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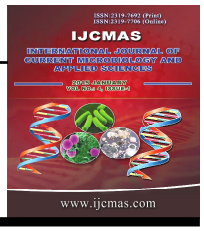

Original Research Article

Antimicrobial activity of a thermotolerant *Aureobasidium pullulans* strain isolated from Faizabad region of Uttar Pradesh in India

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Keywords

Aureobasidium pullulans, Antimicrobial Activity,

A B S T R A C T

A thermotolerant strain of *Aureobasidium pullulans* was isolated from a flower sample during summer season which showed activity against gram-negative bacteria, but could not inhibit *Staphylococcus aureus* and *Bacillus* species. The results clearly indicate that *A. pullulans* has a good antimicrobial activity against some pathogenic microorganisms and can prove to be good antibiotic producing orgainsm in future.

Introduction

Aureobasidium pullulans (De Bary) Arnaud is a cosmopolitan yeast like fungus that occur in diverse habitats, including the phylloshere of many plants and also on various tropical fruits (Gaur et al., 2010). A. pullulans is industrially important because of its capacity to produce the polysaccharide "pullulan" (Singh et al., 2012). It is a linear α-D-glucan, made mainly of maltotriose repeting units interconnected by α-1,6 linkages. The regular alternation of α-1,4 and α-1,6 bonds results in two distinctive properties that is structural flexibility and enhanced solubility (Leathers, 2002). This polysaccharide is of great economic importance with increased applications in food, pharmceutical, agricultural, blood plasma substitute and chemical industries (Cheng et al., 2009, Sena et al., 2006). Pullulan produces a high viscosity solution at a relatively low concentration and can be used for oxygen-impermeable films and fibers, thickening or extending or adhesives or encapsulating agents (Lazaridou et al., 2002). World Health rganization has placed *A. pullulans* within risk "Group I" (W.H.O. Weekly Epidemiological Records, 1994), where there is no possibilty of infection to either society or laboratory workers. A large number of bioactive and structurally diverse fungal metabolites have been isolated and cahracterized and some of these have been

used for the development of valuable pharmaceuticals and pesticides. Of the estimated 1.5 million species of fungi recorded worldwide, approximately 4,000 secondar metabolites of fungal origin are known to possess biological activities (Strobel and Daisy, 2003); the vast majority coming from the species of *Penicillium*, *Aspergilus*, *Acremonium* and *Fusarium* (Schulz et al., 2002). There are relatively few reports on antimicrobial compounds obtained from various yeasts and yeasts-like fungi. Few reports are available on antimicrobial activity of *A. pullulans* (Kalantar et al., 2006, Weilin et al, 2009, Sepcic et al, 2011, Price et al, 2013). McCormack et al., (1994) repoted for the first time the inhibition of P. aeruginosa and Staphylococcus aureus by compounds obtained from *A. pullulans*. Takesako et al., (1993) reported a group of antifungal antibiotics, named aureobasidins, from *A. pullulans*. A. pullulans appears to be a promosing organism for development of newer antimicrobial agents, both for chemotherapy as well as non-medical applications. A large number of bioactive and structurally diverse fugal metabolites have been isolated and characterized and some of these have been used for the development of valuable pharmaceuticals and pesticides.

On the basis of the above observation a thermotolerant strain of A. pullulans, RG-5, (MTCC N0. 9605) was subjected to antimicrobial activity against some pathigenic bacterial and fungal strain.

Materials and Methods

Isolation and Maintenance of Micro-organism:

A. pullulans was isolated from the flower and leaves samples collected nearby university campus. Isolation was done by selective enrichment method as followed by Pollock et al., (1992). Flowers and leaves samples were soaked in sterile water for 3 days at 35^{o}C, and then 0.1 ml was transferred to 10 ml of basal fungal medium (pH 5.0). After 2 days the turbid cultures were allowed to sit undisturbed for 20 minutes to allow filaments and aggregates to settle to the bottom. About 10µl from the upper, partially clarified phase that was enriched for yeast like cells was spread onto agar plates containing Glucose, 2.0%; Ammonium Sulphate, 0.06%; di-Potassium Hydrogen Orthophosphate, 0.5%; Sodium Chloride, 0.1%; Magnesium Sulphate, 0.04% and Yeast Extract, 0.04% with pH - 5.0.(Qualigens Chemicals, Mumbai, Maharastra, India). Isolates were maintained on the same medium at 4^{o}C in slants and sub-cultured monthly. The isolates were identified on the basis of morphological and cultural characteristics using standard identification manuals of fungi (Kurtzman and Fell, 1998) on its respective medium.

Growth Conditions and Fermentation:

For this the method of Kalanter et al., (2006) was followed. A loopful of cells (10^{8} cells/ml) taken from the slant culture of *A. pullulans* were suspended in three ml of sterile distiled water. From this suspension one ml was transferred into 10 ml of Sabouraud Dextrose Broth (SDB), incubated for 48h on a shaker set at 120 rpm, in order to prepare inoculums. One ml of the inoculum was added to 100 ml of malt extract broth and incubated at 28^{o}C on a shaker set at 120 rpm for three days. The entire content of the flask was centrifuged at 5000 rpm for 20 min and the supernatant was extracted three times with 10 ml of ethyl acetate, concentrated up to 2 ml under a stream of nitrogen gas and then used for testing the antimicrobial activity.

Testing the antimicrobial activity: Antimicrobial activity of the above extract was determined by the disc assay procedure (Jacques and Goldstein, 1986). The concentrated ethyl acetate extract (100 µl) was added to an ampoule containing 10 sterile disks (Whatman paper number 1; 5 mm diameter) and kept in a refrigerator for 24 h. Control disks were prepared using an ethyl acetate extract of the uninoculated medum.

Microorgainsms: The antibiotic assays were performed using *Acinetobacter calcoaceticus*, *Bacillus subtilis*, *Eschericia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Trichophyton rubrum*, *Candida albicans*, *Saccharomyces cerevisiae* and *Pichia angusta*. All the microorganisms were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial and fungal strains were maintained on Muller Hilton Agar and Sabouraud Dextrose agar, resrectively.

Result and Discussion

The fungal strain showed activity against gram-negative bacteria, but could not inhibit *Staphylococcus aureus* and *Bacillus* species. Strikeingly, Pseudomonals seem to be apparently much more sensitiv to *A. pullulans*. An emering nosocomial pathogen, *Acinetobacter calcoaceticus* was also inhibited by *A. pullulans*. Similarly, *E.coli*, *S. typhi* and *K. pneumoniae* was also inhibited by this fungus. This particular strain of *A.pullulans* inhibited *P. angusta* but could not inhibit *C. albicans* and *S. cerevisiae* (Table 1).

Production of antibiotic has been reported most often from the eubacteria-like *Bacillus*, Actinomycetes, e.g. *Streptomycetes* and from lower fungi e.g. *Cephalosporium*. It should be pointed out that more than 3000 antibiotics have been isolated from actinomycetes and relatively few from fungi (Giancarlo and Francesco, 1982). Mac William (1995) has found that yeasts and yeast-like fungi, as compared to other microorganisms, are not a promising source of novel antibiotics.

Because of the emergence of pathogens resistant to most currently available antimicrobial agents and a concomitant increase in the number of immunosuppressed patients, screening of antimicrobial compounds is becoming increasigly important. This study provides comprehensive evidence that *A. pullulans* could be a promising producer of antimicrobial compounds. In this study the antimicrobial activity of *A. pullulans* strain was predominantly against gram-negative bacteria and *Pseudomonas* species. In particular and unlike the study of McCormack et al., (1994), no activity was seen against *Staphylococcus aures*. It is worth nothing that *Pseudomonas aeruginosa* has been a notorious organism for chemotherapy. It is highly resistant to β-lactam antibiotics and intractable to treatment with most potent antipseodomonal agents (Vasil et al., 1990).

The toxicity of the fungal metabolites herein screened to *P. aeruginosa* is of biological interest. Some strains causing septicemia and pneumonia in cystic fibrosis and immuno-compromised patients are becoming difficult to treat with available antimicrobial agents (Lory, 1990). Due to multi-resistence of this bacterium, there is a lack of active antibiotics effective against it, resulting in an increase in nosocomial infections and high mortality. Sequel to these facts, fungi as *A. pullulans* effect against *P. aeruginosa*, is proper candidates to search for new principals. Also, the fact that extracts from these fungi exhibit

activities against some of the microorganisms implicated in the pathogenesis of skin diseases (yeast such as *Candida albicans* and Dermatophytes such as *T. rubrum*) provides some scientific basis for the utilization of substances from these two fungi for the treatment of skin disease.The result clearly indicate that *A. pullulans* has good antimicrbial activity against a number of microorganisms and can prove to be good antibiotic producing organism in future.

Table.1 Antimicrobial activity of *A. pullulans* strains jugged as the diameter (mm) of the inhibition zone

Target Organism	Zone of Inhibition (mm)
Eschericia coli	10
Pseudomonas aeruginosa	5
Salmonella typhi	10
Acinetobacter calcoaeticus	11
Klebsiella pneumonia	10
Staphylococcus aureus	0
Bacillus subtilis	0
Candida albicans	0
Saccharomyces cerevisiae	0
Trichophyton rubrum	0
Pichia angusta	5

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