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Effect of Variation Conditions Fermentation to Production Biomass of Endophytic Fungi *Athelia rolfsii* Strain orchid

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Abstract

Endophytic fungi are organisms that live on plant tissues without causing negative effects which the endophyte can produce secondary metabolites probably same with host plant. Research ever done before is isolate the active trace of ethyl acetate extracts of culture media endophytic fungi *Athelia rolfsii Strain orchid* from the stems of red betel shown to have cytotoxic activity against breast cancer cell line T47D with IC_{50} of 9.2 mg/mL and in vero cells with IC_{50} 109 mg/mL. This study aims to determine the effect of variations in the fermentation conditions (type of media, carbon source, nitrogen source, temperature, pH and salinity) on the production of biomass of endophytic fungi *Atheliarolfsii Strain orchid*. Misellium dry weights calculated as the product of the dry weight of the biomass and the supernatant liquid-liquid extraction results from media and ethyl acetate metabolites is calculated as the total. Results of variation in fermentation conditions, the optimum conditions obtained are: biomass production by supplementation with glucose, yeast, SDB, a temperature of 29 °C, pH 5.

Keywords: endophytic *Atheliarolfsii Strain orchid*, biomass production, fermentation conditions, total metabolites, the concentration of bioactive compound

INTRODUCTION

Plants and microbes produce secondary metabolites with various biological activities that can treat various diseases. Biotechnology techniques such as tissue culture and the role of endophytic microbes in enhancing secondary metabolites participate in the development of drugs derived from nature. Most of the chemical components derived from plants with various molecular structures used as drugs are secondary metabolites. (Radji, 2005). Development of endophytes as a source of medicine is one of the non-chemical alternatives that continue to be explored and developed. Endophytic microbes are microbes that live in plant tissues by forming colonies in these plant tissues without endangering their hosts.

The ability of endophytic microbes to produce secondary metabolites according to their host plants is a reliable opportunity to produce secondary metabolites isolated from their host plants. Endophytic fungi are compounds that live in plant tissues without causing negative effects where the endophytes produce secondary metabolites that are likely to be the same as the host plants (Kumar and Sagar, 2007). Mao et al's research (2005) proved that Cordyceps militaris which is one of the endophytic fungi in China produces cordycepin which is a compound that also has anti-cancer and antiviral properties, achieving its maximum production with a modification of carbon sources producing 0.03454% b/v cordycepin per day in glucose 42.0 grams/L. Referring to the research, it is necessary to increase productivity by varying the fermentation conditions by modifying the type of media, carbon source, nitrogen source, temperature, pH and salinity to produce the optimum active compound and isolates containing the active compound as indicators (Rollando, R.,2018).

MATERIAL AND METHOD

To optimize the culture medium in order to select the growth medium of endophytic fungi suitable for use Czapek'sDox Broth, Sabourod's Broth, Potato Dextrose Broth, Triptic Soya Broth and Nutrient Broth, while for optimization of carbon sources using glucose, starch, sucrose, fructose and maltose. For optimization of nitrogen sources using beef extract, yeast extract, peptone, ammonium chloride and sodium nitrate were added to the basal medium as much as 1% respectively. To study the effect of optimum pH for growth and production of metabolites, 0.1 N NaOH and 0.1 N HCl were used as fermentation medium adapters. NaCl with a range of 3-7 grams / liter was added to the basal medium to study the salinity of the growth of endophytic fungi and metabolite production. As a solvent for extraction and fractionation ethyl acetate, chloroform, methanol and n-hexane were used. Silica gel 60 F254 (E. Merck) is used as a stationary phase. The solvents used were methanol, n-hexane, chloroform and ethyl acetate obtained from E. Merck (Darmstat, Germany).

The equipment used in this study were glassware (porcelain funnel, erlenmeyer, TLC chamber, separating funnel, measuring cup, porcelain pipette and cup), 254 nm UV lamp and 366 nm lamp, autoclave (AC-300AE, Tiyoda Manufacturing Co. Ltd.), aseptic boxes, petri dishes, steel wire, plugs, bunsen lamps, shaking incubators, tweezers, ovens, digital cameras, Camag-5 Linomats, and a set of TLC-3 Camag Scanners.

PROCEDURE

Basal Medium

Potato Dextrose Broth (GDP) is used as a basal medium because it is most commonly used on a laboratory scale. One hundred ml of the medium poured into 250 ml of erlenmeyer was then sterilized. Each flask was inoculated with Atheliarolfsii Strain orchid culture at 7 days with 5 mm cork hole with 5 plug diameter and incubated at room temperature. Mycelium production as biomass is harvested every two days and separated from the media with Whatman Filter which has been pre-treated, washed with water. Dry it in an oven at a temperature of 550 then weigh it to a constant weight to get the dry weight of the biomass. Make a fungus growth curve between the time and dry weight obtained in every 100 ml of media. Liquid-liquid extraction of supernatant with 25 ml ethyl acetate 3 times. Separate the ethyl acetate extract from the media with a separating funnel then dry it in a fume hood to dry. Dry extracts included in an ependorf tube are calculated as the total metabolite production. Repetition is carried out as many as 5 replications (Yuniati, Y amd Rollando,R.,2018)

Effect of pH variations

To study the effect of optimum pH, a pH range of 5-7 in liquid culture is used which contains different pH levels for each erlenmeyer. One hundred ml of liquid medium was inserted into 250 ml erlenmeyer under aseptic conditions. The medium was given the desired pH by adding 0.1 N NaOH or 0.1 N. HCl. Erlenmeyer was sterilized at 1210C and 15 psi for 20 minutes. Each different erlenmeyer was inoculated with Atheliarolfsii Strain orchid culture that was 7 days old using 5 plug cork holes with a diameter of 5 mm. Mycelium production as biomass were calculated as dry weight. Each replication was carried out 5 times.

Effect of Carbon Source Variations

To study the carbon source of endophytic fungi Athelia rolfsii Strain orchid used glucose, starch, sucrose, fructose, maltose as much as 1% added to the basalt medium. Each erlenmeyer consists of different carbon sources inoculated with Athelia rolfsii Strain orchid culture that is 7 days old and 5 mm in diameter and 5 plugs using cork holes and incubated at room temperature. Mycelium production as biomass and total metabolite production were calculated as dry weight with the same treatment as in basal medium and bioactive compound levels determined by Densitometry TLC. Each replication was carried out 5 times.

Effect of Nitrogen Source Variations

To study the suitable nitrogen source in this study used nitrogen beef extract, yeast beef extract, peptone, ammonium chloride, and sodium nitrate added as much as 1% in the basalt medium. Each erlenmeyer containing a different nitrogen source was inoculated with Athelia rolfsii Strain orchid culture that was 7 days old using a 5 mm cork hole with a diameter of 5 plug then incubated at room temperature. Mycelium production as biomass and total metabolite production were calculated as dry weight with the same treatment as in basal medium and bioactive compound levels determined by Densitometry TLC. Each replication was carried out 5 times.

Effect of Culture Media Variations

In order to select a suitable growth medium, Athelia rolfsii Strain orchid from PDA solid media was grown in different culture media such as Czapek's Dox Broth, Sabourod's Broth, Potato Dextrose Broth, Triptic Soya Broth, and Nutrient Broth. Each flask was inoculated with Athelia rolfsii Strain orchid culture at 7 days with 5 mm cork holes with 5 plug diameter and incubated at room temperature. Mycelium production as biomass and total metabolite production were calculated as dry weight with the same treatment as in basal medium and bioactive compound levels determined by Densitometry TLC. Each replication was carried out 5 times.

Effect of Incubation Temperature Variations

To study the effect of the optimum temperature required for metabolite growth and production, a temperature range of 25-300C and room temperature for the basal medium was used. A total of 100 ml of the basal medium was sterilized at 121°C at 15 psi for 20 minutes. In an aseptic condition into the culture, inoculated with endophytic fungi culture Athelia rolfsii Strain orchid using 5 mm cork holes with a diameter of 5 mm and then incubated in the specified temperature range. Mycelium production as biomass and total metabolite production were calculated as dry weight with the same treatment as in basal medium and bioactive compound levels determined by Densitometry TLC. Each replication was carried out 5 times.

Effect of NaCl Concentration Variation

To study the effect of salinity on the metabolite growth and production of Athelia rolfsii Strain orchid was incubated at various concentrations at 3-7 grams/Liter with 1% carbon and nitrogen sources while all parameters were also in optimum conditions. Each erlenmeyer was inoculated with Athelia rolfsii Strain orchid culture that was 7 days old using 5 cork holes with a diameter of 5 mm and then incubated at room temperature. Mycelium production as biomass and total metabolite production were calculated as dry weight with the same treatment as in basal medium and bioactive compound levels determined by Densitometry TLC. Each replication was carried out 3 times (Yuniati Y, Alfanaar R, Rollando R,.2018).

RESULT AND DISCUSSION

Basal Medium

The maximum biomass production is on day 14 (773.3 mg / 100 mL media), while the lowest production is on day 4 with a weight of 501.2 mg / 100 mL media. The results of the statistical analysis in Appendix 2 shows that there is a significant difference in the weight of total metabolites dissolved in ethyl acetate between harvests on day 2 ie 25.8 mg / 100 mL media and day 12 ie 13.7 mg / 100 mL media.Based on the profile of TLC UV254 in Figure 1 shows that on the 10th day the profile of other metabolites expressed including bioactive compounds were observed with thicker stains, whereas on the 12th and 14th day TLC profiles did not indicate the presence of other metabolites that clearly separated, it is suspected that other metabolites may accumulate on the stain because in rough extracts it still contains other metabolites. Day 10 is determined as a fermentation period on the basis of selection that the day is considered a fungus that has entered the stationary phase because the total production of biomass and metabolites begins to decline on the 12th day, even though on the 14th day it rises. The fermentation was then carried out by variations in pH, carbon source, nitrogen source, media type, incubation temperature and salinity under fermentation conditions.

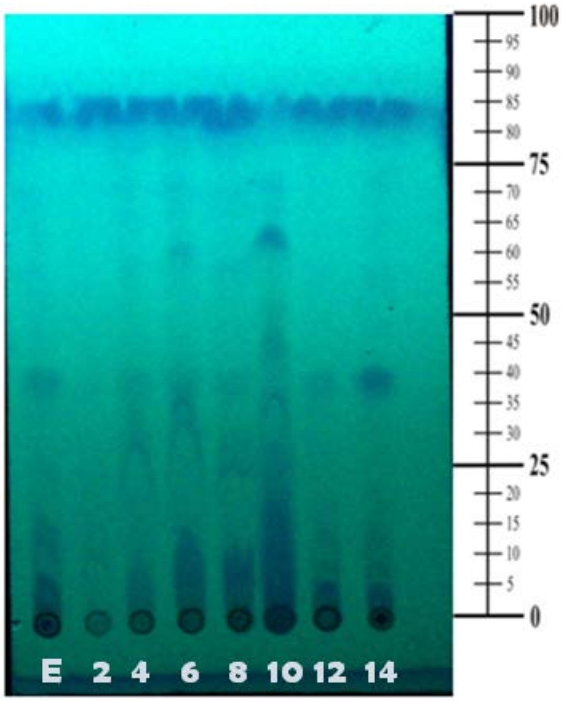

Figure 1. Profile of Total Metabolite TLC Dissolved in Ethyl Acetate at Basal Medium

Effect of pH variations

The result of fermentation shows that pH 5 is the optimal pH for maximum growth of biomass endophytic fungi cell RL1 that is 621.2 mg / 100 mL media GDP even though its weight is lower when compared to the weight of biomass in basal medium, then followed by pH 7, 6, 5.5 while pH 6.5 is pH which produces the lowest biomass with a weight of 550.3 mg / 100 mL media

The highest production of total metabolites dissolved in ethyl acetate which is 29.2 mg / 100 mL media is in pH 6. The same thing was reported by Rizk et al., 2007 that pH 6 is the best pH for the production of antimicrobial metabolites from endophytic fungi Athelia rolfsii, fungi the same also produces the production of anti-influenza metabolites at pH 6.3-6.4 (Nishihara et al., 2001).

Effect of Carbon Source Variations

From the results of data analysis showed no significant difference in the weight of biomass produced from fermentation with variations of carbon sources compared to the weight of biomass at the basal medium on the 10th day. However, the weight of total metabolites dissolved in ethyl acetate showed a significant difference in the weight of total metabolites in the basal medium. That is the carbon source of fructose, glucose, maltose, and sucrose. This indicates that for the growth of mycelia and endophytic fungi metabolites RL1 prefers simple carbon compared to complex carbon like starch.

Carbon sources are used by fungi as a source of energy for normal growth ranging from simple hexose sugars such as glucose to polysaccharides such as starch, a carbon source that is generally glucose is a structural element for fungal cells along with hydrogen, oxygen and nitrogen. Carbon metabolism is an important component in Glutamine Synthetase (GS) which is the first step in the synthesis path of various important macromolecular compounds (Kavanagh, 2005).

Effect of Nitrogen Source Variations

Fermentation results with a variety of nitrogen sources showed that yeast extract was the best nitrogen source for the production of RL1 fungi biomass of 1026.9 mg / 100 mL of GDP media followed by peptone, beef, ammonium chloride, and finally sodium nitrate was the source of nitrogen with the lowest biomass production of 832.4 mg / 100 mL of GDP media. Peptone and yeast are also the best organic nitrogen sources for cellulase enzyme production by Athelia rolfsii fungi (Padmavathi et al., 2012), whereas in the production of total metabolites dissolved in ethyl acetate, the maximum production is from media supplemented with peptone (37.2 mg / 100 mL of media) followed by beef extract (36.4 mg / 100 mL medium), ammonium chloride, sodium nitrate and yeast. The results of the calculation of total biomass and metabolite production can be seen in appendix 5. Similar things were reported in the study (Mathan et al., 2013) which stated that maximum biomass production and antimicrobial metabolites of Athelia rolfsii endophytic fungi were produced from the GDP media supplemented with nitrogen sources yeast extract with a weight of 56 mg / 25 mL medium and the lowest production of biomass is from the source of nitrogen sodium nitrate (30 mg / 25 mL media).

Effect of Media Type Variations

Sabourod Dextrose Broth (SDB) is the best medium for the production of endophytic fungi biomass RL1 with the results of the constant weight of 848.3 mg dry mycelium in 100 mL media. SDB is a liquid peptone medium enriched with dextrose carbon sources (Atlas, 2010). In the variation of fermentation conditions with nitrogen sources, peptone produces the second highest biomass after yeast extract so that it is probable that SDB media containing peptone composition in it can trigger the production of endophytic RL1 biomass fungi. The PDB media which is also a basal medium produces 757.1 mg of biomass in 100 mL of media, while Nutrient Broth media gives the lowest biomass yield of 116.7 mg in 100 mL media, presumably because NB is a medium that is generally preferred by bacteria than fungi because of the composition of Broth Nutrient which is very complex and favored by bacteria.

Effect of Fermentation Temperature Variations

Fermentation observation results with temperature variations of 250-300 C and at room temperature up and down. Fermentation temperature of 29 °C is the optimum temperature for biomass production with a weight of 769 mg / 100 mL media and the lowest biomass production is produced in fermentation with an incubation temperature of 27°C which is 458 mg / 100 mL media. Biomass production results with fermentation temperature variations showed that biomass at 29°C mycelia from endophytic fungi RL1 appeared uniform and the amount was more crowded with the media compared to mycelia in the media at other temperatures, and the color of the media that changed from the beginning of fermentation showed nutrients spent during incubation time. The temperature of 270 C in the mycelia looks smaller and does not overload the media. The color of the media also does not change because it still looks clear and yellow and does not thicken. The production of total metabolites dissolved in ethyl acetate, temperature of 26 °C is the optimum temperature needed for endophytic fungi fermentation of RL1 which produces 21.5 mg / 100 mL medium, and the lowest temperature that produces a total metabolite is 300 C with extract weight 11.4 mg / 100 mL media .

CONCLUSION

pH 5, glucose, yeast, Sabourod Dextrose Broth (SDB), at room fermentation temperature and saline concentration of 7 grams / L have an influence on the production of biomass fungi.

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