Analysis antifungal compounds produced by endophytic bacteria from oil plant using Bioautography

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Abstract. This study was undertaken to evaluate the antifungal activities of the bioactive compounds produced by endophytic bacteria isolated from oil palm plant using TLC-Bioautography. Antifungal compounds used in this study have been extracted from potential endophytic bacteria of previous studies using ethyl acetate solvent. The antifungal compounds was tested for its activity against the pathogenic fungus Fusarium oxysporum f.sp. by using TLC-Bioautography method. The results showed that there are 3 spots on the TLC plate for samples of B11 antifungal compound with an incubation time of 54 hours and B11 with an incubation time of 24 hours. Observation of bioautographic antifungal activity test showed the presence of inhibition zones with the largest average percentage given by B11 with an incubation time of 24 hours is 11.59%. The inhibition zone provided B11 with an incubation time of 54 hours is only 11.08%.

1. Introduction

Oil palm plant diseases are generally caused by two main causes, fungus and bacteria. Fungal attacks are more common than bacterial attacks. One fungus is known to attack oil palm plants is Fusarium oxysporum f. sp. Fusarium are considered very harmful because they can infect plants [1]. The fungus becomes a pathogenic fungus that causes vascular wilt and yellow lines in oil palm plants. Wilt vessels diseases is very deadly to oil palm plants, while yellow line disease causes oil palm growth to be imperfect.

Various chemical control efforts have been made to tackle wilt vessels disease caused by the pathogen Fusarium oxysporum f.sp., but the results are not effective. One of the efforts to control fusarium wilt in oil palm which can be used as an alternative is biological control by utilizing endophytic bacteria. The ability to provide resistance to host plants by endophytic bacteria because these microbes are able to produce secondary metabolites that can inhibit the growth rate of pathogenic fungi [2].

Candrawati [3] has conducted research on the ability of extracts of active compounds of endophytic bacteria in inhibiting the growth of Fusarium oxysporum f.sp. on oil palm with the agar diffusion method. The results of this study indicate that the secondary metabolite compound B11 endophytic bacteria with an incubation time of 54 and 24 hours provide a significant inhibitory effect on Fusarium.
oxysporum f.sp. with percentages of 43.85% and 29.23%, respectively. Although this research has succeeded in showing the effectiveness of endophytic bacterial active compounds in inhibiting fungal pathogens, but the research has not been able to provide information about the characteristics of endophytic compounds produced. For this reason, further research is needed to identify endophytic bacterial active compounds from oil palm plants that have the ability to inhibit the pathogen Fusarium oxysporum f.sp.

This research uses TLC bioautography method to directly track antifungal compounds extracted from palm oil endophytic bacteria. With this method, we can find out the antifungal activity of endophytic bacteria by observing the inhibition zone around the spot of the TLC plate that has been affixed to the agar media.

2. Material and methods

2.1. Place
This research was conducted at Agromicro Laboratory, Biotechnology Center, Agency for Assessment and Aplication of Technology, Puspiptek Serpong Area, Indonesia.

2.2. Samples
The research sample used is an antifungal compound from potential endophytic bacteria produced in our earlier work. The sample was B11 with an incubation time of 24 hours and 54 hours.

2.3. Materials
The materials used in this study include pure culture of Fusarium oxysporum f.sp., Potato Dextrose Agar (PDA), silica gel, aguadest, ethyl acetate, anhydrous sodium sulfate, and n-hexane.

2.4. Detection of antifungal activities via TLC-Bioautography
The activity test of the active compound of endophytic bacteria in the form of antifungal in this study used the TLC-contact bioautography method or agar diffusion. TLC-Bioautography testing of secondary metabolite extracts from the most active endophytic bacteria of palm oil as an antifungal using activated GF-254 silica gel TLC plates. The extract was bottled on the TLC plate then eluted with hexane: ethyl acetate ratio = 1: 4. After that, the Rf value is visualized and calculated.

Stains on plate I chromatogram visualized under UV light with a wavelength of 254 nm. Plate II is placed on the surface of the PDA media that has been inoculated with the fungus F. oxysporum f.sp. Petri dishes were incubated at 28.3°C for 15 days. The fungal growth was observed daily and the formation of inhibition zones was recorded [4].

3. Results and discussion
TLC results using eluent n-hexane : ethyl acetate (1: 4) with UV light display at a wavelength of 254 nm showed three spots for each B11 bacterial active compound with incubation time of 24 hours and 54 hours. The Rf value for B11 with a 24-hour incubation time was 0.62; 0.68; and 0.78, while the Rf value for B11 with an incubation time of 54 hours is 0.17; 0.46; and 0.89.

TLC-Bioautographic observations showed the presence of inhibition zones with the largest average percentage given by B11 with a 24-hour incubation time of 11.59%. The inhibition zone provided by B11 with an incubation time of 54 hours is only 11.08%.
### Table 1. Percentage of inhibition in vitro of secondary metabolites of endophytic bacterial compounds against Fusarium oxysporum f.sp. Isolate 1 (Fo1).

| Extract Code for Antifungal Compound | Inhibitory (%) | Sample 1 | Sample 2 | Average |
|--------------------------------------|----------------|----------|----------|---------|
| B11/24                               |                | 0.10     | 23.08    | 11.59   |
| B11/54                               |                | 20.69    | 1.47     | 11.08   |
| control (+)                          |                | 23.08    | 41.18    | 32.13   |
| control (-)                          |                | 0.00     | 0.00     | 0.00    |

Tests with the TLC Bioautography method, showed that there are secondary metabolites which are antagonistic towards *F. oxysporum* f.sp. with a clear zone around the former TLC plate. The biggest inhibitory zone was shown by extracting secondary metabolites from B11 bacteria with an incubation time of 24 hours a second repeat, which was 23%. However, the percentage of inhibition zones is still smaller than the positive control inhibition zone of the second test which is 41.18% (see Figure 1). Based on Figure 1, the growth of the fungus *F. oxysporum* f.sp. in the B11 / 54 test sample the second test showed a clear zone around the third spots on the TLC plate, even though the overall percentage of inhibition was only 1.47%. This shows that the third spot contains compounds that are antagonistic to the fungus *F. oxysporum* f.sp. The antagonistic nature of *F. oxysporum* f.sp was also demonstrated by secondary metabolite compounds extracted from B11 bacteria with an incubation time of 54 hours in the presence of a clear zone at the edge and above the TLC plate. The presence of this clear zone shows that the three stains on the former plate give antagonistic properties to *F. oxysporum* f.sp. This means that the three compounds that have been successfully extracted from B11 bacteria with an incubation time of 24 hours are active compounds.

![Figure 1. Antifungal activities of active compounds using TLC-Bioautography.](image)

The results of this study also showed that negative control did not provide inhibitory zones to the growth of *F. oxysporum* f.sp. so that this could prove that the solvent used to dissolve the extract had no antifungal activity or was not antagonistic to *F. oxysporum* f.sp. The positive control used is antifungal in the form of Nystatin with a concentration of 10,000 ppm giving a large enough inhibition zone against *F. oxysporum* f.sp, this indicates that the test fungus is not yet resistant to positive control so that positive control is still feasible to use.

### 4. Conclusion

The results of TLC-Bioautography extract of antifungal compounds showed the presence of 3 spots on the chromatogram, but the results of inhibition test of the antifungal compounds by TLC-Bioautography did not provide significant inhibition.
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