Production of Antifungal Compounds by Andalas Endophytic Bacteria (Morus macroura Miq.) Isolate ATB 10-6 at Fermentation Medium with Optimum Carbon and Organic Nitrogen Source

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Abstract. Andalas (Morus macroura Miq.) is one of the endemic plants in West Sumatera, which is known to have bioactive compounds that have the potential as antifungal. One of the effective ways to produce bioactive compounds from the plants is by utilizing endophytic bacteria through a fermentation process. One of the factor that influence fermentation is the medium. The purpose of this study was to see the effect of carbon and organic nitrogen sources on the growth of the Andalas endophytic bacteria ATB 10-6 isolate and its ability to produce antifungal compounds. The carbon sources used were glucose, lactose, maltose, sucrose, and starch. The types of organic nitrogen sources used were yeast extract, malt extract, beef extract and peptone water. Bacterial growth was observed by measuring optical density (OD), while antifungal activity was carried out by diffusion method. The result showed that a starch and yeast extract was the best carbon and organic nitrogen for the Andalas endophytic bacteria growth. These two types of nutrients are also the best sources of organic carbon and nitrogen to produce antifungal compounds.

1. Introduction

The occurrence of fungal resistance to antifungals has significantly increa. Therefore, the discovery of new antifungal bioactive compounds is important. One of the sources of antifungals that has been developed is utilizing endophytic bacteria. Endophytic bacteria are bacteria that live in plant tissue that are able to colonize without disturbing their host [1]. Endophytic bacteria that live on plants, are able to produce the same secondary metabolites as those produced by their hosts [2].

Previous research has succeeded in isolating endophytic bacteria from the Andalas plants (Morus macroura Miq.) [3-5]. Andalas is a plant that has several types of antimicrobial active compounds, such as stilbene derivatives (oxyresveratrol and andalisin A), 2-arylbenzouran derivatives, morasin M, coumarin derivatives (umbeliferon and β-resolsilaldehyde) [6]. Based on research conducted, Andalas endophytic bacteria ATB 10-6 isolate is one of the isolates that have high antifungal activity [5].

The production of active compounds by endophytic bacteria is carried out through a fermentation process. Carbon and nitrogen are important components in the fermentation medium. There are reports...
that the use of organic nitrogen sources in bacterial growth affects the pH value of the medium [7]. The fermentation medium containing 0.5% trypton and 2% beef extract produces the best production of antimicrobial compounds by Lactobacillus fermentum [8]. Furthermore, bacteria use carbon to build cell mass. There are reports that glucose is the best carbon source in producing antifungal compounds [9].

Employing a suitable production medium for the fermentative production of an antifungal is a very crucial requirement of the present. The aim of this study was to determine the effect of carbon and organic nitrogen sources on the growth of endophytic bacteria of Andalas ATB 10-6 plant isolate and its ability to produce antifungal compounds.

2. Materials and Methods
2.1 Preparation of endophytic bacterial isolates
Andalas endophytic bacteria isolate ATB 10-6 was isolated from the roots of the Andalas plant [5]. Isolates were maintained on nutrient agar at 4 °C. For this study, endophytic bacterial isolates were grown and maintained in LB-agar at 26 °C.

The fermentation starter was prepared in LB-broth. Andalas endophytic bacterial cells were inoculated in a 250 mL Erlenmeyer flask containing LB broth at 26°C for 24 hours. The culture is incubated in an incubator shaker at 250 rpm.

2.2 Carbon and organic Nitrogen Source
The optimized carbon sources in this study consisted of glucose, sucrose, lactose, maltose and starch. The carbon concentration used is 0.5%. Furthermore, the optimized organic nitrogen sources are yeast extract, beef extract, malt extract and peptone water with a concentration of 1% each.

2.3 Fermentation Conditions
The fermentation was carried out in 5 mL volumes (respectively). 0.1 g of carbon, 0.2 g of organic nitrogen and 0.1 g of NaCl are dissolved in distilled water to a volume of 20 mL. Furthermore, 2 mL of starter culture was inoculated into each fermentation medium. The fermentation culture was aliquoted into three test tubes (each with a volume of 5 mL). The same process is carried out for all organic carbon and nitrogen combinations. The cultures were incubated in an incubator shaker at a speed of 150 rpm at room temperature for 12 hours.

2.4 Analysis of Bacterial Growth
Bacterial growth was measured based on the OD value. 2 mL of fermentation medium is taken and then put into the cuvet. OD was measured using a spectrophotometer at a wavelength of 600. The absorbance values were recorded.

2.5 Analysis of Antifungal Activity
The antifungal activity was tested based on the diffusion method. the fermentation medium is centrifuged as much as 1 mL at 4000 rpm for 10 min. The supernatant was used for the antifungal activity test. The supernatant is dropped as much as 20 µL onto disc paper. Furthermore, the disc paper was placed on NA medium that had been inoculated with the test microbe (C. albicans). The cultures were incubated for 24 hours. The clear zone formed around the disc is measured using a slide.

3. Results and Discussion
A series of experiments was carried out to investigate the effect of various types of carbon and organic nitrogen sources on the growth of endophytic bacteria Andalas isolate ATB 10-6 and their ability to produce antifungal compounds. The results obtained from the experiment show that, the differences in the carbon source used have influenced the growth of endophytic bacteria andalas isolates ATB 10-6. The OD value of each culture can be seen in Table 1.
Based on the data in Table 1, it can be seen that starch and yeast extract provide good endophytic bacterial cell growth compared to other sources of carbon and organic nitrogen. There was no interaction between types of carbon and organic nitrogen in influencing the growth of endophytic bacteria isolates ATB 10-6.

Table 1. Average of Optical Density (OD) value of Andalas Endophytic Bacteria ATB 10-6 isolate were grown on a medium containing different sources of carbon and organic nitrogen

| Carbon Sources | Organic Nitrogen Sources | Malt Extract | Yeast Extract | Beef extract | Pepton Water | Carbon |
|----------------|--------------------------|--------------|---------------|--------------|--------------|--------|
| Glucose        |                          | 0.741        | 0.987         | 0.842        | 0.943        | 0.878^H |
| Lactose        |                          | 0.826        | 1.039         | 0.849        | 1.063        | 0.944^D |
| Maltose        |                          | 0.743        | 1.155         | 0.808        | 1.062        | 0.942^C |
| Sucrose        |                          | 0.746        | 1.059         | 0.751        | 0.890        | 0.861^A |
| Starch         |                          | 0.839        | 38.398        | 0.920        | 0.954        | 10.277^E |
| Organic Nitrogen | 0.779^A                  | 8.527^B      | 0.834^B       | 0.982^C      |

In this study also observed the effect of carbon and organic nitrogen sources on the ability of Andalas endophytic bacteria isolate ATB 10-6 to produce antifungal compounds. The antifungal activity is determined from the value of the formed inhibition zone diameter (Fig 1). As well as the influence of carbon and organic nitrogen sources on bacterial growth, starch and yeast extracts play a greater role in increasing the production of antifungal compounds than other sources of nutrients. The interaction between types of carbon and nitrogen has a different effect on the resulting antifungal activity (Table 2)

![Figure 1](image_url)

Table 2. Average of antifungal activity value of Andalas Endophytic Bacteria ATB 10-6 isolate were grown on a medium containing different sources of carbon and organic nitrogen

| Carbon Source | Average Diameter of Inhibition Zone (cm) | Organic Nitrogen Sources | Carbon |
|---------------|----------------------------------------|--------------------------|--------|
| Glucose       | 1.100^bc                           | 2.240^dhi                | 1.147^fhi | 1.847^ghi | 1.583^h |
| Lactose       | 1.090^ab                           | 2.117^dhi                | 1.880^fhi | 2.233^fhi | 1.830^c |
| Maltose       | 2.570^bcd                           | 2.997^ghi                | 2.270^ghi | 2.810^fhi | 2.661^e |
| Sucrose       | 1.740^bcd                           | 3.020^ghi                | 0.000^a   | 1.400^g   | 1.540^a |
| Starch        | 0.000^a                            | 3.263^hi                | 2.273^ghi | 2.803^hi | 2.084^d |
| Organic Nitrogen | 1.300^A                         | 2.727^D                 | 1.514^B   | 2.218^C   |
The results in this study contradict with the research conducted by\cite{10} showing that the best carbon source in the growth of the probiotic bacteria *Pseudomonas sp.* is glucose. The same results were also found in research conducted by\cite{11}, where glucose is the best carbon source in the growth of the *Bacillus pumilus* bacteria. Lisdiyanti et al., (2012)\cite{12} revealed that glucose in fermentation media serves as an initiation to accelerate cell division in the exponential phase. However, according to\cite{13}, the carbon source in the form of glucose can reduce the pH of the solution, so that the formation of metabolite compounds will be disrupted, and decreased antibiotic production. In certain cases, glucose as a carbon source is able to suppress enzymes involved in the biosynthesis of antibiotics, such as phenoxazinone synthetases and N-acetyl kanamycin.

The correct type of carbon and nitrogen source is essential to optimize the production of antifungal compounds. Medium containing the optimum concentration of peptone, yeast extract and NaCl concentration has been reported to increase enzyme production 1-2 times. Further research on the optimization of other fermentation factors (such as pH, aeration temperature and others) needs to be done to obtain a more efficient fermentation condition in producing antifungal compounds by endophytic bacteria andalas isolates ATB 10-6 for commercial purposes.

4. Conclusion
Fermentation medium containing starch and yeast extrack is the best condition in influencing the growth of endophytic bacteria Andalas isolate ATB10-6 and its ability to produce antifungal compounds.

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