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Research Article

SENSITIVITY DETERMINATION OF ANTIFUNGAL DRUGS AGAINST LIPASE PRODUCING FUNGI ISOLATED FROM OIL CONTAMINATED SOIL OF UJJAIN CITY

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A R T I C L E I N F O

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A B S T R A C T

This research aims to evaluate the antimycotic activity of the few common antibiotics against screened lipase producing fungi. Six lipase producing fungal isolates as belonging to *Aspergillus niger, Alternaria alternata, Aspergillus flavus, Trichoderma harzianum and Penicillium sp., 1 and 2* genera out of 35, showing maximum lipolytic activity were taken. These fungi were isolated from the different oil contaminated soil sites of the Ujjain City. Four antifungal antibiotics viz., Nystatin, Amphotericin B, Clotrimazole, and Fluconazole discs were used for the assessment of the fungal response. Potato Dextrose Agar medium was used and inoculated with fungal isolates and discs were placed and incubated for 5 days at 27˚C. Zone of Inhibition measured around the antibiotic disc recorded as indication of activity. Experiments were conducted in triplicates. Results reveal that most susceptible fungi for all four antifungal antibiotics is *Aspergillus flavus* and *Penicillium sp*.1 , while other four fungal isolates show different growth response for these antibiotics. All fungi were resistant for the fluconazole as no zone of inhibition was observed around the disc. This study suggests that effect of antibiotics on the growth of lipolytic fungi must be taken into consideration as these fungi were being isolated from the environmentally polluted sites and for the implementation of these fungi to the field level because antibiotics can affect the lipase activity which leads to the poor bioremediation capacity.

INTRODUCTION

Fungi are a group of heterogeneous organism which is ubiquitous in nature. One of the most important role of fungi in the ecosystem is in the form of bioremediation in which fungi-based technology is used to decontaminate the environment (https://en.wikipedia.org/wiki/Mycoremediation). Fungi are well known for their enzymes as they can synthesize extracellularly and very useful industrially (Dariush Norouzian, 2008) and these non-specific enzymes, are able to break down many kinds of substances including oils, fats and lipids (Strong and Burgess 2007; Prazeres *et al*., 2006; Babu and Rao, 2007). Lipids and fats are main pollutants of the ecosystem, produced and released by various ways such as petro-chemical, dairy and automobile industries. Due to its hydrophobic nature, lipids are difficult to remediate, but Lipases are the enzymes which catalyze the hydrolysis of triacylglycerols to glycerol and free fatty acids and are ubiquitous in nature. Lipases are widely distributed in nature and also produced by various organisms such as animals, plants, bacteria, and fungi (Saeed *et al*., 2005; Abada, 2008).

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Production of lipases has increased since the 1980s and used as industrial biocatalysts because of their properties like bio-degradability, highly specific nature and high catalytic effectiveness. Recent works have shown that lipase is closely related with the organic pollutants present in the soil (Freire & Castilho, 2008; Patil *et al*., 2011; Balaji *et al*., 2013; Carvalho *et al*., 2015; Godfrey Omare Mauti *et al*., 2016). Lipase activity was responsible for the drastic reduction of total hydrocarbon from contaminated soil. In the recent past the lipases have gained importance to other enzymes commercially, specifically in the area of organic synthesis (Sumathy *et al*., 2012). The growth of lipase producing fungi can be affected by the various factors such as temperature (Ferreira Costa and Peralta, 1999; Venkateshwarlu and Reddy, 1993), pH (Ammar and McDaniel, 1984; El-Gamal and El-Sheikh, 1989; Abd-Alla, 1999; Rani and Panneerselvam 2009), toxic substances, antibiotics which ultimately affect the fungal growth and yield of the lipase enzyme (Shimaa *et al*., 2017). It is also studied that the complexity of the soil system is determined by the numerous and diverse interactions among its physical, chemical, and biological components, as modulated by the prevalent environmental conditions (Buscot, 2005). Fungi grow, multiply and produce active lipase in soil in the optimum and physio-friendly conditions.

Naturally various antibiotics are produced by microorganisms in the soil which affect the activity of the lipase producing fungi. As clear from the above literature that information about the effect of antifungal agents on lipase producing fungi are not well studied and needs an attention due to its importance in the bioremediation. Keeping this parameter in mind, present laboratory work was conducted where four antibiotics (discs) were tested for their sensitivity against six fungal isolates. These fungi were isolated from the oil contaminated soil sites of Ujjain City.

MATERIALS AND METHODS

Collection of soil samples*:* For this purpose, the samples were collected from different oil contaminated soil sites of Ujjain city like near dairy effluents, garages, petrol stations and temples like Shani temple etc. All the soil samples collected in the sterilized polythene bags using sterilized spatula and brought to the laboratory (Wadia and Jain, 2017).

Isolation and screening of lipase producing fungi: Ten gram samples (from each site) were suspended in 250 ml Erlenmeyer flask containing 100ml sterile physiological saline. This was followed by constant and vigorous stirring for 30 min. at 120 rpm to dislodge soil clumps before allowing settling. The supernatant was decanted and a 10–fold serial dilution made from it. Serial dilutions were prepared and 0.1 ml from 10^{-2} and 10^{-3} dilution was cultivated on Potato Dextrose Agar (PDA) plates and incubated at 27^{o}C for 5 days. Fully grown fungal colonies were then purified on PDA plates. These fungal isolates were further screened for lipase production by Cup - plate method (Seirra, 1957) using tributyrin agar medium for cultivation and to obtain potent lipase producing fungi. For this purpose tributyrin agar (HiMedia) was used having following composition: Peptone: 5.0gm; Yeast extract: 3.0gm; Agar agar: 15.0gm; Tributyrin (Glycerol Tributyrate): 10.0ml; Distilled water: 990 ml; pH: 7.5. All the isolated fungal culture were inoculated on the TBA plates and incubated at 27°C up to 15 days. The formation of clear zones around the colonies is an indication of lipase production by the organisms.

Identification of screened fungi: After screening, six potent fungal cultures were used for further study and their identification was done by microscopic examination using Lactophenol cotton blue staining technique. Identified fungi were maintained on PDA (Potato Dextrose Agar) medium. Colony morphology identification of selected fungal culture was followed by laboratory manuals.

Antibiotic Sensitivity Testing

Growth of fungal isolates: All six screened fungi were grown in the potato dextrose broth and incubated for 5 days at 27^{o} C. After proper incubation, these cultures were used for disc diffusion method.

For the antibiotic testing, disc diffusion method is used. Prepared disc of the HiMedia of all four antibiotics (Nystatin, Amphotericin B, Clotrimazole and Fluconazole) were used. The diameter of the disc was standard (6 mm) and the amount of the antibiotic is 10 mcg/disc. Potato Dextrose Agar medium was prepared and about 20 ml of autoclaved molten agar medium poured into each sterilized Petri Dish. Plates were kept for solidification. After solidification, broth culture of each fungus spreaded on the surface of PDA medium plates with the help of sterilized cotton swabs. Filter paper discs of antibiotic (HiMedia) were placed on the surface of the plates. All plates were incubated for 5 days at 27^{o} C for observation of zone of inhibition around the disc. Experiments were conducted in triplicates.

RESULTS AND DISCUSSION

The present study deals with a preliminary work of antibiotics effect on the growth of six lipase producing fungi isolated from the different oil contaminated soil sites of Ujjain city. Nwuche and Ogbonna (2011**),** isolated and worked on lipase producing fungi from the Palm Oil Mill Effluent (POME). Microbial lipases have already established their vast potential regarding usage in numerous applications (Davranov, 1994; Jaeger and Reetz, 1998; Pandey *et al*., 1999; Rathi *et al*., 2001; Burkert *et al*., 2004; Houde *et al*., 2004; Kumar *et al*., 2005; Ebrahimipour *et al*., 2017). The effect of nystatin antibiotic on six fungal isolates is represented in the Figure 1. As indicated in the figure that maximum zone of inhibition is observed against *Penicillium sp.*2 (29 mm) and *Aspergillus flavus* also shown similarity to the maximum value (28.0 mm) while minimum zone of inhibition is observed against *Trichoderma harzianum* (11.5 mm). Other three fungi showed intermediate effect for Nystatin.

The literature of growth response of lipase producing fungi against antibiotics is not up to the mark in the central India. It is suggested by previous researchers that fungi show different growth for the antibiotics and also lipase production can be influenced by the type and concentration of carbon and nitrogen sources (Shaukat Ali *et al*., 2009). Although, the literature on antibiotic response for lipolytic fungi activities are meager. Physiology of lipase production varies widely in different microorganisms (Sharma *et al*., 2001).

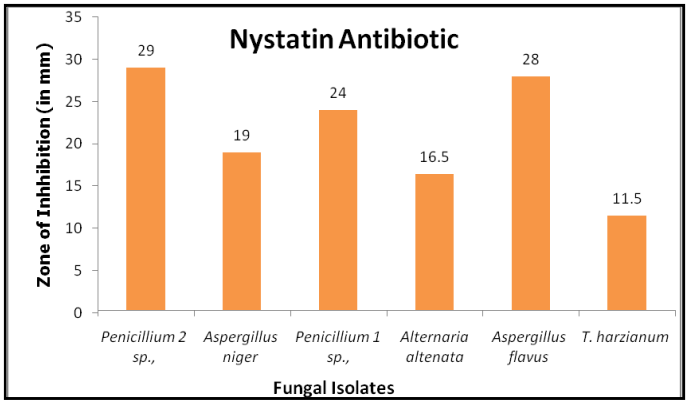


Figure 1 Effect of Nystatin on fungal growth in the form of zone of inhibition

Figure 2 represents Amphotericin B effect on studied fungal isolates and maximum zone of inhibition is observed against *Penicillium sp.,*2 and *Aspergillus flavus* (29.5 mm) while minimum zone of inhibition is observed against *T. harzianum* (only 1 mm). This indicates that these two fungi are sensitive for Nystatin while *T. harzianum* is resistant to the Nystatin while other three isolates are considered as intermediate. Two other antibiotics viz., Clotrimazole and Fluconazole also tested for their effect on lipase producing fungi. All fungi show resistant behavior against Fluconazole as mentioned in the Table.1.

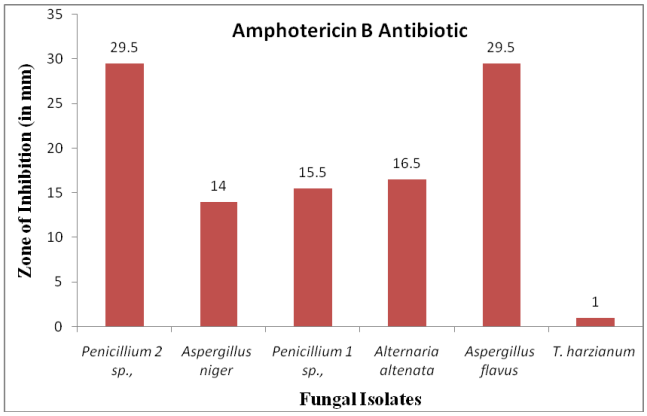


Figure 2 Effect of Amphotericin B on fungal growth in the form of zone of inhibition

Surprisingly, Clotrimazole gives maximum zone of inhibition against *Penicillium sp.,1* (36.2mm), two other fungi show intermediate zone around the disc (*Penicillium sp*.,2 shows 13.34 mm of zone, while *Aspergillus flavus* show 13.71 mm zone around disc). *Aspergillus niger, Alternaria alternata* and *T. harzianum* don't show zone of inhibition means these are resistant for the Clotrimazole (Table.1).

S.No.	Name of the fungal isolates	Nystatin	Amphotericin B	Clotrimazole	Fluconazole
1	*Penicillium* sp., 2	29.5±0.76	29.5±1.52	13.34±0.40	NO
2	*Aspergillus niger*	19±2.11	14±2.0	NO	NO
3	*Penicillium* sp., 1	24±2.0	15.5±1.8	36.2±1.2	NO
4	*Alternaria alternata*	16.5±2.08	16.5±0.28	NO	NO
5	*Aspergillus flavus*	28±0.57	29.5±1.5	13.71±1.7	NO
6	*Trichoderma harzianum*	11.5±0.76	NO	NO	NO

NO = Not Observed (No Zone Formation); ± SD = Standard Deviation

For the observation of the zone of inhibition against growth of fungi are presented in Photo plates 1-8. Photo plate 1 shows growth of fungal isolates in the Potato Dextrose Broth after incubation of 5 days, ready to use for disc diffusion method. In Photo plates 2 and 3 top and bottom view of the fungal growth with zone of inhibition on agar medium.

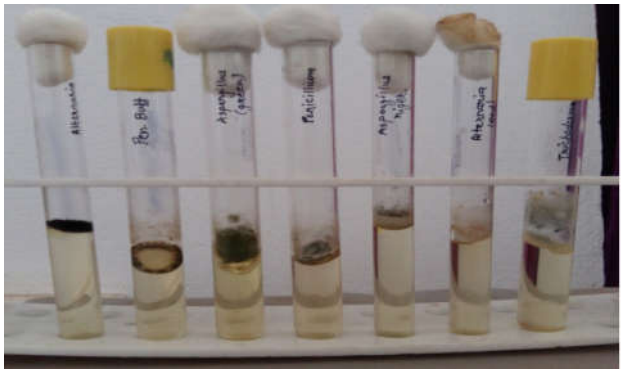


Photo Plate 1 Fungal isolates in Potato Dextrose Broth

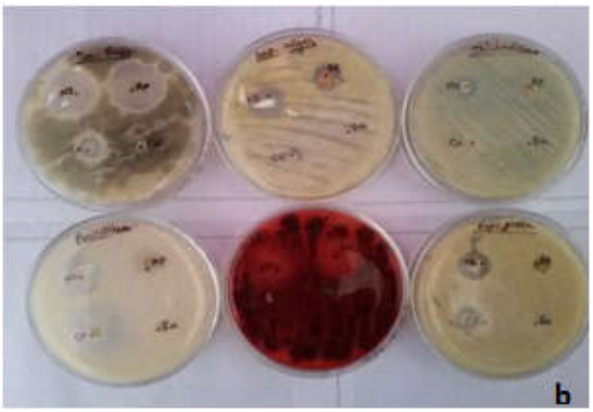


Photo Plate 2a and b: Fungal isolates Showing Zone of inhibition; a) Top View b) Bottom View

a)

b)

Photo Plate 3: Effect of four antibiotics (antimycotic) against *Penicillium sp.,* 2 [(a) Top & (b) Bottom]

a)

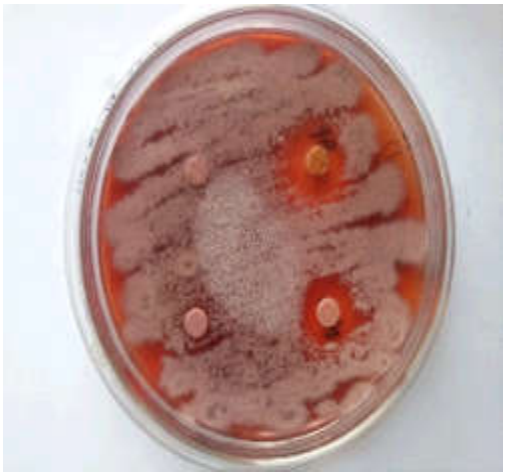
b)

Photo Plate 4 Effect of four antibiotics (antimycotic) against *Aspergillus niger* [(a)Top & (b) Bottom]

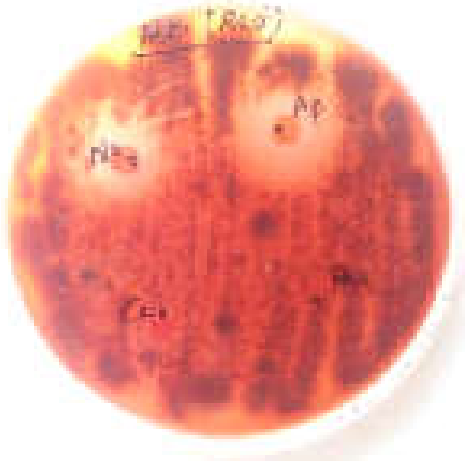
a)

b)

Photo Plate 5 Effect of four antibiotics (antimycotic) against *Penicillium sp.,* 1 [(a) Top & (b) Bottom]



a)



b)

Photo Plate 6 Effect of four antibiotics (antimycotic) against *Alternaria alternata* [(a) Top & (b) Bottom]

a)

b)

Photo Plate 7 Effect of four antibiotics (antimycotic) against *Aspergillus flavus* [(a) Top & (b) Bottom]

a)

b)

Photo Plate 8 Effect of four antibiotics (antimycotic) against *Trichoderma harzianum* [(a)Top & (b) Bottom]

CONCLUSION

It is concluded from the present study that six potential lipase producing fungal isolates *viz., Aspergillus niger, Alternaria alternata, Aspergillus flavus, Trichoderma harzianum Penicillium 1 and 2 sp.,* were tested for antifungal resistance. As evident from the results that antibiotic resistance is a very important parameter during the optimization of media for large scale production of extracellular lipase from fungi. Since these fungi were isolated from environmentally polluted areas, so it is necessary to have knowledge about their pathogenicity towards other organisms and about the antibiotics that could be used against them to inhibit their harmful effects. And also if the fungi are unable to cope up with antibiotics secreted by other microorganisms present in the soil it is difficult to take them for mass scale enzyme production. Although, present work is a preliminary type but its results are quite promising for the further research due to its applied role in bioremediation.

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