Preliminary Study on Antimicrobial Activity of Fermentation Broths of Oudemansiella Mucida

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Abstract. Oscillation culture was carried out on Oudemansiella mucida, the obtained fermentation broths were extracted with ethyl acetate, n-BuOH in succession to give three sections: extract of ethyl acetate, n-BuOH and the remained fermentation broths. Their antimicrobial activities against five common phytopathogenic funguses were determined by filter paper method and solid culture medium method. The result indicated that the fermentation broths markedly inhibited five phytopathogenic funguses. The metabolites with antimicrobial activity were mainly congregated in EtOAC extract, with the minimal inhibiting concentration (MIC) for Alternaria brassicae, Alternaria longipes and Gloeosporum fructigenum being 10 mg/mL, the MIC for Fusarium graminearum and Alternaria alternata being 5mg/mL.

1. Introduction

The application of biopesticide is a considerable method for sustainable agricultural development and a significant part of the green food revolution. The replacement of chemical pesticides by biological pesticides is an inevitable trend. Microbial pesticides, which are important components of biological pesticides, have been one of the research hotspots [1]. Microbial-derived pesticides mainly search for new agricultural active lead compounds from microorganisms, and use them as templates to develop new pesticides [2].

Oudemansiella mucida, commonly known as porcelain fungus, is a basidiomycete fungus of the Physalacriaceae family and native to Europe. Recent studies have shown that Oudemansins contained in Oudemansiella mucida can significantly inhibit most aerobic saprophytic pathogens [3]. Musilek et al. previously isolated an antifungal active substance mucidin from Oudemansiella mucida, then obtained strobulurin and oudemansin [4]. In this paper, the antibacterial activity of Oudemansiella mucida was studied with plant pathogen. It is expected to find the active ingredients of bacteriostasis, and provide a theoretical basis for the application of Oudemansiella mucida in biopesticide.
2. Experiment

2.1. Reagents
Oudemansiella mucida, Alternaria longipes, Alternaria brassicae, Gloeosporum fructigenum, Fusarium graminearum, Alternaria alternate were purchased from Institute of Botany, Kunming, Yunnan. Ethyl acetate, n-butanol, acetone PDA medium were purchased from Xi'an shunda chemical reagent instrument co. LTD, China. All reagents were analytical-reagent grade.

2.2. Instrument
LS-B50L pressure steam sterilizer (Beijing Boya instrument co. LTD), YJ-875SA medical purification device (Thermo Scientific co. LTD), LRH-250A biochemical incubator (Xi'an Keyi instrument co. LTD) were applied in our experiment.

2.3. Sample pretreatment
The plant pathogenic bacteria were activated with a slanted PDA medium, and bacterial suspensions (10² to 10³ cfu/mL) were prepared with sterile physiological saline water solution. The Oudemansiella mucida strain was inoculated on a bevel-modified PDA medium, and cultured at 28 °C for 7 days, then inoculated into a 1000 mL Erlenmeyer flask containing 400 mL of liquid culture medium, shake on a rotary shaker at 28 °C for 10 days' fermentation [5].

The fermentation broth was centrifuged at 5000 r/min to obtain 25 g (dry weight) mycelium and 10 L solution. 1 L of solution was extracted and concentrated to obtain 3.6 g extract, which was then prepared to 40 mg/mL (in water) as Sample I. The remaining 9 L of solution was concentrated to 2 L, then was extracted with ethyl acetate and n-butanol respectively. The ethyl acetate extract was concentrated to 1.2 g; the n-butanol extract was concentrated to 2.3 g. They were prepared to 40 mg/mL (in acetone) as Sample II and Sample III. Finally, the raffinate was concentrated 25 g then was prepared to 40 mg/mL (in water) as sample IV .

Qualitative test for antibacterial activity: The filter paper was made into a small disc (diameter: 6 mm), dried at 160 °C for 2 h, and these filter paper were immersed into sample I, sample II, sample III, and sample IV respectively. Then, the plant pathogen was evenly spread on the PDA medium, and the above-mentioned impregnated circular filter paper was placed on the surface of the culture medium. Sterile water was used as control. The size of the inhibition zone was observed and measured after cultivate [6].

The sample II (Ethyl Acetate Extract from Fermented Broth) was diluted with acetone to a series of concentrations: 40 mg/mL, 20 mg/mL, 10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL. 1 mL of each dilution was separately injected into a sterile Petri dish and mixed uniformly with 9 mL of PDA medium. After solidification, the plant pathogen was spread on the surface of the medium and incubated. The lowest concentration of complete aseptic growth is the minimum inhibitory concentration (MIC) of sample II [9-10], with sterile water and acetone as controls.

3. Results and discussion

3.1. Qualitative test of antibacterial activity of the fermentation broth of Oudemansiella mucida
The five tested strains selected in this experiment are common and representative pathogens in plants. The results were shown in Table 1. It is generally believed that the inhibition zone is resistant between 6 and 10 mm; 10 to 11 mm is mildly sensitive; 11 to 15 mm is moderately sensitive; and 16 to 20 mm is highly sensitive [11]. The fermentation broth of Oudemansiella mucida exhibited good antibacterial activity. It indicated that the fermentation broth of Oudemansiella mucida contains active substances, which can inhibit the common plant pathogenic bacteria.
Tab. 1 Inhibitory activity of Broths of *Oudemansiella mucida*.

| samples | Alternaria longipes | Alternaria brassicae | Gloesporum fructigenum | Fusarium graminearum | Alternaria alternata |
|---------|---------------------|----------------------|------------------------|----------------------|---------------------|
| CK      | 11.36               | 12.02                | 11.79                  | 13.11                | 13.05               |

Note: CK — sterile water; The data in table show average diameter of antimicrobial circle with 3 replications (units: mm). “-” shows these samples have no effect on the phytopathogenic fungus.

3.2. Qualitative test of antibacterial activity of fermentation broth extract of *Oudemansiella mucida*

The antibacterial activity test were performed with three samples respectively (the ethyl acetate extract, the n-butanol extract and the extracted fermentation broth of *Oudemansiella mucida*). The results were shown in Table 2. The antibacterial activity of the ethyl acetate extract was significantly stronger than that of the n-butanol extract, while the raffinate had few bacteriostatic effects. Thus it was indicated that the substances with bacteriostatic activity were mainly concentrated in the ethyl acetate extract.

Tab. 2 Inhibitory activity of extracts of *Oudemansiella mucida*

| samples | Alternaria longipes | Alternaria brassicae | Gloesporum fructigenum | Fusarium graminearum | Alternaria alternata |
|---------|---------------------|----------------------|------------------------|----------------------|---------------------|
| II      | 15.39               | 15.52                | 15.81                  | 14.96                | 16.01               |
| III     | 9.58                | —                    | 8.92                   | 7.13                 | 9.03                |
| IV      | —                   | —                    | —                      | —                    | —                   |
| CK1     | —                   | —                    | —                      | —                    | —                   |
| CK2     | —                   | —                    | —                      | —                    | —                   |

Note: The concentrations of II, III, IV are 40mg/mL; CK1 — sterile water, CK2 — acetone; The data in table show average diameter of antimicrobial circle with 3 replications (units: mm); “-” shows no effect on these phytopathogenic fungus.

3.3. Determination of Minimum Inhibitory Concentration (MIC)

The results of MIC with ethyl acetate extract of fermentation broth of *Oudemansiella mucida* were shown in Table 3. The ethyl acetate extract significantly inhibited the five plant pathogens, *Alternaria longipes*, *Alternaria brassicae*, *Gloesporum fructigenum*, *Fusarium graminearum*, *Alternaria alternata* and the minimum inhibitory concentration against black spot, tobacco red star and grape anthrax. The MIC of *Alternaria longipes*, *Alternaria brassicae* and *Fusarium graminearum* was 10 mg/mL, while the minimum inhibitory concentration (MIC) of *Gloesporum fructigenum* and *Alternaria alternata* was 5 mg/mL. The results illustrated that the ethyl acetate extract of *Oudemansiella mucida* has higher antibacterial effect on *Alternaria longipes*, *Alternaria brassicae* and *Fusarium graminearum*. 

Tab. 3 Inhibitory activity of extracts of *Oudemansiella mucida*
Tab. 3 Inhibitory activity of ethyl acetate Extract of Different Concentrations

| concentration(mg/mL) | Alternaria longipes | Alternaria brassicae | Gloesporum fructigenum | Fusarium graminearum | Alternaria alternata |
|----------------------|---------------------|----------------------|-----------------------|----------------------|---------------------|
| 40                   | -                   | -                    | -                     | -                    | -                   |
| 20                   | -                   | -                    | -                     | -                    | -                   |
| 10                   | -                   | -                    | -                     | -                    | -                   |
| 5                    | +                   | +                    | +                     | -                    | -                   |
| 2.5                  | + + +               | + ++                 | + ++                  | + + +                | + + +               |
| 1.25                 | + + + +             | + + + +               | + + +                 | + + + +              | + + + +             |
| CK1                  | + + + +             | + + + +               | + + +                 | + + + +              | + + + +             |
| CK2                  | + + + +             | + + + +               | + + +                 | + + + +              | + + + +             |

Note: CK1—sterile water, CK2—acetone; The effect is average of 3 replications; “-”, “+”, “++” and “+++” show respectively of no growth, lower, strong and stronger growth.

3.4. Conclusion

The fermentation broth of *Oudemansiella mucida* had good antibacterial activity. The antibacterial active substances were mainly concentrated in the ethyl acetate extract. The n-butanol extract also exhibited certain antibacterial activity. However, the raffinate has few bacteriostatic activities. The antibacterial activity of the ethyl acetate extract was stronger than that of the n-butanol extract, indicating that the ethyl acetate extract contained high amount or high activity of secondary metabolites. The ethyl acetate extract showed significant antibacterial effect on plant pathogenic bacteria with a minimum inhibitory concentration (MIC) at 5 mg/mL. This study could provide theoretical guidance for the application of higher fungi such as *Oudemansiella mucida* as a biological pesticide. In order to clarify the antibacterial active ingredients of *Oudemansiella mucida*, it is necessary to isolate and purify the ethyl acetate extract and the n-butanol extract in further research.

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