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Antifungal Potential of *Actinomycetes* isolated from Soil against Pathogenic Fungi

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A B S T R A C T

Due to overuse of antibiotics the microbes are becoming resistant to them, so new antimicrobial should be explored which have low MIC and higher efficiency.The present study was conducted to determine the antifungal potential of *Streptomyces*. Six actinomycetes viz. *Streptomyces*, *Micromonospora*, *Nocardia sp*-1, *Nocardia sp*-2, *Nocardia sp*-3 and *Streptosporangium* were isolated from the soil of KGM Garden and Ganges River Bed, Haridwar. For the evaluation of antimicrobial potential of *Streptomyces* culture, fermentation of *Streptomyces* culture was carried out by Shake flask method. Further *Streptomyces* was taken for antifungal screening against lab fungi *Mucor* and *Aspergillus*. *Streptomyces* was found to inhibit the growth of *Mucor* 18.3 mm ZOI and *Aspergillus* 17.6 mm ZOI. After observing the characteristics shown by *Streptomyces* culture, it can be concluded that the antimicrobial potential in actinomycetes culture may lead to the discovery of new antifungal drugs as it show maximum inhibition against *Mucor* and *Aspergillus* comparable to the standard antifungal Nystatin potential.

Introduction

Microbes are present in vast number in nearly every environment i.e. soil, water, food, air, etc. This include bacteria, fungi, protozoa, viruses, algae, etc. They causes diseases like small pox, Anthrax, AIDS, etc. On the other hand they help in food production, pollution control, energy production, control and treatment of diseases by producing drugs like antibiotics to kill their own kind. One of the microbial products of secondary metabolites i.e antibiotics are special chemotherapeutic agents. These are mainly produced by fungi, actinomycetes and few bacteria.

Actinomycetes are Gram positive bacteria which comprises a group of branching unicellular micro-organisms, are best known for their ability to produce antibiotics. Among actinomycetes the *Streptomyces* are the dominant. Actinomycetes are rod shaped to filamentous, aerobic and speculation with DNA rich in G+C from 55-75%. The name actinomycetes derived from (greek akis- a ray of beam, mykes- fungus) and was given to this organism because of their morphologically resemblance with fungi. Actinomycetes produce branching mycelium which may be of two kind viz. substrate

mycelium and aerial mycelium. They form a mycelium that grows on the agar and in some cases; the primary mycelium is short lived and soon breaks up with bacillary or coccoid elements (Star *et al.,* 1981). Among 80% of the world's antibiotics are known to come from the genera *Streptomyces* and *Micromonospora*. Different *Streptomyces* species produce about 75% of commercially and medically useful antibiotics. Antibiotics are special chemotherapeutic agent, which have lethal or inhibitory effect on microbes but in therapeutic concentration have little or no toxic action on the tissue and usually produced a large scale microbial process. The first antibiotic Penicillin was discovered by Fleming in 1928. The antibiotics inhibit synthesis of cell wall peptidoglycan, damage to permeability of cell membrane, inhibition of nucleic acid synthesis and protein synthesis. Some antibiotics are used as antifungal agents. The fungi are eukaryotic protest without chlorophyll. The molds have leathery to velvety, powdery, granular, or cottony colonies made up of tubular cells, the hyphae. Mucormycosis is an invasive disease caused by phycomycetes, mainly by species of *Mucor* and *Rhizopus.*

The present study indicate that the higher number of actinomycetes were isolated from Ganges River bed soil active against bacteria and fungi, where the human activities is very less for agriculture and other purpose and these actinomycetes can be useful for many applications such as control of infectious diseases and drug discovery.

Materials and Methods

Collection of soil sample

Soil sample for the isolation of actinomycetes were collected from depth of 10-20cm from KGM Campus, Jwalapur and Ganges river bed, Haridwar, India. These sample were placed in polythene bags, closed tightly and analyzed for actinomycetes.

Isolation of Actinomycetes

Labeled sterile water blanks test tube with 1 gm of finely pulverized, air dried soil in 10 ml sterile distilled water. It was used as stock solution and then plating out low dilution 10^{-3} to 10^{-6} of sample onto glycerol yeast extract agar medium. The inoculated plates were then incubated at 26^0C – 28^0C for 5-7 days.

Identification of Actinomycetes

The identification of actinomycetes was done on the basis of morphology of spore chain, pigment production, color of aerial mycelium, color of substrate mycelium, consistency, Gram's staining, growth on actinomycetes media, growth of streptomyces media, etc. Biochemical characterization of actinomycetes was done by methyl red test, voges proskauer test, catalase test, starch hydrolysis, etc. The potent actinomycetes selected for further studies were characterized by morphological and biochemical methods described in the Identification key by Bergey's Manual of Determinative Bacteriology (1957).

Shake flask method for metabolite production

Starch nitrate agar slant culture of actinomycetes were inoculated in 250 ml flask containing 100 ml medium and incubated in shaking incubator at 250 rpm. The flask is then allowed to proceed in the device which continuously shakes or swirls the culture.

In vitro screening of isolates for antagonism by Well diffusion method

Well Diffusion Method

The procedure for estimating the

bioactivities and concentration of antibiotics to inhibit the growth of test organism. It can be measured by the diameter of inhibition zone. This can be done by Well diffusion method (for fungi).

In 150 ml flask sterile solution was taken and a piece of fungal mycelia mat was suspended and vortexed. The tubes were kept in shaker at 28^0C for 24 hrs. Poured sterile CDA on sterile plate and allow it to solidify. After solidification 1 ml of fungal culture solution was transferred to the plate. Make well by the help of sterile cork borer and added 0.8 ml actinomycetes culture by a sterile pipette. The plates were kept for diffusion of solution at room temperature for 30 mins. Then incubated at 28^0C $\pm$2 for 2-4 days. The plates were examined and measure the zone of inhibition around the wells.

Results and Discussion

The actinomycetes were isolated from the soil and minimum CFU found in 10^{-5} dilution whereas maximum in 10^{-3} dilution as the number of colonies decreases with the increase in dilution (Table 1). Six actinomycetes were identified on the basis of morphological appearance and Gram staining. They were *Streptomyces*, *Micromonospora*, *Nocardia sp* 1, *Nocardia sp* 2, *Nocardia sp* 3, *Streptosporangium*. On the basis of various characteristics as noticed by Gram staining, actinomycetes which was further isolated from different dilution of soil sample, resemble with *Streptomyces sp*. (Table 2,3) was taken for antifungal screening against given lab fungi *Mucor* and *Aspergillus* (Table 4 and Figure 1).

The actinomycetes culture resemble with *Streptomyces*

The colonies were aerobic, globular and folded with aerial and substrate mycelia of different color, white smooth embedded in agar colony. The culture characteristics (pigment production) chains of spores are often spirally coiled sporophores may be simple or branched, formed by a segregation of protoplasm within the hypha into a series of round oval or cylindric bodies (Table 2).

Actinomycetes from natural source are widely recognized to produce secondary metabolites, including many antimicrobial such as streptomycin, erythromycin and tetracycline with original and ingenious structure and potent biological activities. These actinomycetes are considered to be the resource of few leading compounds in drug development. Antibiotic are commonly used for treatment of infectious disease caused by various microbes. Once the causative organism is identified for specific disease and isolated, it is very important to test the sensitivity of organism to the effective antibiotics. Use of this antibiotic for curing microbial diseases is known as chemotherapy. Some antibiotics which are in current use are as follows:-

- Penicillin, vanomycin etc mainly active against Gram positive bacteria.
- Streptomycin, neomycin etc mainly active against Gram negative bacteria.
- Ampicillin, tetracycline etc mainly active against both Gram positive and Gram negative bacteria.
- Griseofulvin, nystatin etc mainly active against fungi.

Now a day's drug resistances among microbes have increased. Kitamato *et al* (1956) were first to discover transferable drug resistance in *Shigella* isolated from dysentery cause in Japan. Igarahi *et al* (1997) obtained Resormycin, novel herbicidal and antifungal antibiotic isolated from cultured broth of *Streptomyces platensis.*

Table.1 Enumeration of *Actinomycetes* in each dilution plate from two soil sample soil (Average of Triplicate)

S.no.	Dilution	KGC Garden Soil	Ganges River bed Soil
		Average ± SE	Average ± SE
1.	10^{-3}	10.3± 0.57	11 ± 0.57
2.	10^{-4}	8.6 ± 0.57	7.6 ± 1.52
3.	10^{-5}	7.6 ± 1.73	7 ± 1.15

Table.2 Morphological characterization of isolated *Actinomycetes*

Organism	Mycelium and nature of colony	Color of colony	Type of spore	Pigmentation	Gram stain
Streptomyces	Smooth embedded colony in agar plate	White	Long chain of spore	Pale yellow	+ve
Micromonospora	Branched mycelium powdery colony	White to grey	Monosporophore	-	+ve
Nocardia sp-1	Hairy	Pinkish white	Long chain of spore	Pink	+ve
Nocardia sp-2	Smooth colony	White	Long chain of spore	Yellow	+ve
Nocardia sp-3	Powdery colony	Creamish white	Long chain of spore	Wine red	+ve
Streptosporangium	Buttery colony branched mycelium	Reddish white	Long chain of spore	Red	+ve

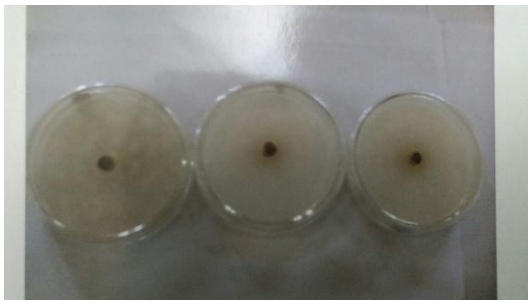
Table.3 Biochemical characterization of *Streptomyces*

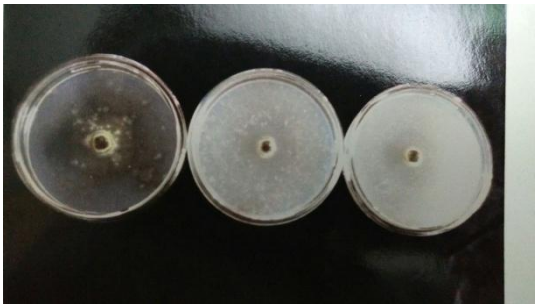
S.no.	Biochemical characterization	*Streptomyces*
1.	Catalase test	+ve
2.	Starch hydrolysis	-ve
3.	Indole test	-ve
4.	Methyl red test	+ve
5.	Voges proskauer	-ve
6.	Citrate utilization	+ve

Table.4 Antifungal activity of test *Streptomyces* against *Aspergillus* and *Mucor* species (Average of Triplicate + SE)

S.no	Antifungals	*Aspergillus*	*Mucor*
1.	*Streptomyces* (Test organism)	17.6 ± 0.565	18.3 ±0.577
2.	Nystatin	21 ± 1.00	22 ± 1.00
3.	Distilled water	Nil	Nil

Fig.1 Plates showing ZOI against (A) *Aspergillus* and (B) *Mucor*





Ogawa *et al.*, (1998) screened *Streptomyces sp* isolated from soil sample of Iwakuni city, Japan for their bacteriophage activities which also exhibited antimicrobial activity against Gram positive organisms. Singh and khan (2001) isolated the actinomycetes from the river bed of Ganga and KGM college garden resembles on the basis of culture characteristics with *Streptomyces sp*. Chaudhary and Singh (2015) reported fungicidal potency of *Cinnamomum tamala* leaves against common food borne pathogens. Chaudhary and Singh (2015) reported control of food pathogenic fungi by oil derived from seeds of Indian spice plant, *Foeniculum vulgare*. During the present investigation the actinomycetes culture showed good antimicrobial activity against fungus viz. *Mucor* and *Aspergillus* 18.3 and 17.6mm respectively whereas known antifungal drug nystatin showed susceptibility against test fungus *Mucor* and *Aspergillus* by means of ZOI 22 and 21 respectively.

In conclusion, in recent years microorganisms especially Actinomycetes become important in pharma industry for its novel microbial products exhibiting antimicrobial, antiviral antitumor as well as anticoagulant and cardioactive properties. The active compound may serve as model system in discovery of new drugs and becomes a major thrust area in modern medicine. The search for new drugs against fungal infections is a major challenge to current research in mycotic disease. During the present study "*Streptomyces*" were found to have excellent antifungal potential and hence merit further studies concerning purification characterization and identification of the active secondary metabolites.

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