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PRODUCTION OF LOVASTATIN AND SULOCHRIN BY *Aspergillus terreus* USING SOLID STATE FERMENTATION

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Abstract

Lovastatin is an anti-cholesterol agent that was produced by *Aspergillus terreus* using solid state fermentation (SSF). During this fermentation process, sulochrin is also produced as an unwanted co-metabolite. However, our previous result showed that sulochrin had potential as antidiabetes because it is an inhibitor agent of α-glucosidase. In this paper, we reported our observation on lovastatin and sulochrin production pattern in relation with inhibitor α-glucosidase activity during eleven days fermentation of *A. terreus* koji (SSF) ethyl acetate extract. Koji obtained from solid state fermentation with rice as the substrate and incubated at room temperature, sample is taken daily for eleven day (D-1 to D-11). Lovastatin and sulochrin production was measured by Liquid Chromatography- Mass Spectrometer based on their molecular weight m/z 404.5 and 332.3 respectively. The inibitory activity is measured by inhibition model of koji extract against α-glucosidase (EC 3.2.1.20) from *Saccharomyces cereviceae*. The results show that lovastatin production was started on the day 2 (0.04 mg/g) and achieving the optimal production on day 7 (11.46 mg/g), while sulochrin production was started on day 4 (0.60 mg/g) and keep produced until the end of fermentation period at Day 11 (3.11 mg/g). Koji extract was started to show inhibitory to α-glucosidase activity on Day 5 (IC_{50}= 23.34 µg/mL) and keep showed activity until Day 11 (IC_{50}=3.33 µg/mL). These results suggest that inhibitory activity of koji extract to α-glucosidase activity have relation with sulochrin biosynthesis production.

Keywords: α-glucosidase inhibitor, Aspergillus terreus, lovastatin, SSF, sulochrin

1. Introduction

Lovastatin (Mevinolin, Monocolin K, and Mevacor™) is potent drug for lowering blood cholesterol. Lovastatin act as competitively inhibition to the enzyme 3-hidroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) with catalyzes the rate limiting step of cholesterol biosynthesis [1]. In the recent years, lovastatin has also been reported as a potential therapeutic agent for the treatment of various types of tumors because it suppresses tumor growth in vivo by inhibiting the synthesis of non-sterol isoprenoid compounds [2]. Lovastatin is produced as a secondary metabolite by the fungi Penicillium sp, *Monascus ruber*, and *Aspergillus terreus* [3].

A. terreus is widely used lovastatin producer of the industrial importance [4], which is extensively excreted from fungal cells into the medium in the form of β–hydroxy acid-mevinolinicacid [5]. As a kind of secondary metabolite of fungi, lovastatin is an intracellular product and mostly accumulated in mycelia. Submerged fermentation processes for large-scale lovastatin production have been developed using *A. terreus* [3,5,6]. In submerged fermentation, its yield is proportional to the amount of biomass, with the high cell density causing the increase of the fermentation broth viscosity and the difficulty in stirring and oxygen mass transfer; an alternative strategy to produce lovastatin is by solid state fermentation (SSF) [7].

In recent years, reseacher have show an increasing interest in solid state fermentation (SSF) as potential alternative of submerged fermentation, because it uses economical substrate, requires fewer processing and down-streaming stages, utilizes lesser power and generates lesser effluent [8]. Moreover, SSF has higher product yield and offers better product stability [2]. Except the low substrate cost and low energy consumption, the SSF process can offer a good environment for fungi to grow, therefore high mycelia density and high lovastatin production can be expected [2,7,9].

Nevertheless, such a microorganism of the rich secondary metabolism as *A. terreus* is also capable of

biosynthesis of other compound, such as sulochrin [10]. This compound is also a product of the polyketide synthase (PKS) pathway [11] and, as mevinolinic acid, is formed from malonyl-CoA and acetyl-CoA [4]. For the purpose of lovastatin production, sulochrin is considered as an unwanted co-metabolite due to the toxicities associated with the contaminants and/or due to difficulties in removing the contaminants during downstream processing [10].

In previous result, sulochrin isolated from the ethyl acetate extract of *A. terreus* by SSF using rice as substrate (koji), showed potential as α-glucosidase inhibitor and demonstrated depressed postprandial blood glucose level in mice [12]. Hence, *A. terreus* not only could produce anti cholesterol agent but also have possibility to produce anti diabetes. Therefore, the aim of this work was to do observation on lovastatin and sulochrin production pattern in relation with inhibitory activity to α-glucosidase during seven days fermentation of *A. terreus* koji (SSF) ethyl acetate extract.

2. Methods

Microorganism and growth condition. A wild-type strain *A. terreus* is a collection of Research Center for Chemisrty-Indonesian Institute of Sciences, was used in the present study. It was grown on maintained media slant containing: yeast extract (0.4%), malt (1%), dextrose (0.4%) and agar (2%), spores were collected after 7 days at 28 ^{o}C.

Solid state fermentation. Solid substrate (1 kg rice) washed twice with water, drained, and autoclaved at 121 ^{o}C for 15 min with addition of water (1:1). After cooling, media was inoculated with 50 ml (5%) of *A. terreus* culture grow on sporulation medium [13]. Media was thoroughly mixed, placed in sterile alummunium tray (10x10 cm) and incubated for 11 day at 28 °C. Every day, one tray was harvested to analyse lovastatin and sulochrin content, inhibition assay for α-glucosidase activity, reduced sugar were assayed by Somogyi Nelson (DNS) method, and organic nitrogen was determinant by Kjedahl method.

Inhibition assay for α-glucosidase activity. The reaction mixture consisting 250 µL of 20 mM *p*-nitorpehenyl α-D-glucopyranoside (Sigma Chemical Co), 495 µL of 100 mM phosphate buffer (pH 7.0) adding to flask contain 5 µL of sample dissolved in DMSO at various concentrations (3.125 to 25 µg/mL). The reaction mixture was pre-incubated for 5 min at 30 °C, the reaction was start by adding 250 µL α-Glucosidase (0.075 unit) (EC 3.2.1.20 from Wako Pure Chemical Industry) incubation was continued for 15 min. The reaction stopped by adding 2 mL 0f 0.1 M Na_2CO_3. Enzymatic activity was quantified by measuring absorbance at 400 nm. IC_{50} value was defined as the concentration of α-glucosidase inhibitor that inhibited 50% of α-glucosidase activity.

Extraction method. Fermented sample was extracted by 300 mL of ethyl acetate in 250 mL Erlenmeyer flask, shaked at 150 rpm for 1 day. The etyl acetate extract was concentrated *in vacuo* to give brown pasta.

Analytical methods. The concentration of lovastatin and sulochrin was determined with Liquid Chromatography-Mass Spectrometer (Mariner Biospectrometry), system ESI (Electrospray Ionisation), positive ion mode, using Supelco C18 column (150 mm x 2 mm 1.d), acetonitril:water (95:5) was used as mobile phase, flow rate 1 mL/min.

3. Results and Discussion

The main composition of rice is used as substrate in SSF of *A. terreus* is starch. Its must be hydrolyzed into glucose to acetate, which is one of the compounds involved in biosynthesis of lovastatin. Although, *A. terreus* can produce amylase to hydrolize starch, in the beginning of the SSF process, there is not enough amylase secreted by cells. The amount of reduced sugar increased in the first three days due to the hydrolysis of starch by amylase which is secreted through the rapid growth of fungi [7]. After five days the reduced sugar was kept almost constant because of the dynamic balance between glucose formation and consumption (Fig. 1). Similiar patern also occur for protein content in the substrate after fermentation processed N content was increased as metabolism processed during fermentation of *A. terreus*.

Our experimental results showed that sulochrin in ethyl acetate extract appeared at 1.7 min in a liquid chromatography, earlier than lovastatin which appeared

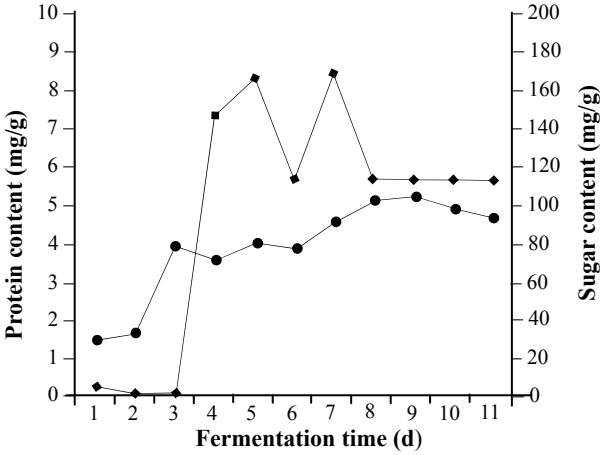

Figure 1. Time Courses of Reduced Sugar (♦) and Protein Content (●) during SSF Process

at 2.4 min (Fig. 2) with their molecular weight *m/z* 333.13 $[M+H]^+$, with *m/z* 405.17 $[M+H]^+$ respectively (Fig. 3). In Fig. 4, the evolution curved for lovastatin and sulochrin production in SSF were presented. The formation of lovastatin was start earlier on Day 2 (0.04 mg/g), which explained that although lovastatin is a kind of secondary metabolite, its accumulation in mycelia seems growth related, which is different with the phenomena in submerged fermentation [7]. The maximum lovastatin yield was achieved on day 7 (11.46 mg/g), after that, the lovastatin yield was decreasing slightly. While sulochrin production was started on day 4 (0.60 mg/g) and keep produced until the end of fermentation period at Day 11 (3.11 mg/g).

In our previous study on sulochrin isolation, we found that sulochrin showed α-glucosidase inhibitory activity not only at *in vitro* assay but also at *in vivo* experiment as measured in plasma glucose levels after sucrose administered to mice [12,13]. This result is similar with other published results which showed that several α-glucosidase inhibitors have been isolated from the fermentation broth and solid of certain microorganisms,

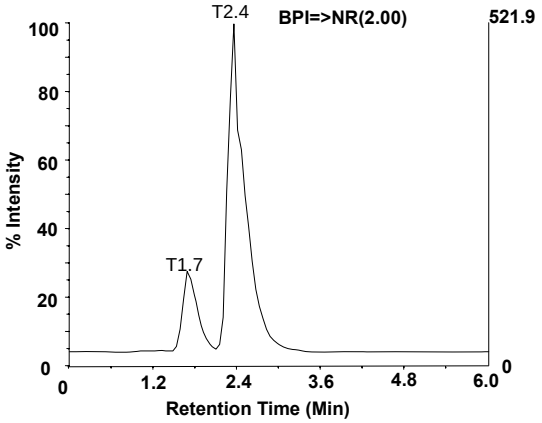

Figure 2. The Chromatogram Sulochrin (Rt; 1.7) and Lovastatin (Rt;2.4) Standar

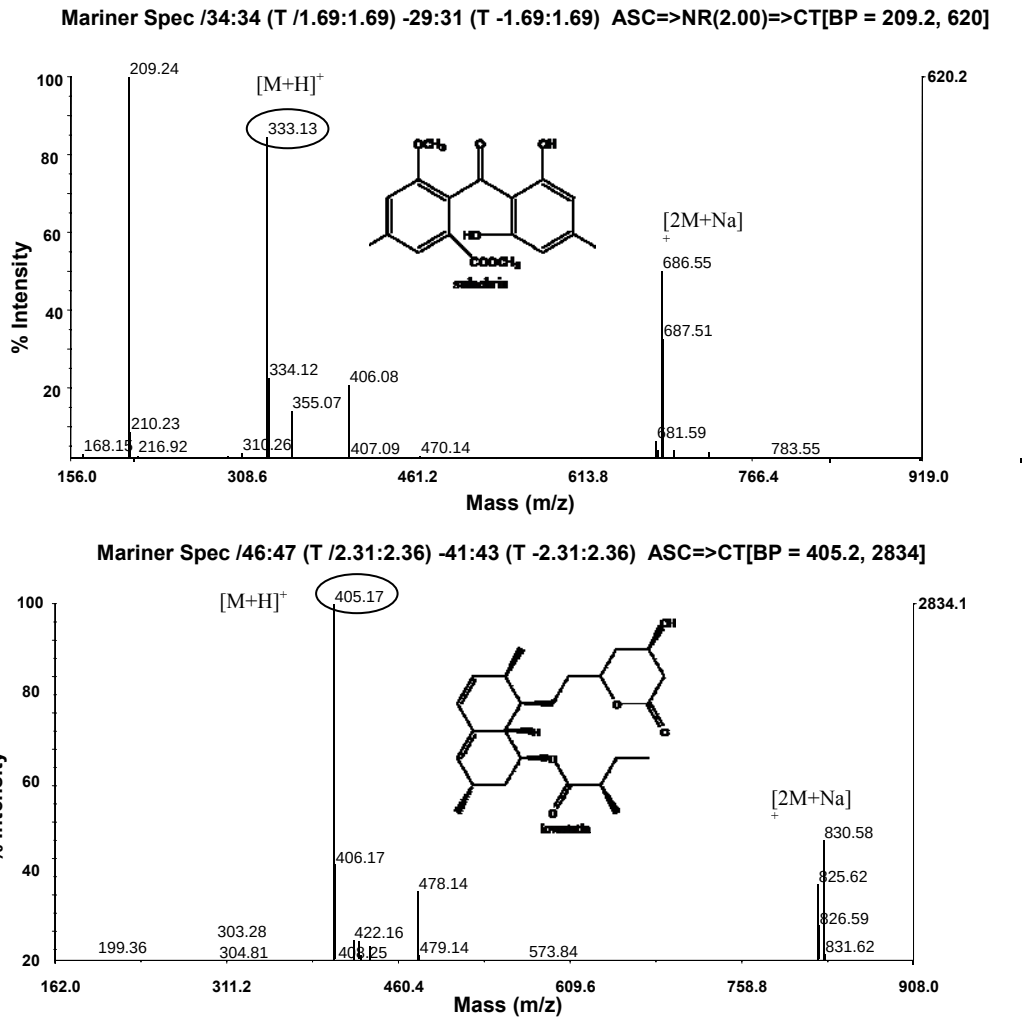

Figure 3. The Mass Spectrum of Sulochrin and Lovastatin

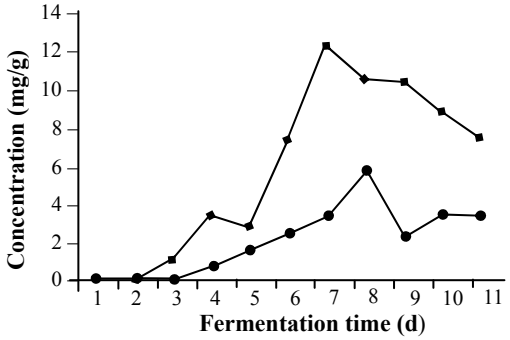

Figure 4. Time Course of Lovastatin (♦) and Sulochrin (•) Production during *A. terreus* SSF Process

such as validamycin A from growth of *Streptomyces hygroscopicus*, Acarbose isolated from fermentation broth of *Actinoplanes* spp., reported traditionally fermented soybean product (Touchi) was found to possess strong α-glucosidase inhibitory activity [14,15]. Although, screening α-glucosidase inhibitory compound from solid state fermentation with rice as substrate have not reported yet.

The results of α-glucosidase inhibitory activity assay during the process of *A. terreus* with rice (koji) presented in Tabel 1. Koji extract was started to show inhibitory to α-glucosidase activity on day 5 (IC_{50}= 23.34 µg/mL), which sulochrin produced was 1.46 mg/g. Activity of α-glucosidase inhibitory increasing rapidly during the first 7 days of fermentation process, this phenomenon might be due to the increase of secondary metabolite production during fermentation. However, during the end of fermentation process, the activities are varied, this might be due to the present of metabolite(s) other than sulochrin that also responsible for inhibitory activity of α-glucosidase.

Although, it is suggest that the metabolites might still have correlation with sulochriin biosynthetic pathway. During lovastatin fermentation process, this compound is present at polyketide pathway that originate from malonyl-CoA and acetyl-Co-A, which as same as sulochrin biosynthesis pathway [4]. Sulochrin may be further modified to form geodin ($C_{17}H_{12}Cl_2O_7$; *m/z* 399.183) [11] this suggestion is supported with the LC-MS result (*m/z* $[M+CH_3CN+H]^+$ = [M+42] = 441.95). Geodin is known to have ability to enhance (stimulate) glucose uptake by rat adipocytes and sulochrin is the precursor of geodin [16]. However, the present of other inhibitor compound(s) that has no relation with sulochrin production is still possible, since as mention earlier other α-glucosidase inhibitors by other microbes have been reported [14,15].

4. Conclusion

The maximum yield of lovastatin was achieved on day 7 (11.40 mg/g), while the maximum sulochrin was achieved on day 8 (5.26 mg/g) at SSF using rice as a substrate of *A. terreus* α-glucosidase inhibitor activity is assumed to relate to the biosynthesis sulochrin. Therefore, SSF of *A. terreus* is a potential system to be used for production of anti-cholesterol and/or antidiabetes compounds.

References

[1] A.W. Alberts, J. Chen, G. Kuron, V. Hunt, J. Huff, C. Hoffman, Proc. Natl. Acad. Sci. 77 (1980) 3957.
[2] H.R. Valera, J. Gomes, S. Lakshmi, R. Gururaja, S. Suryanarayan, D. Kumar, Enzyme. Microb. Technol. 37/5 (2005) 521.
[3] H. Hajjaj, N. Peter, D. Philippe, App. Environ. Microbiol. 67 (200) 2596.
[4] M. Bizukojc, S. Ledakowicz, J. Biotechol. 132/4 (2007) 453.
[5] J.L. Casas Lopez, J.A. Sanches Perez, J.M. Fernandez Sevilla, F.G. Acien Fernandez, E. Molina Grima, Y. Christy, Enzyme. Microb. Technol. 33/8 (2003) 270.
[6] M.S. Kumar, S.K. Jana, V. Senthil, S. Shashanka, S.V. Kumar, A.K. Sadhukhan, Process Biochem. 36 (2000) 363.
[7] P.L. Wei, Z.N. Xu, P.L. Cen, J. Zhejiang Univ. Sci A. 8/9 (2007) 1521.
[8] D. Kumar, V.K. Jain, G. Shanker, A. Srivastava, Process Biochem. 38 (2003) 1725.
[9] B.J. Xu, Q.J. Wang, X.Q. Jia, C.K. Sung, Biotechnol. Bioprocess Eng. 10/1 (2005) 78.
[10] V.A. Vinci, T.D. Hoerner, A.D. Coffman, T.G. Schimmel, R.L. Dabora, A.C. Kirpeker, C.L. Ruby, R.W. Stieber, J. Ind. Microbiol. 8 (1991) 113.
[11] R.D. Couch, G.M. Gaucher, J. Biotech. 108 (2004) 171.
[12] R.T. Dewi, A. Darmawan, S.D.S. Banjarnahor, H. Mulyani, M. Angelina, Minarti, Proceeding of the International Conference on Pharmacy and Advanced Pharmaceutical Sciences, Yogyakarta, Indonesia, 2009. p. 44.
[13] R.T. Dewi, Y. Iskandar, M. Hanafi, L.B.S. Kardono, M. Angelina, D.I. Dewijanti, S.D.S. Banjarnahor, Pak. J. Biol. Sci. 10/18 (2007) 3131.
[14] Y.P. Zhu, L.J. Yin, Y.Q. Cheng, K. Yamaki, Y. Mori, Y.C. Su, L.T. Li, Food Chem. 109 (2008) 737.
[15] F. Hiroyuki, Y. Tomohide, Elsevier 70 (2001) 219.
[16] S. Sato, N. Okusa, A. Ogawa, T. Ikenoue, T. Seki, T. Tsuji, J. Antibiot. 58 (2005) 583.