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Research Article

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Endophytic fungi isolated from Sambiloto (*Andrographis paniculata* Nees) as a source of fungal lipid production

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ABSTRACT

Endophytic fungi isolated from sambiloto (Andographis paniculata), were investigated for fungal lipid production as a biodiesel precursors. The fungi Aspergillus sp1 (SbD3), Aspergillus sp2 (SbD5), Aspergillus sp3 (SbD6), and Penicillium sp (SbD4) were cultured in flasks with cultivated in Potato Dextrose Broth (PDB) medium for 8 days in static. Dried fungal biomass (30 g) were subjected to addition of each solvent in chloroform, methanol, water (1:2:1 (v/v/v). The reaction mixture was left to reflux for 24 h. After that period, the reaction mixture was filtered and concentrated in a rotatory evaporator. The lipid compounds were then extracted by liquid-liquid extraction using n-hexane, and concentrated in a rotatory evaporator. The fungal lipid were determined on the basis of spectroscopic analysis (FTIR and ^{1}H-NMR). In conclusion, this work revealed the possibility of using the promising Aspergillus sp2 (SbD5) in biodiesel production.

Keywords: endophytic fungi, fungal lipid, *Andrographis paniculata*

INTRODUCTION

Biodiesel is a product obtained from transesterification of triglycerides or esterification of fatty acids with mono-alcohols [1]. Some of the microorganisms such as endophytic fungi are reported can accumulate significant amounts of lipids [2]. Microorganisms metabolically transform the external carbon into carbohydrate or hydrocarbon and then to lipids. Lipids are considered to be important storage compounds in the form of triacylglycerols (TAG) and esters. If the lipid content in the cell exceeds 20%, then microorganism can be called as oleaginous microorganism [3].

In this concern, fungal lipids can represent a valuable alternative feedstock for biodiesel production, and a potential solution for a bio-based economy. Recently, the development of processes to produce single cell oil (SCO) by using oleaginous microorganisms has triggered significant attention. These organisms accumulate lipids, mostly in the form of triacylglycerols. The occurrence of TAG as reserve compounds is widespread among all eukaryotic organisms such as fungi, plants and animals, where it was rarely described in bacteria [4].

EXPERIMENTAL SECTION

Source of endophytic fungi

Aspergillus sp1 (SbD3), *Aspergillus* sp2 (SbD5), *Aspergillus* sp3 (SbD6), and *Penicillium* sp (SbD4) of sambiloto (*Andographis paniculata*), obtained from stocks fungus stored in Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University. The sambiloto were collected on January 2014 from the Indralaya, Ogan Ilir, South Sumatra.

Preparation of Inoculum

Each of these endophytic fungal from stocks fungus were inoculated into Potato dextrose Agar (PDA) medium. Furthermore, each culture was incubated at room temperature for 2 x 24 hours. Each of fungal spores inoculated into 1 L potato dextrose broth (PDB) medium, then homogenized and spore population was calculated using the Counting Chamber. One milliliter of fungal culture containing 5×10^5 number of spores was inoculated in 100 mL culture medium in 250 mL conical flasks [5; 6] The inoculum from four endophytic fungus in the form of a suspension of spores is ready for use in the culture of endophytic fungi.

Cultivation of endophytic fungi

Potato dextrose broth (PDB) medium containing 300 ml of medium for the cultivation of endophytic fungi was placed into 30 flasks (1 L each). Fungal suspension was inoculated under sterile conditions to each 300 mL PDB medium (ratio 1:10). The cultures were incubated for eight days in static conditions at room temperature [7].

Production of biomass

After completion of incubation to harvest the mycelium from culture medium were passed through a filter paper (Whatman No.1) and the harvested mycelium was washed several times. The mycelium were dried in an oven at 60^oC until constant weight was achieved. Furthermore, each the biomass dry weight (BDW) were weighed (Kumar dan Banerjee, 2013).

Extraction of fungal lipid

Furthermore, the dried biomass was ground and extracted with $CHCl_3$ and MeOH with a ratio of 2: 1 (v/v). The lipid extract was washed with 150 ml of NaCl 1% and continued with the distillate water 150 ml. The $CHCl_3$ layer was filtered using Whatman No. 1 and placed into a dry container (w1). Evaporation is done until all the solvent evaporates. The residue is heated at a temperature of 104^oC for 30 minutes. The weigh of the container recorded w2. Lipid content is calculated by subtracting from the w1 and w2 expressed as % BDW [8].

Identification of lipid fungal

The FTIR was analysed by Shimadzu-Spectrum One. Analyses by NMR spectroscopy were performed on Agilent DD2 spectrometer operating at 500 MHz (^1H). The lipid was then dissolved in 99.9% $CDCl_3$ (MERCK).

RESULTS AND DISCUSSION

Four endophytic fungi including *Aspergillus* sp1 (SbD3), *Aspergillus* sp2 (SbD5), *Aspergillus* sp3 (SbD6), and *Penicillium* sp (SbD4) of sambiloto (*Andographis paniculata*), were cultured in 9 L of PDB medium (30 bottles (1 L) containing 300 mL PDB medium per bottle) for 8 days at room temperature. Brief procedures of fungal lipid production is showed in Figure 1. The fungal biomass and lipid productions are shown in Table 1.

Table 1. Fungal biomass and lipid production in 9 L of PDB medium

Strain	biomass dry production (g)	Biomass dry weight (gL^{-1})	lipid production (g)	%Total lipids to biomass dry weight (%)
Aspergillus sp1 (SbD3)	33.6	3.73	2.1	6.25
Penicillium sp (SbD4)	51.8	5.76	1.2	2.32
Aspergillus sp2 (SbD5)	36.3	4.03	5.4	14.87
Aspergillus sp3 (SbD6)	26.1	2.90	1.8	6.90

The results demonstrated that *Penicillium sp (SbD4)* showed the highest biomass production by the biomass dry weight (BDW) of 5.76 g L^{-1} and *Aspergillus sp3 (SbD6)* showed the lowest biomass production by BDW of 2.90 g L^{-1}. The total lipids dry weight varied among different strains and ranged from 2.32% to 14.87% of biomass dry production. *Aspergillus sp2 (SbD5)* showed the highest fungal lipid by BCW of 14.87% and 4.03 gL^{-1} respectively.

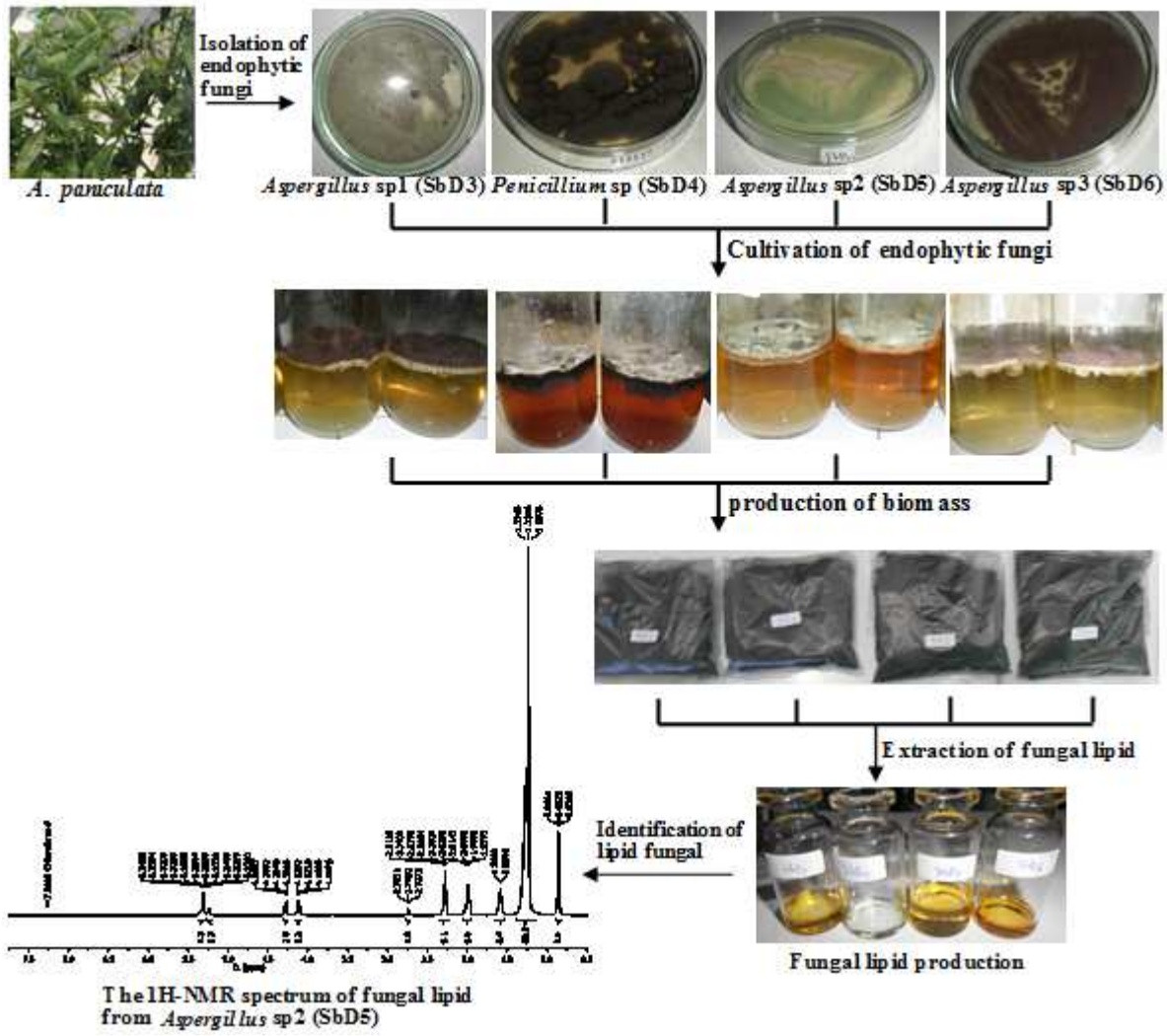

Figure 1. Brief procedures of fungal lipid production

The IR spectrum of fungal lipid exhibit the characteristics of triacylglycerol. This is indicated by the presence of C-H aliphatic (2854.6-2924.1 cm^{-1}), carbonyl ester (1712.8 cm^{-1}), and C-O ester (1172.7 cm^{-1}).

The fungal lipid were subjected to ^{1}H NMR analysis, allowing for their characterization. The analysis revealed several signals indicating the proton characteristic of the triacylglycerols. Three signals seen at δ 4.26 ppm (2H, d), 4.12 ppm (2H, d) and 5.24 ppm (1H, m) is related to the presence of methylene and methyne proton from the triacylglycerols. Three triplet signals at δ 0.84 (3H, t), 0.84 (3H, t), and 0.86 ppm (3H, t) indicates the presence of the terminal methyl group of triacylglycerols. The signals at 5.25-5.50 ppm (4H, m) indicates that the fungal lipid has five vinyl protons, which confirms the presence of unsaturated carbon in the long chain of the triacylglycerols. Furthermore, the signals that appear at 1.00 - 2.50 ppm is a signal of the methylene protons of long chain hydrocarbon. The same NMR profile was observed in all of the extracts of fungi examined.

Zheng et al. (2012) [9] reported that the strains of *Aspergillus* when cultured on glucose and xylose could produce biomass with a dry weight 4.6-7.2 gL^{-1}, and the lipid contents varied among different strains of Aspergillus from 8.0% to 37.4% of DCW. Elreesh and Haleem, (2013) [10] examined among 30 fungal isolates screened for lipid production using Nile-red staining assay, an isolate designated fungal sp. strain DGB1 was recorded as the highest lipid producer with lipid content up to 40% (w/w) and biomass dry 7.2 g/L after four days of incubation. Subhash and Mohan, (2011) [3] reported that *Aspergillus* sp using sabourauds dextrose broth medium and corncob waste liquor as substrates showed improvement in both biomass production 13.6 g/L and fungal lipid 23.3% was registered at 48 h.

In this study, biomass production and % total lipid to biomass dry weight were more less than the literature. The incubation period is 8 days, after this period of time the lipid yield dramatically decreased. Increment in lipid productivity was found to be dependent on the influence of biomass growth and substrate availability.

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REFERENCES

[1] S Ebrahimi; G Amini; H Younesi; GD Najafpour. *Middle-East Journal of Scientific Research*, **2012**, 11 (10), 1323-1327.
[2] Santos-Fo; C Floriscaldo; TP Fill; J Nakamur; MR Monteiro; RF Edson. *J. Microbiol Biotechnol*, **2011**, 21 (7), 728-722.
[3] CV Subhash; SV Mohan. *Bioresource Technology*, **2011**, 102, 9286-9290.
[4] GA Elreesh; DA Haleem. *European Journal of Experimental Biology*, **2014**, 4(1): 576-582.
[5] P Dey; J Banerjee; MK Maiti. *Bioresource Technology*, **2011**, 102, 5815–5823.
[6] Elfita; Munawar; Muharni; Suprayetno. *Indonesian Journal of Chemistry*, **2013**, 13(3): 209-215.
[7] Elfita; Munawar; Muharni; MA Sudrajat. *Hayati Journal of Biosciences*, **2014**, 21 (1): 15-20.
[8] SPJ Kumar; R Banerjee. *Indian Journal of Experimental Biology*, **2013**, 51, 979-983.
[9] Y Zheng; X Yu; J Zeng; S Chen. *Biotechnology for Biofuels*, **2012**, 5:50. 1-10.
[10] GA Elreesh; DAE Haleem. *African Journal of Biotechnology*, **2013**, 12(34), 5347-5353.