Biological and Enzymatic activity of *Aureobasidium pullulanus* Isolated from pigeon dropping

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**ABSTRACT**

**Objective:** The aim of this study was detect the biological activity of *Aureobasidium pullulanus* isolate that isolated from pigeon dropping and its extract.

**Methods:** The extract was tested against two bacterial isolates *Escherichia coli* and *Staphylococcus aureus* and two yeast isolates *Candida albicans* and *Cryptococcus neoformans*, in addition to measure enzymatic activity of this isolate and its cytotoxicity effect against human blood solution.

**Results:** *Aureobasidium pullulanus* isolate and its crude extract revealed that inhibition activity against all tested isolates. The cytotoxicity assay of *Aureobasidium pullulanus* extract was showed there is no cytotoxic affecting human solution that using in different concentrations. The studying *Aureobasidium pullulanus* isolate also have the ability to produce two important enzymes hemolysin and lipase.

**Conclusion:** *Aureobasidium pullulanus* have the ability to produce secondary metabolites and these metabolites have power of inhibition against bacteria and yeasts also have enzymatic activity.

**INTRODUCTION**

*Aureobasidium pullulanus* is a yeast-like ascomycetes with polymorphic ability in its life cycle. It was used to produce many compounds such as cyclic peptide, which has specific antifungal activity called aureobasidin A⁴. In addition, *A. pullulanus* was used as a biocontrol agent in agriculture by means of its strong antagonistic activity against other microorganisms⁵. Furthermore, a recent study revealed that some of *A. pullulanus* strains can produce an antibacterial compound called exophilin A, in addition to high yield of liamocins and heavy oils which have possible industrial applications as surfactants⁶. The fungal capacity of hydrolyzing different organic and inorganic compounds to obtain enough nutrients and energy by production of different extracellular enzymes. Moreover, it is known that 60% of enzymes used in industrial processes are produced by a few genera of fungi ubiquitous and of worldwide distribution⁷. The objectives of our study included the evaluation of the biological activity of *A. pullulanus* extract against some pathogenic bacteria and yeast isolates with cytotoxicity assay of the fungal extract, in addition, the study aimed to determination of fungal ability to produce hemolysine and lipase *in vitro*.

**MATERIALS AND METHODS**

*Aureobasidium pullulanus* isolate which was isolated by Inaam et al.⁸ was used in this study to evaluate the
biological and enzymatic activity.

Fermentation: For fermentation test the Potato Dextrose Broth (PDB) medium was prepared, five disc of activated colonies of the fungus were inoculated within 300 ml of the fermentation medium and incubated at 25°C for 14 days.

Extraction: After incubation period the fermentation medium was centrifuged 6000 cycle/ minutes for 10 minutes and filtered on Whatman No. 1 filter paper, the pH was adjusted at 3 using 2N HCl. The filtrate was extracted with ethyl acetate (1:1 vol) using a separating funnel. The organic layer was collected in Petri dishes and dried at room temperature.

Biological activity: In vitro antibacterial and antifungal activity were determined for both A. pullulanus isolate and A. pullulanus extract against two bacterial isolates namely Escherichia coli and Staphylococcus aureus along with two isolates of yeasts, Candida albicans and Cryptococcus neoformans.

A suspension of each isolate was prepared and standardized to a turbidity equivalent to that of a 0.5 MacFarland scale (1x10⁸ cfu/ml) for bacteria and (1x10⁷ cfu/ml) for yeasts.

The test was performed according to Perez et al. by adding 10µl of each microorganism inoculums on the surface of Muller-Hinton agar (MHA) for bacterial isolates and Sabouraud dextrose agar (SDA) for yeast isolates, then spread with sterile L-shape glass rod, after drying, 9mm diameter pore was made in the center of each plate by using cork borer. The same cork borer used to cut a portion of A. pullulanus colony and placed in a pore of plate. While biological activity of A. pullulanus extract was carried out by adding 100µl of fungal extract in the central pore of plates. All tests were done with duplicate and control plates.

All inoculated plates were incubated at 37°C for one day to bacterial isolates and at 30°C for 3-5 days to yeast isolates. After incubation period the diameter of the inhibition zones were evaluated in millimeters.

Cytotoxicity test: Biocompatibility test was carried out for prepared A. pullulanus extract against human fresh blood according to Nair et al. method, briefly, Different concentrations of extract (10,50,100 and 200 ppm) were prepared, then 100µl from each concentration was added to each tube of human blood solution. The tubes were incubated at room temperature and formation of turbidity of blood solution was tested at 15, 30 and 60 min.

Enzymatic study: Hemolysin activity was evaluated with blood plate assay according to Luo et al. A standard inoculum of A. pullulanus (10 µl of 1x10⁸ cfu/ml) was deposited onto the medium. The plates were then incubated at 37°C in 5% of CO₂ for 3-5 days, after incubation period, the presence of a distinctive translucent halo around the inoculums sit indicated positive hemolytic activity.

Lipase activity was determined with tween 80 agar according to Schoofs et al. The portion of A. pullulanus colony grown on SDA was transferred to the center of tween 80 agar medium. The inoculated agar plates were incubated at 30°C for 3-7 days, the presence of a precipitate zone around the inoculated site on the tween 80 medium viewed with transmitted light indicates the production of lipase.

RESULTS

The present work elicits that Aureobasidium pullulanus isolate and its crude extract showed biological activity against both E. coli and S. aureus in addition to yeast isolates represented by C. albicans and Cr. neoformans and the resulting zone of inhibition diameter values are given in Table 1.

Table 1: The inhibition zone towards pathogenic bacteria and yeast isolates by used Aureobasidium pullulanus extract.

<table>
<thead>
<tr>
<th>Target microorganism</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>18</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>8</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10</td>
</tr>
<tr>
<td>Cryptococcus neoformans</td>
<td>11</td>
</tr>
</tbody>
</table>

Figure 1: Agar-well diffusion assay of A. pullulanus crude extract against two bacterial and fungal isolates. (A: Escherichia coli, B: Staphylococcus aureus, C: Candida albicans and D: Cryptococcus neoformans)

The cytotoxic activity of A. pullulanus extract was assessed by using different concentrations of human blood solution are presented in Figure 2, the result showed no turbidity formation after 15, 30 and 60 min from adding 100µl of A. pullulanus extract to all concentrations of human blood solution, indicated no cytotoxic effect of fungal extract.

Figure 2: Cytotoxicity test showed that no formation of turbidity after adding 100µl from A. pullulanus extract to all concentrations of human blood solution during 15, 30 and 60 min. at room temperature (1: 200 ppm, 2: 100 ppm, 3: 50 ppm, 4: 10 ppm and 5: control tube)
Further test revealed that the ability of A. pullulanus isolate to produce two important enzymes, hemolysin and lipase (Figure 3 and Figure 4).

Discussion

Aureobasidium pullulanus has been well studied ecologically 11, taxonomically 12, physiologically 13, and structurally 14. Its ability to produce extracellular enzymes has been investigated for pectolytic activity 12,15 urease production 12, and proteolysis 12,16. A. pullulanus strain that tested by Singh et al 17 reported inhibition activity against gram-negative bacteria but could not inhibit S. aureus, C. albicans and Saccharomyces cerevisiae isolates. The effectiveness A. pullulanus against bacterial and yeast isolates, which known as common pathogenic agents for many infection, indicates the possibility of using this fungal product as a candidate antibiotic in the future, this result is in agreement with by Singh et al 17 study. The cytotoxicity result gives impression of the possibility to use fungal extract as anti-infectious drug because there is no cytotoxic effect against human blood solution. The microbial lipases, which are normally produced from some bacteria and filamentous fungal species, have a tremendous potential for several bioprocess. The reason is that microbial lipases do not require cofactors, have a wide range of suitable substrates, and remains stable under organic solvents 18. Therefore, the search of novel microorganisms with the capacity of producing lipases with physicochemical properties appropriate for industrial applications is useful. In the present investigation, A. pullulanus isolate showed lipase activity, and the lipolytic activity of A. pullulanus and some fungal genera has been reported before 19,20,21,22. Hemolysins lyses RBCs by creating pores or holes in red blood cell membranes resulting in the release of iron that promotes microbial growth 23. There are two main types of hemolysins, designated alpha (α) and beta (β). Alpha hemolysins cause partial lyses of the RBCs, resulting in a dark zone surrounding the fungal colony on sheep’s blood agar (SBA). The beta hemolysins cause complete lyses of the RBCs, resulting in a clear zone around the colony growing on SBA 24. In our study, Figure 3 revealed that A. pullulanus isolate produce beta hemolysin as halo zone around colony was cultured on SDA supplemented with 7% sheep blood and 3% glucose after the incubation period. In a study performed by Van Emon et al 25 to measure the ability of 90 species of common indoor fungi to produce hemolysin in different time and temperature, they found that has the ability to secret hemolysin at 37°C after two days of incubation on SBA.

Conclusions

In conclusion, the results of our study indicated that A. pullulanus isolate that grow in unfavorable environment like pigeon dropping has ability to produce novel secondary metabolite with potent activity and has high enzymatic activity.

REFERENCES


