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Research Paper

EVALUATION OF MICROBIAL ANTIBIOTIC AND COMMERCIAL ANTIBIOTICS

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Abstract

Bacillus spp. were isolated from soils samples collected from differentsites. From these 48 samples, 23 isolates of bacteria of interest (*Bacillus* spp.) were isolated which were differ in their colony morphology. The isolated strains were then subjected to tentative identification which was carried out by their morphological and staining behavior. Overall, strain MC3 presented a good response against the test fungal species/ strains. Out of the sixteen test fungi, strain MC3 caused zone of inhibition from 20mm to 32mm range in eleven fungi and from 10mm to 17mm in three fungi Only two fungi *were* unaffected from the antifungal antibiotic produced by strain MC3. An attempt was also made to work out the possibilities to decide the minimum inhibitory concentration of the isolated antifungal antibiotic against the test fungus *i.e., Curvularia* lunata*.* The isolated antibiotic extract of 10^{-4}dilution exhibited the MIC in the range of about 10 µg/ml. The isolated antibiotic extract showed a good inhibition of test fungus and reduced the dry weight about 55% in comparison to control, while nystatin, chotrimazole and amphotericin inhibiting the growth of test fungus at a high rate and reduced the dry weight at a greater extent (about 67%, 62% and 57%) in comparison to control.

Key words: Antibiotic, MIC, Bacillus, Fungus.

INTRODUCTION

Antibiotic resistance is the ability of bacteria or other microbes to resist the effects of an antibiotic. The presence of antibiotics in waterways leads to an increase in bacterial resistance to those antibiotics through selective pressure and horizontal gene transfer (Alonso *et al.,* 2001). *Bacillus* species are known to produce various antibiotics and antifungal compounds that inhibit phytopathogenic fungi (Cho *et al.,* 2003; Thaniyavarn*et al.,* 2003;)*Bacillus* spp., *Pseudomonas* spp. and *Streptomyces* spp. are widely used as biocontrol agents. *Bacillus* spp. has been reported to produce several antibiotics Bacteria are able to synthesis a wide range of metabolites with fungicidal capabilities. The antagonistic potential of soil bacteria strains (*E. coli, Baccillusfortis, B. faragiris Pseudomonas fluorescence* and *P. malaphilla*) was assessedby the dual culture technique against some food pathogenic fungi, *i.e Aspergillusflavus, A. niger,*

Penicilliumitalicum and P. simplicissmum. The result indicatedthat bacterial species exhibited varying degree of biocontrol potential against all tested fungi (Sobia *et al.,* 2010) Nystatin is a polyene antibiotic derived from *Streptomyces noursei* (Hazwn*et.al* 1951). It is active against a broad spectrum of fungi in vitro and in vivo, including *Aspergillus fumigatus*, *Candida albicans*, *Coccidioidesimmitis*, *Cryptococcus neoformans*, and *Histoplasma capsulatum*(Stanley 1965). However, nystatin is not well absorbed from the gastrointestinal tract, and its parenteral administration results in dose-limiting toxicities and harmful infusion-related reactions (Procknow*et.al* 1958). For this reason, the clinical application of nystatin has largely been limited to topical use in mucosal and cutaneous forms of candidiasis (Solotorovsky*et.al* 1958).
Like nystatin, amphotericin B is a polyene antibiotic with a broad spectrum of antifungal action. It remains the most effective agent currently available for the treatment of many systemic fungal infections, despite the fact that its clinical use is seriously limited by nephrotoxicity and other side effects (Gallis*et.al* 1990). Clotrimazole is a broad-spectrum antifungal agent that is used for the treatment of dermal infections caused by various species of pathogenic dermatophytes, yeasts, and Malassezia furfur. The primary action of clotrimazole is against dividing and growing organisms. In vitro, clotrimazole exhibits fungistatic and fungicidal activity against isolates of Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophytonfloccosum, Microsporumcanis, and Candida species including Candida albicans. In general, the in vitro activity of clotrimazole corresponds to that of tolnaftate and griseofulvin against the mycelia of dermatophytes (Trichophyton, Microsporum, and Epidermophyton), and to that of the polyenes (amphotericin B and nystatin) against budding fungi (Candida). Using an in vivo (mouse) and an in vitro (mouse kidney homogenate) testing system, clotrimazole and miconazole were equally effective in preventing the growth of the pseudomyceliaand mycelia of Candida albicans. Strains of fungi having a natural resistance to clotrimazole are rare. Only a single isolate of Candida guilliermondi has been reported to have primary resistance to clotrimazole. Amphotericin B (AMB) is an antifungal drug with a mechanism of action that involves interaction with membrane sterols, such as ergosterol. The interaction results in membrane pore formation, ion leakage and, ultimately, cell death.(Brajtburg*et.al* 1990) AMB belongs to the family of macrolide polyene antimycotics, produced by *Streptomyces* spp. and widely used for treatment of opportunistic fungal infections (for example, those caused by *Aspergillus, Fusarium* or *Candida*)(Idemyor 2003). Clotrimazole is a broad-spectrum antifungal agent that inhibits the growth of pathogenic yeasts by altering the permeability of cell membranes. The action of clotrimazole is fungistatic at concentrations of drug up to 20 mcg/mL and may be fungicidal in vitro against Candidaalbicans and other species of the genus Candida at higher concentrations. In the present study *Bacillus* spp. were isolated from soils samples collected from differentsites. From these 48 samples, 23 isolates of bacteria of interest (*Bacillus* spp.) were isolated which differed in their colony morphology.Selected Bacillus species was carried out by studying the activity of both antifungal antibiotic produced by *Bacillus* strain and commercially available preparations of antifungal antibiotics (Nystatin, Clotrimazole and Amphotericin B) by following both broth dilution technique and disc diffusion method.

MATERIAL AND METHODS

Soil sample were collected from different states of India in sterilized plastic bags .The samples were processed using the soil dilution plate .For soil dilution, one gram of soil dilution in 10 ml. of sterilized distilled water. The dilution was spread with sterilized spreader and plates were placed in an incubator at 370 C for 24 hours. Distinct individual colonies purified by streaking on a new nutrient agar plates. Pure cultures were identified according to the literature (Bergy*et.al* 1993). Selected bacterial species were Bacillus species**.**This was carried out by studying the activity of both antifungal antibiotic produced by *Bacillus* strain and commercially available preparations of antifungal antibiotics (Nystatin, Clotrimazole and Amphotericin B) by following both broth dilution technique and disc diffusion method.
For broth dilution assay, a stock of 1000 µg/ ml of the commercial antibiotic preparation was prepared in 0.1 M sodium phosphate buffer (pH- 7.0).Further dilutions (10^{-1}X to 10^{-4}X) of commercial antibiotic and antifungal antibiotic in the culture filtrate were prepared in pH- 7.0

phosphate buffer. The percent inhibition of growth of *C. lunata* obtained after performing broth dilution assay were compared for each antibiotic sample and effectivity of these against *C. lunata*was assessed.
For agar disc diffusion assay, discs was prepared by commercially available Nystatin (100 units/ disc), Amphotericin B (100 units/ disc) and Clotrimazole (10 units/ disc) were used.

RESULT AND DISCUSSION
Antifungal activity of the antibiotic isolated from selected bacterial strain MC3 was studied and the results were compared to that of standard antifungal antibiotic nystatin, Clotrimazole and Amphotericin B. The results of the inhibition of fungal growth and radial mycelial growth are summarized in Tables 1 and2. It appeared that the isolated antibiotic inhibited radial mycelial growth of the test fungus at a concentration of 1000 µg/ ml medium to varying degrees that ranged from about 55–69%. The MIC values of the crude extract ranged between 10^{-4}µg/ml against *Curvularialunata*.
The results of minimum inhibitory concentration (MIC) of the isolated antibiotic crude extracts against the *Curvularialunata* are presented in Table 1. The isolated antibiotic extract of 10^{-4} dilution exhibited the MIC in the range of about 10^{-4} µg/ml. The isolated antibiotic extract showed a good inhibition of test fungus and reduced the dry weight about 55% in comparison to control, while nystatin, clotrimazole and amphotericin inhibiting the growth of test fungus at a high rate and reduced the dry weight at a greater extent (about 67%, 62%and 57%) in comparison to control. The commercial antifungal antibiotic (nystatin, clotrimazole and amphotericin) inhibit the growth of test fungus at a varying range between 67%-82%, 62%-77% and 57%-76%, respectively, while the isolated antifungal antibiotic could caused only 55%-69% inhibition range.
The results of the inhibition was also confirmed by agar diffusion method and revealed that the antifungal antibiotic isolated from the MC3 strain found effective to inhibit the growth of test fungus (Table 2). The isolated antifungal antibiotic caused inhibition zone of 1.9 cm. While the commercial antifungal antibiotics caused inhibition zone of 2.37 cm, 1.93 cm and 1.92 cm, respectively, in comparison to control. The results of the agar diffusion method showed that the isolated antifungal antibiotic was proved best against test fungus, in the present study, in comparison to the clotrimazole and amphotericin, while nystatin showed best in all the test antifungal antibiotics (including antifungal antibiotic produced in present study).

Table. 1 Effect of different concentrations of isolated antifungal antibiotic and commercial antibiotics on the growth of *Curvularialunat*

Broth Dilution	Dry Weight of the Fungal Biomass (mg)								
	Control Sample	Nyst.	% Inhibition	Clotri.	% Inhibition	Ampho-B	% Inhibition	MC3	% Inhibition
10^{-1}	745	134	82.01	174	76.64	177	76.24	232	68.85
10^{-2}		145	80.53	197	73.55	214	71.27	257	65.50
10^{-3}		254	65.90	245	67.11	243	67.38	269	63.89
10^{-4}		246	66.97	286	61.61	318	57.31	332	55.43

Nyst.- Nystatin, Clotri.- Clotrimazole, Ampho. B- Amphotericin-B, MC3- Antibiotic produced in present study

Table Effect of different concentrations of isolated antifungal antibiotic and commercial antibiotics onthe growth of *Curvularialunata*

Type of Antibiotic	Inhibition Zone (diameter in cm)			
	Set- 1	Set -2	Set- 3	Mean diameter
Nystatin	2.53	2.35	2.23	2.37
Clotrimazole	2.00	1.87	1.93	1.93
Amphotericin B	1.92	2.01	1.84	1.92
MC3 Antibiotic*	2.11	2.01	1.80	1.97

MC3 Antibiotic* produced in present study

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