

Conclusion

Adding phytohormones increased the lovastatin content in the fruiting bodies and mycelia of *P. ostreatus*. A hormone combination with the appropriately aged culture maximized the increase in lovastatin. Adding phytohormones also increased the expression of the *CYP450* gene related to lovastatin biosynthesis. These phytohormone treatments have the potential to be applied further for developing local functional foods to alleviate hypercholesterolemia in Indonesia and are recommended for oyster mushroom cultivators.

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References

[1] Nofiartika, F., Prasetyaningrum, Y.I. 2019. Pengaruh pemberian jus jamur tiram terhadap kadar kolesterol, trigliserida, dan malondialdehyde penderita hiperkolesterolemia. Jurnal Gizi Klinik Indonesia. 16(3): 122–128, https://doi.org/10.22146/ijcn.40813.

[2] Alarcón, J., Aguila, S. 2006. Lovastatin Production by *Pleurotus ostreatus*: Effects of the C:N Ratio. Z. Naturforsch. 61c: 95–98, https://doi.org/10.1515/znc-2006-1-217.

[3] Seenivasan, A., Subhagar, S., Aravindan, R., Viruthagiri, T. 2008. Microbial production and biomedical applications of lovastatin. Indian J. Pharm. Sci. 70(6): 701–709, https://doi.org/10.4103/0250-474X.49087.

[4] Tandirerung, W.Y., Haryati, B.Z. 2022. Respon Produksi Jamur Tiram Putih (*Pleurotus ostreatus*) terhadap Penggunaan Limbah Daun Kakao Sebagai Media Tumbuh dan ZPT Air Kelapa. AgroSainT. 12(1): 48–53.

[5] Chaurasia, A., Biswas, S.K., Husain, A., Kumar, S. 2020. Investigations of growth hormone on growth parameter and biological efficacy of Pleurotus florida (Mont.) Singer. IEEE-SEM. 8(9): 51–61.

[6] Ambhure, S.D., Patil, V.S., Shewale, S.A., Nakhate, P.R., Barge, M.S. 2021. Effect of growth regulators on yield of oyster mushroom (*Pleurotus sajor-caju*). Pharm. Inno. Int. J. 10(1): 403–405.

[7] Aryantha, I.N.P., Putra, W. 2014. Effect of Magnetic Field Induction to Lovastatin Production in Fruiting Body and Mycelium of Oyster Mushroom (*Pleurotus ostreatus*). Proceeding Indonesian Student Scientific Conference "Towards Sustainability and Quality of Life". Wageningen. Netherlands. pp. 6–14.

[8] Gulyamova, T.G., Ruzieva, D.M., Nasmetova, S.M., Sattarova, R.S., Lobanova, K.V., Abdulmyanova, L.A., *et al*. 2013. Lovastatin production by *Aspergillus terreus* in solid state and submerged fermentations. Int. J. Eng. Sci. Technol. 5(3): 19–24, http://dx.doi.org/10.4314/ijest.v5i3.2.

[9] Livak, K.J., Schmittegen, T.D. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta Ct}$ Method. Methods. 25(4): 402–408, https://doi.org/10.1006/meth.2001.1262.

[10] Shen, X.T., Mo, X.H., Zhu, L.P., Tan, L.L., Du, F.Y., Wang, Q.W., *et al*. 2019. Unusual and Highly Bioactive Sesterterpenes Synthesized by *Pleurotus ostreatus* during Coculture with *Trametes Robiniophila* Murr. Appl. Environ. Microbiol. 85(14): 00293-19, https://doi.org/10.1128/AEM.00293-19.

[11] Castanera, R., López-Varas, L., Pisabarro, A.G., Ramírez, L. 2015. Validation of Reference Genes for Transcriptional Analyses in *Pleurotus ostreatus* by Using Reverse Transcription-Quantitative PCR. Appl. Environ. Microbiol. 81: 4120–4129, http://dx.doi.org/10.1128/AEM.00402-15.

[12] Barrios-González, J., Pérez-Sánchez, A., Bibián, M.E. 2020. New knowledge about the biosynthesis of lovastatin and its production by fermentation of *Aspergillus terreus*. Appl. Microbiol. Biotechnol. 104: 8979–8998, https://doi.org/10.1007/s00253-020-10871-x.

[13] Raina, S., De Vizio, D., Palonen, E.K., Odell, M., Brandt, A.M., Soini, J.T., *et al*. 2012. Is quorum sensing involved in lovastatin production in the filamentous fungus *Aspergillus terreus*? Proc. Biochem. 47(5): 843–852, https://doi.org/10.1016/j.procbio.2012.02.021.

[14] Sorrentino, F., Roy, I., Keshavarz, T. 2010. Impact of linoleic acid supplementation on lovastatin production in *Aspergillus terreus* cultures. Appl. Microbiol. Biotechnol. 88: 65–73, https://doi.org/10.1007/s00253-010-2722-0.

[15] Berger, R.G., Bordewick, S., Krahe, N.K., Ersoy, E. 2022. Mycelium vs. Fruiting Bodies of Edible Fungi—A Comparison of Metabolites. Microorganisms. 10(7): 1379, https://doi.org/10.3390/microorganisms10071379.

[16] Chen, S.Y., Ho, K.J., Hsieh, Y.J., Wang, L.T., Mau, J.L. 2012. Contents of lovastatin, γ-aminobutyric acid and ergothioneine in mushroom fruiting bodies and mycelia. LWT. 47(2): 274–278, https://doi.org/10.1016/j.lwt.2012.01.019.

[17] Gong, Q., Li, Z., Wang, L., Dai, T., Kang, Q., Niu, D. 2019. Exogenous of Indole-3-Acetic Acid Application Alleviates Copper Toxicity in Spinach Seedlings by Enhancing Antioxidant Systems and

Nitrogen Metabolism. Toxics. 8(1): 1, https://doi.org/10.3390/toxics8010001.

[18] Agboola, D.A., Ogunyale, O.G., Fawibe, O.O., Ajiboye, A.A. 2014. A Review of Plant Growth Substances: Their Forms, Structures, Synthesis, and Functions. J. Adv. Lab. Res. Biol. 5(4): 152–168.

[19] Prakash, S., Mahajan, G., Sharma, N., Sardana, V. 2015. Enhancing Grain Yield and Nitrogen-Use Efficiency in Rice Through Foliarly Applied Gibberellic Acid in Dry-Direct-Seeded Rice. J. Crop Improvement. 29(1): 65–81, https://doi.org/10.1080/15427528.2014.976693.

[20] Yu, X.J., Sun, J., Sun, Y.Q., Zheng, J.Y., Wang, Z. 2016. Metabolomics analysis of phytohormone gibberellin improving lipid and DHA accumulation in *Aurantiochytrium* Sp. Biochem. Eng. J. 112: 258–268, https://doi.org/10.1016/j.bej.2016.05.002.

[21] Francois, J.M., Alkim, C., Morin, N. 2020. Engineering microbial pathways for production of bio-based chemicals from lignocellulosic sugars: current status and perspectives. Biotechnol. Biofuel. Bioprod. 13: 118, https://doi.org/10.1186/s13068-020-01744-6.

[22] Mulder, K.C.L., Mulinari, F., Franco, O.L., Soares, M.S.F., Magalhães, B.S., Parachin, N.S. 2015. Lovastatin production: From molecular basis to industrial process optimization. Biotechnol. Adv. 33(6 Part 1): 648–665, https://doi.org/10.1016/j.biotechadv.2015.04.001.

[23] Khalili, Z., Jalili, H., Noroozi, M., Amrane, A. 2019. Effect of linoleic acid and methyl jasmonate on astaxanthin content of *Scenedesmus acutus* and *Chlorella sorokiniana* under heterotrophic cultivation and salt shock conditions. J. Appl. Phycol. 31: 2811–2822, https://doi.org/10.1007/s10811-019-01782-0.

[24] Porcel, E.R., López, J.L.C., Ferrón, M.A.V., Pérez, J.A.S., Sánchez, J.L.G., Chisti, Y. 2006. Effects of the sporulation conditions on the lovastatin production by *Aspergillus terreus*. Bioprocess. Biosyst. Eng. 29(1): 1–5, https://doi.org/10.1007/s00449-006-0048-1.

[25] Lv, Z.Y., Sun, W.J., Jiang, R., Chen, J.F., Ying, X., Zhang, L., *et al*. 2021. Phytohormones jasmonic acid, salicylic acid, gibberellins, and abscisic acid are key mediators of plant secondary metabolites. World J. Tradit. Chin. Med. 7(3): 307–325, http://dx.doi.org/10.4103/wjtcm.wjtcm_20_21.