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Research Article

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Fermentation and thin layer chromatography characterization of natural pigment from *Aspergillus niger* isolated from corncob

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ABSTRACT

Aspergillus niger is saprophyte fungi that live in organic material such as corncob. A. niger was then isolated from corncob to obtained natural pigment from A. niger. The fermentation process was done using corncob as medium fermentation (liquid medium). The result showed that the optimum condition of fermentation of A. niger was of 8 days after cultivation with temperature was of 30^0C and agitation condition of 150 rpm. Further, characterization of natural pigment produced by using Thin Layer Chromatography (TLC) found that there are two compounds with Rf 0,61 and 0,5.

Keywords: *A. niger, fermentation, TLC characterization*

INTRODUCTION

Colour is a vital constituent and is probably one of the first characteristics perceived by the senses [1]. Synthetic colourant has been used widely in industrial process. Synthetic colourant is known as stable colourant that obtained from synthetic compound. However, synthetic colourant has negative effect for health. The use of synthetic colourant can be replaced by natural colourant or natural pigment. Natural pigment has less side effect than synthetic colourant. Moreover, it can be used as colourant as food colourant, cosmetic colourant and as colourant in pharmaceutical formulation. Natural pigment are non-toxic, non-polluting, and less hazardous. Moreover, their antioxidant and antimicrobial nature further adds to their positive effect [2].

Natural pigment can be obtained from various sources like plants, animals and microbes. Production of natural pigment from microbes can be alternative issues to obtained natural pigment. Fungi is found to be an interesting ecological source of natural pigment as several fungal species are rich in stable colourant such as anthraquinone, carbixcylic acids, pre-anthraquinone, etc.[3].

Aspergillus niger is one of fungal species that can be used to obtained natural pigment. The used of *A. niger* as source to obtained natural pigment has potentially develop. *A. niger* is saprophyte fungi that live in organic material such as corncob. Corncob is commonly regarded as a waste product in agricultural processing. Fermentation technology that used agricultural waste as medium of fermentation has been done by many researches. The fermentation process of *A. niger* to obtained natural pigment was conducted using corncob as medium of fermentation. The use of corncob as medium of fermentation is cost effective and environmental friendly. Moreover, corncob can be us directly as medium of fermentation without having to add any other minerals [4, 5].

A study about fermentation of *A. niger* using solid and liquid medium to obtained chitosan has been reported. The result was fermentation of *A. niger* in solid medium has yield 15 – 20 times larger than fermentation of *A. niger* in liquid medium [6]. A study about the use of corncob and kernel of corn as medium fermentation of *Neurospora sp* to obtained pigment has been done. The result was the growth of *Neurospora sp* in corcob medium wa higher than kernels of corn [4]. Moreover, a study about the use of corncob as medium fermentation of *Neurospora sitophila* to obtained lacasse has been report [7].

In this paper, we reported that the optimum condition of *A. niger* fermentation to obtained natural pigment using corncob as medium fermentation and TLC characteristic profile of natural pigment obtained from the isolated *A. niger*.

EXPERIMENTAL SECTION

Collecting of fungal sample

Fungal sample obtained from corncob as agricultural waste at Limau Manis Village, Padang, West Sumatra Indonesia.

Isolation and purification of *A. niger*

Fungal was isolated from corncob as agricultural waste. Fungal was then isolated into PDA medium in a Petri dish using sterile needle. Fungus incubation at a temperature of 240 - 280C for 5-7 days. Make observations after two days of incubation, in the event of contamination by other fungi, do insulation back to obtain pure cultures of fungi.

Identification of *A. niger*

Identification of A. *niger* in the Central Regional Veterinary II, Baso West Sumatra.

Fermentation of *A. niger*

Fermentation in liquid media using corn cob boiled water. A total 100 ml of water boiled corn cobs was pour into Erlenmeyer. Add glucose 3%, 0.5% CaCO3, 0.1% FeSO4, 0.2% MgSO4, ZnSO4 0.01% and 0.02% NaNO2. Adjust initial pH of t fermentation medium to 6 by addition of NaOH. Inoculation of *A. niger* into the fermentation medium as seed culture. Incubation for 72 hours at temperature 30^0C with continuous agitation conditions using rotary shaker at speed of 150 rpm [6].

A total 100 mL of fermentation medium was pour into 250 mL Erlenmeyer flask as test culture. 5 mL of seed culture incorporated into the test cultures aseptically. Incubation at temperatures of 30^0C under agitation condition using rotary shaker incubator at 150 rpm. Observes on 2, 4, 6, 8, 10 and 12 days after fermentation.

Dry cell weight and pH of medium fermentation measurement

Measurements of fungal cell dry weight on 2, 4, 6, 8, 10 and 12 days after fermentation. Separate the yeast cells from the fermentation medium using filter paper. Move the yeast cells, which exist on filter paper in a Petri dish. Dry aired for one night, then measure dry cell weight of the yeast cells.

Measurement of pH value performed on 2, 4, 6, 8, 10 and 12 days after fermentation. Measurement of pH value was conducted using pH meter pHepTester®.

Fractionation of natural pigment obtained from *A. niger*

Fractionation performed on days 2, 4, 6, 8, 10 and 12 after fermentation using ethyl acetate. Separate the ethyl acetate fraction of the fermentation medium using a funnel. Ethyl acetate was evaporate using rotary evaporator to obtained thick fraction. This was done 5 – 6 times.

Determination of natural pigment yield

Fraction that was obtained on days 2, 4, 6, 8, 10 and 12 after fermentation was then evaporated to obtain a dry fraction. Measure the weigh of dry fraction (g) compared with volume of fermentation medium (ml) multiplied by 100%. Compare the yield obtained from each day of fermentation.

Characterization of isolate compound

Thin Layer Chromatography (TLC) pattern

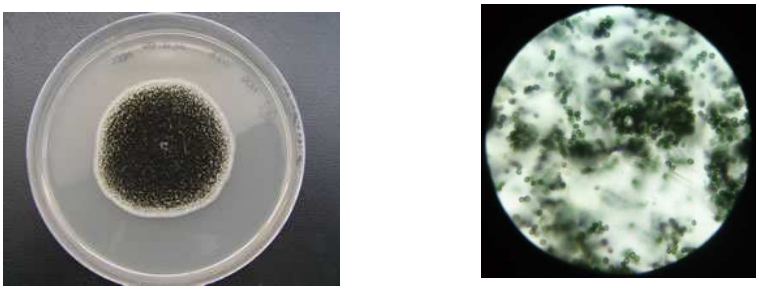
Fraction obtained from *A.niger* was then characterization using Thin Layer Chromatography

(TLC). Sample was spotted on TLC plate at stance of 0.5 cm. Enter TLC plate into a chamber that has been saturated by mobile phase, let the mobile phase elution rise to the limit. Observe the nodes under UV light with wavelength of 254 nm.

RESULTS AND DISCUSSION

Isolation and identification of *A. niger*

Samples of *A. niger* was isolated from corn cobs as agricultural waste. Isolate were then identified macroscopically and microscopically. Based on identification by Balai Veteriner Regional II showed the result was *Aspergillus niger*.



(a) **(b)**

Figure 1. Macroscopic (a) and microscopic (b) profile of the isolated *A. niger*

Table 1. Characterization of Isolated of *A. niger*

Observation	Explanation
Macroscopic	A. niger is composed of a collection of fine threads, called hyphae, have a basic white colored fur with black colored conidia [8].
Microscopic	Microscopic observation using methylene blue Lacto phenol shows conidiophores of *A. niger* are round with black colored conidia is split by age fungal cells [8].

Fermentation of *A. niger*

The optimum time for fermentation of *A. niger* in liquid medium to obtained natural pigment was 8 day after fermentation at pH value was 4,5. The yield of natural pigment was 0.1196% (w / v).

Fermentation of *A. niger* in agitation conditions was conducted using rotary shaker. This aims to accelerate the growth of the fungal so that the production of natural pigment became much more. Agitation speed is a very important factor in the fermentation process since it will increase the amount of dissolved oxygen in the fermentation medium [9]. At lower agitation speed insufficient in fermentation medium usually affect microbes growth, whereas higher agitation speeds sometimes also lowering secondary metabolites production [10]. The optimum agitation speed for fermentation *A. niger* was known by 150 rpm in producing lignin peroxidase [9].

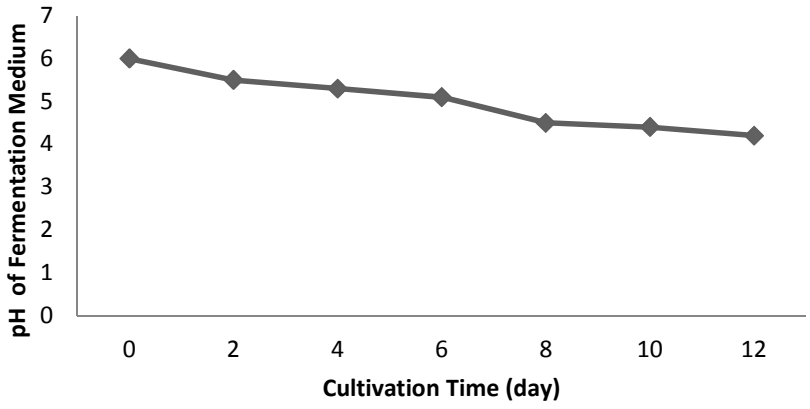


Figure 2. Relationship between cultivation time and pH of fermentation medium

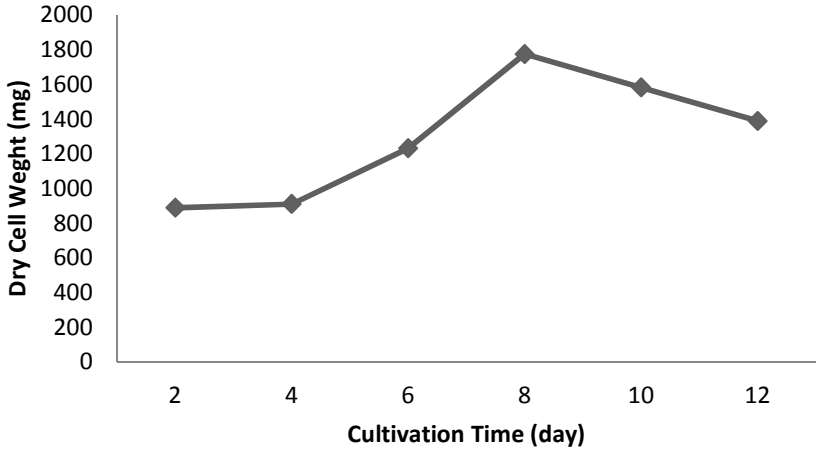


Figure 3. Relationship between cultivation time and dry cell weight of *A. niger*

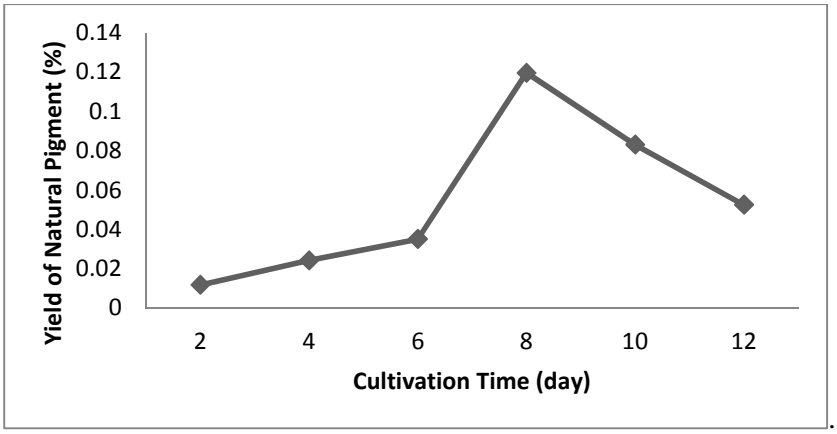


Figure 4. Relationship between cultivation time and yield of natural pigment

Based on this study, it was known that the growth of *A. niger* was influenced by pH of fermentation medium. It can be seen that from the result that growth of fungal affected at lower and high pH. High or low pH of the fermentation medium was affected by the chemical constituents present in fungal cells. In addition, a decrease in pH of the fermentation medium associated with the production of metabolites produced by fungi. An increase in dry cell weight of fungal also affected by pH of fermentation medium. At optimum pH value fungal growth would be increased so that the number of fungal cells produced even more. Moreover, the production of natural pigment obtained from by *A. niger* which gave the highest yield results at optimum pH of fermentation medium [11].

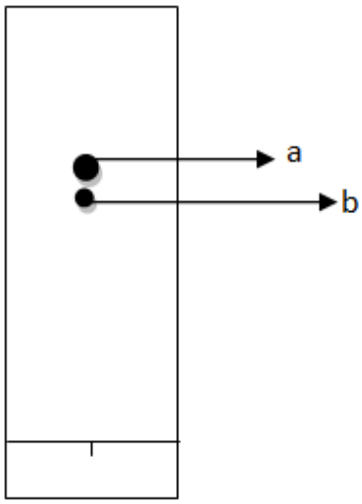
Characterization of natural pigment

Characterization of natural pigment obtained from *A. niger* was conducted using Thin Layer Chromatography (TLC). The natural pigment from *A. niger* has two nodes.

Table 2. Retention factor (Rf) natural pigment obtained from *A. niger*

Compound	Rf
a	0,61
b	0,5

a: first isolated compound produced by A. niger
b: second isolated compound produced by A. niger



Gambar 6. Thin Layer Chromatography (TLC) patterns of natural pigment from *A. niger* after 8 days cultivation

CONCLUSION

The optimum condition for fermentation *A. niger* to obtained natural pigment was 8 days after fermentation with temperature was 30^0C and shaking condition at 150 rpm. The yield of natural pigment was 0, 1196 5 (w/v). Characterization of TLC pattern has two nodes with Rf values are 0, 61 for first node and 0, 5 for second node.

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