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ANTAGONISTIC ACTIVITY OF SOIL ACTINOMYCETES AGAINST COMMON HUMAN PATHOGENS

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ABSTRACT

Antimicrobial activity of actinomycetes isolated from the soil was studied for the production of novel secondary metabolites. A total of 93 isolated actinomycetes were subjected to primary screening against human pathogenic *Escherichia coli*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger*. Out of 93 actinomycetes, only 25 isolates showed the antagonistic activity against the test pathogens. Six isolates were effective against *Escherichia coli*, 10 were effective against *Bacillus subtilis*, 6 were effective against *Candida albicans* and 19 were effective against *Aspergillus niger*. Among them four isolates were effective against all the test pathogens. These four isolates were subjected to the secondary screening to test the broader spectrum activity. These isolates were identified by morphological and biochemical methods, 3 isolates were identified as *Streptomyces* spp. and one isolate as *Micromonospora* sp.

Key words : Actinomycetes, *Streptomyces*, Agar well diffusion, Antagonistic activity, Broad spectrum antibiotics

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INTRODUCTION

We are living in the age of microorganisms as they have significant positive and negative impact on human population. A variety of infections are caused by microorganisms (bacteria, virus, fungi, and protozoan's) which are very harmful to both animals and plants. For the treatment of such diseases antibiotics are being used from ancient time. After more than 50 years of widespread use, however, many antimicrobials are not as effective as they used to be[1]. Over time, some bacteria have developed ways to circumvent the effects of antibiotics. Widespread use of antibiotics is thought to have spurred evolutionarily adaptations that enable bacteria to survive these powerful drugs. Antimicrobial resistances provide a survival benefit to microbes and make it harder to eliminate infections from the body[2]. Pathogens are gaining resistance to existing antibiotics, hence; there is a desperate need of screening actinomycetes for antimicrobial compound[3]. Actinomycetes have the capability to synthesize many different biologically active secondary metabolites such as antibiotics and enzymes[4, 5]. Actinomycetes produce 60% of total antibiotics and *Streptomyces* cover around 80% of these[3, 6]. Actinomycetes are Gram-positive aerobic bacteria with high G + C content DNA (>55%). Phenotypically they are highly diverse, free living, saprophytic, widely distributed in soil, water and colonizing plants showing marked chemical and morphological diversity[7]. The present study was mooted out to explore the antimicrobial potential of soil actinomycetes.

MATERIALS AND METHODS

Sample collection and pretreatment

For the isolation of actinomycetes, soil samples were collected from different sites of C.C.S. University Campus, Meerut. Samples were collected 5 cm below the soil surface where most of the microbial activity takes place. Samples were taken aseptically using sterile spatulas, forceps, and scalpels into sterile polyethylene bags. Samples were brought to the laboratory in aseptic condition and stored in the refrigerator at 4 °C until use. Just before isolation, soil samples were heated for 1 hour at 50 - 55 ºC in oven to remove the bacterial and fungal contaminants[8].

Isolation of actinomycetes

After pretreatment, soil samples were subjected to isolation of actinomycetes by pour plate method. One gram of pretreated soil was added in 99 ml of sterilized physiological saline and stirred vigorously for 10 minutes on a rotatory shaker. The suspension was serially diluted up to 10^{-5} level and then 100 µl of each dilution was added in each flask. Thereafter, the molten and cooled selective medium was poured into sterilized Petri plates and incubated at 28±2 ºC for 7 days. After incubation, powdery colonies were isolated on the basis of the color and morphological differences and transferred on fresh Bennet's agar media.

Test organisms

Human pathogenic bacteria of representative groups such as Gram negative *Escherichia coli,* Gram positive *Bacillus subtilis,* unicellular fungi *Candida albicans* and filamentous fungi *Aspergillus niger* obtained from culture collection unit of Department of Microbiology, C.C.S. University Campus, Meerut, were used in this study.

Primary screening

The antagonistic activity of actinomycetes was tested by perpendicular cross streak method[9]. Single streak of 4-6 mm diameter of the actinomycetes strains were streaked on the surface of the modified nutrient agar medium[10] and incubated at 28±2 ºC for 5-7 days. Fresh cultures of test organisms were streaked perpendicular to the actinomycetes streak. These perpendicularly cross streak plates were then incubated at 28±2 ºC for 48 hrs. Control plates were also maintained without inoculating actinomycetes to assess the normal growth of bacteria and fungi.

Secondary screening

Promising antagonistic strains were then subjected to the further study by shake flask culture method to assess their antimicrobial activity by agar well diffusion method. Actinomycetes cultures were inoculated in nutrient broth and placed on rotatory shaker for 72 hrs. Thereafter, the broth was centrifuged at 6000 rpm for 10 minutes and supernatant was collected. The inoculums of test organisms (10^6 cells/ml) with 0.6% O.D. at 530 nm with UV visible spectrophotometer was prepared. The microbial suspension was then spread on the solidified Muller Hinton agar plates with sterilized cotton swab. Wells were made in agar medium by 6 mm sterilized borer and 100 µL of each supernatant was poured in the wells. All the plates were incubated at 28±2 ºC for 48 hrs and the zone of inhibition around the well was measured.

Identification of potential actinomycetes

The potent actinomycetes selected from secondary screening were then identified up to generic level by morphological, physiological and biochemical methods. Morphological methods consist of macroscopic and microscopic methods. The structure of mycelium, color, spore and arrangement of conidiophores was observed under the microscope. Various biochemical tests were performed for their identification. All these observations were then compared with Bergey's Manual of Systematic Bacteriology[11].

RESULTS AND DISCUSSION

For the screening of novel actinomycetes, a total of 93 isolates were isolated from the collected soil samples. Primary screening of the isolates were done by perpendicular cross streak method. No growth of the test organisms after 48 hrs adjacent to the streaking of actinomycetes indicates positive antimicrobial activity of the isolates. If the growth of the test organisms occurred in the entire streak line, then antimicrobial activity of the isolates was recorded as negative. Six (6.45 %) isolates were found effective against Gram-negative bacteria, 10 (10.76 %) against Gram- positive bacteria, 6 (6.45 %) against unicellular fungi and 19 (20.40 %) against filamentous fungi (Table 1). The same findings were also reported by other scientists[12-15]. Among the isolates, 4 isolates DOM 3, DOM 8, DOM 19 and DOM 21 exhibited broad spectrum activity against all the test pathogens. Secondary screening was performed with these promising strains and zone of inhibition were measured. The isolate DOM 3 showed the zone of inhibition of 10.25 mm, 14.73 mm, 12.27 mm and 23.13 mm against *E. coli*, *B. subtilis*, *C. albicans* and *A. niger* respectively, DOM 8 showed the zone of inhibition of 9.27 mm, 10.56 mm, 8.32 mm and 19.31 mm against *E. coli*, *B. subtilis*, *C. albicans* and *A. niger* respectively, DOM 19 showed the zone of inhibition of 8.55 mm, 10.35 mm, 11.18 mm and 19.31 mm against *E. coli*, *B. subtilis*, *C. albicans* and *A. niger* respectively and DOM 21 showed the zone of inhibition of 10.43 mm, 13.23 mm, 12.87 mm and 21.54 mm against *E. coli*, *B. subtilis*, *C. albicans* and *A. niger* respectively (Graph 1). The result of primary and secondary screening reveled that isolates were more active against Gram-positive bacteria (*B. subtilis*) than Gram-negative bacteria (*E. coli*). However, filamentous fungi (*A. niger*) are more susceptible against actinomyctes isolates than unicellular fungi (*C. albicans*).

The identification of the potent antibiotic producing strains on the basis of morphological and biochemical observations revealed that three isolates DOM 3, DOM 8 and DOM 19 under investigation were belonged to the genus *Streptomyces* and one isolate DOM 21 belonged to the *Micromonospora* species (Table 2).

Table 1
Antimicrobial susceptibility of antagonistic actinomycetes against test organisms in primary screening

Isolates	Test organisms			
	Escherichia coli	*Bacillus subtilis*	*Candida albicans*	*Aspergillus niger*
DOM 1	-	-	+	++
DOM 2	-	+	-	-
DOM 3	+	+	+	++
DOM 5	-	-	-	+
DOM 6	-	+	-	+
DOM 8	+	+	+	++
DOM 9	-	-	-	+
DOM 10	+	-	-	++
DOM 11	-	-	-	++
DOM 12	-	-	-	+
DOM 14	-	-	-	++
DOM 15	-	-	-	++
DOM 16	-	+	-	-
DOM 18	-	-	-	+
DOM 19	+	+	++	++
DOM 20	-	-	-	++
DOM 21	+	+	++	++
DOM 25	-	+	-	-
DOM 29	-	-	-	++
DOM 31	-	-	++	-
DOM 34	-	+	-	-
DOM 36	-	+	-	-
DOM 37	-	-	-	+
DOM 38	-	-	-	+
DOM 39	+	-	-	++

+ show good inhibition, ++ show excellent inhibition, - show no inhibition

Graph 1
Antimicrobial susceptibility of antagonistic actinomycetes against test organisms in secondary screening

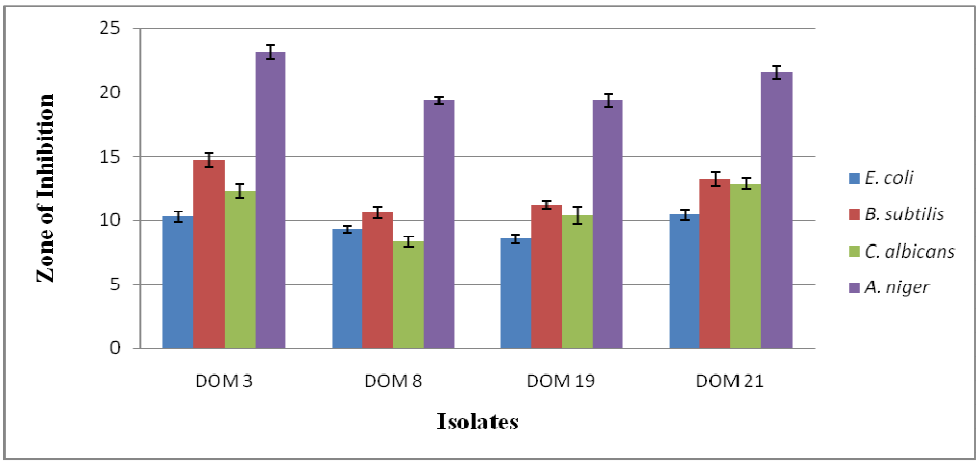

Table 2
Generic level identification of potential strains of actinomycetes

Characteristics	Name of actinomycetes species			
	DOM 3	**DOM 8**	**DOM 19**	**DOM 21**
True mycelium	Present	Present	Present	Present
Shape	Filamentous	Filamentous	Filamentous	Filamentous
Colony elevation	Small, raised	Small, raised	Large, raised	Large concentric ring, raised
Sporulation	Good	Good	Good	Moderate
Shape of spore	Oval	Oval	Oval	Spherical
Motility	Negative	Negative	Negative	Negative
Spore chain morphology	Recti-flexible	Recti-flexible	Recti-flexible	Single spore at tip
Spore surface	Smooth	Smooth	Smooth	Smooth
Color of mycelium	White brown	White	White	White
Reverse side color	Negative	Negative	Negative	Negative
Odor	Earthy odor	Earthy odor	Earthy odor	Earthy odor
Gram staining reaction	Positive	Positive	Positive	Positive
Acid fast staining	Negative	Negative	Negative	Negative
Melanin pigment	Negative	Negative	Negative	Negative
Growth at 28 ºC	++	++	++	++
Growth at 45 ºC	+	+	+	+
Catalase	Positive	Positive	Positive	Positive
Oxidase	Negative	Negative	Negative	Negative
Aesculin hydrolysis	Positive	Positive	Positive	Positive
Gelatin liquefaction	Negative	Negative	Negative	Negative
H_2S production	Positive	Negative	Positive	Positive
Urea hydrolysis	Positive	Positive	Positive	Negative
Nitrate reduction	Negative	Negative	Positive	Positive

Peptone	Positive	Positive	Positive	Positive
Caesin	Positive	Positive	Positive	Positive
Citrate utilization	Positive	Positive	Positive	Positive
Sucrose	Positive	Positive	Positive	Positive
Mannitol	Positive	Positive	Positive	Positive
Glucose	Positive	Positive	Positive	Positive
Lactose	Negative	Negative	Negative	Negative
Cellulose	Negative	Negative	Negative	Negative

+ = moderate growth, ++ = good growth

CONCLUSIONS

It is concluded from the above study that four investigated isolates exhibited antimicrobial activity against the tested human pathogenic bacteria and fungi. The highest activity was shown by the strain DOM 3. Three isolates DOM 3, DOM 8 and DOM 19 were identified as *Streptomyces* spp. and one isolate DOM 21 belonged to the as genus *Micromonospora*. It is confirmed from the present study that soil actinomycetes are good source of broad spectrum antibiotics for pharmaceutical interest.

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