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SCREENING OF POTENTIAL ANTIBIOTIC PRODUCING ACTINOMYCETES FROM THE SOIL OF GANGA RIVER BED OF HARIDWAR

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Abstract:

Actinomycetes are the mostly widely distributed group of microorganism in nature which is of high commercial value and thus are routinely screened for new antibiotics. We have undertaken this study to resolve the problem of antibiotic resistance. 6Actinomycetes were isolated from the soil by serial dilution method. Based on the biochemical characterization Nocardia sp. was chosen to check antibacterial potential. Optimization of growth was carried out at 29^0C for 5-7 days on Glycerol Yeast Extract Agar Medium. Antibacterial activity was studied against E.coli and Bacillus sp.. Antibacterial activity was determined using disc diffusion method. Antibacterial metabolite extracted using ethyl acetate was effective and gave zone of inhibition of 13mm and 20mm against the used pathogenic Bacillus and E.coli strains. As most microorganisms have developed resistance to existing antibiotics. So it has provoked the need of constant research like ours on the production of newer antibiotics.

Keywords: *Actinomycetes, antibiotics, Nocardia sp., E.coli, Bacillus, disc diffusion, GYEAM, Antibacterial potential.*

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INTRODUCTION:

Screening of microorganism for the production of novel antibiotics have been intensively pursued for many years by scientists. Actinomycetes are capable of synthesizing many different biologically active secondary metabolites. Out of these compounds, antibiotics predominate in therapeutic and commercial importance [1]. A substance which is produced by one kind of living organism which is injurious to another is called an antibiotic. Actinomycetes are the most widely distributed group of microorganism primarily inhabit the soil. Actinomycetes are a group of prokaryotic organism belonging to a sub division of Gram positive bacteria, characterized by high G+C content in their DNA [2]. In natural habitats, *Streptomyces* are common and are usually a major component of total actinomycetes population. Some actinomycetes genera such as *Actinoplanes, Amycolatopsis, Kineospora, Microbispora, Nocardia, Micromonospora* are often very difficult to isolate and cultivate due to their slow growth are called rare actinomycetes [3]. But in last two decades, there has been a decline in the discovery of novel metabolites from *Streptomyces* on the culture extracts usually yield disappointingly high number of previously described molecules [4,5]. That's why new sources of bioactive metabolites from another group of atinomycetes, known as rare actinomycetes have promoted recent advances in the discovery of new antibiotic molecules [6]. Among the rare actinomycetes, numerous interesting biologically active compounds have been reported from the genus *Nocardia* such as nargenicin [7] transvalencin [8] nocardithiocin [9] etc. Genus *Nocardia* is a member of the family Nocardiaceae have broad bioactive metabolites profile as it includes a variety of antibacterial, antifungal, antitumor, immunosuppressive compounds. Investigation can possibly reveal actinomycetes species that produce noval antibiotics. It is anticipated that the isolation, characterization and the study of actinomycetes can be useful in the discovery of new antibiotics. In the present era, bioactive metabolites from pathogenic actinomycetes particularly *Nocardia* sp. have been focused due to their unique and diverse metabolic pathway against the life threatening resisting pathogens [10]

MATERIAL AND METHODS:

Sampling Two soil samples were collected from Ganga river bed and garden of Kanya Gurukul Campus with the help of sterile spatula in a sterile polythene bags and stored at 4^0C upto use.

Isolation of actinomycetes Isolation was performed by serial dilution method. 1gm of dried soil was taken in 10ml of sterilized distilled water and agitated vigorously in vortex. Various serial dilution from 10^{-1} to 10^{-7} were prepared. From these dilutions 10^{-3}, 10^{-4}, and 10^{-5} were taken. 1.0ml suspension were applied onto the plates containing Glycerol Yeast Extract Agar Medium (Glycerol – 5.0 ml, Yeast extract – 2.0 gm, K_2HPO_4 – 1.0 gm, Agar – 15.0 gm, Distilled water – 1000ml, pH – 7.0) (Waksman, 1961). After gently rotating the plates, they were incubated at 29$\pm$ 2^0C for 5-7 days or more. Gram staining and various biochemical tests were implemented for the identification and characterization of *Nocardia* sp. (Table 3). Antibacterial activity of *Nocardia* sp. were tested *in-vitro* against two pathogenic bacteria *E.coli* and *Bacillus* obtained from the Deptt. of Microbiology, Kanya Gurukul Campus, Haridwar.

Screening of antibacterial activity of *Nocardia* sp.

a) **Primary screening** A modified cross streak method was used. Single steak of *Nocardia* culture was made on NAM and incubated at 28^0C. After observing a good ribbon like growth, overnight culture of test organism were streaked at right angle to original streak and incubated at 28^0C. Control plate was also maintained without *Nocardia* sp. to access the normal growth of test organism.

b) **Extraction of antibacterial metabolite** *Nocardia* culture was grown in liquid GYEA medium. The filterate were then subjected to solvent extraction by using actetae in the ratio 1:1 (v/v) and then placed on shaker for 1hr. Ethyl phase contains antibiotic substance which is then evaporated to dryness.

c) **Secondary screening** Antibacterial activity was determined by disc diffusion method. Test organism were swabbed on Muller Hinten Agar plate and then discs of the antibiotic substance evaporated were evenly placed on its surface. Plates were incubated at 37$\pm$ 2^0C for 18 to 24 hrs and then examined for zone of inhibition. All the experiments were reported thrice so as to obtained average.

RESULT AND DISCUSSION:

The present study focused on isolation and identification of active actinomycetes having promising activity against drug resistant bacterial pathogens. Actinomycetes were isolated from soil and maximum CFU was found in the 10^{-3} dilution (Table 1). Five different types of the actinomycetes were identified on the bases of gram staining and culture characterization (Table 2). They were *Nocardia* spl, *Nocardia* sp2, *Streptomyces* sp1, *Streptomyces* sp2 and *Streptomyces* sp3. On the basis of biochemical tests, out of five actinomycetes only *Nocardia* sp was chosen for further antibacterial

potential against *E.coli* and *Bacillus* sp. (Table 3). In recent years the natural antagonistic action of one microbe has been stuied intensively. A substance which is produced by one kind of living organism is injurious to another is called an antibiotic. Determination of antibacterial potency against selective pathogen is essential for proper therapy. The inhibition of microbial growth under standardized condition may be utilized for demonstrating the therapeutic efficiency of antibiotics. Actinomycetes isolate was screened for the antibacterial properties both by the primary and secondary screening in crowded plate technique and disc diffusion method [11]. Antibacterial metabolite extracted by ethyl acetate was effective and gave a zone of inhibition of 13mm and 20mm against the test organism *Bacillus* and *E.coli* strains (Table 4). Gentamicin as standard control which showed zone of inhibition of 29mm diameter in case of *E.coli* and 20mm in case of *Bacillus.*

The result in the present investigation is not only comparable but better in some cases as compared to previous reports showed only against the *Pseudomonas, Mycobacterium, Bacillus* [12,13]. Present strain of *Nocardia* was found to be effective against *E.coli* better as compared to *Bacillus.* Singh and Sharma, 2013 reported the 2 strains of *Nocardia* are active against *Streptococcus* (Na1 29.6mm, Na2 26.6mm), *Mucor* (Na1 12.5mm, Na2 22.5mm) and *Aspergillus* (Na1 50%, Na2 60%). They also degrade Petrol very effectively, decrease in total organic carbon of the medium was observed during the degradation of petrol. Similar reports on actinomycetes was carried out by [14] in *Micromonospora*.

Table 1: Enumeration of Actinomycetes in each dilution from soil sample (average of triplicates) :-

S. NO	Dilution	K.G.C. garden soil	Ganga river beds soil
		Average ± SE	Average ± SE
1	10^{-3}	10 ± 4.001	9 ± 1.002
2	10^{-4}	10 ± 3.008	9 ± 5.014
3	10^{-5}	10 ± 4.011	7 ± 3.008

Table 2: Morphological characteristic of actinomycetes.

Organism	Mycelium and nature of colony	Colour of colony	Type of Spore	Pigmentation	Gram Stain
Nocrdia sp1	Smooth colony	White	Long Chain of spore	Yellow	+
Nocardia sp2	Powdery Colony	Creamish white	Long Chain of spore	Wine Red	+
Streptomyces sp1	Arial mycelium from white to grayish white	Greyish white	Long Chain of spore	Brown	+

Table 3: Biochemical characterization of *Nocardia* sp.

S. No	Biochemical characterization	Nocardia
1	Catalase	+
2	Starch hydrolysis	-
3	Indole production	-
4	Methyl red	+
5	Voges Proskauer	-
6	Citrate utilization	+

Table 4: Antibacterial activity of *Nocardia* sp. against *E.coli* and *Bacillus* sp.

S. No.	Test Organism	Zone of Inhibition	
		Nocardia disc	Gentamicin disc
1	*E. coli*	20(S)	29(S)
2	*Bacillus*	13(S)	20(S)

CONCLUSION:

Many microorganism have developed resistance to existing antibiotics. So it provoked the need of constant research on the production of newer antibiotics to overcome this problem. Result obtained from this work are promising and further studies will be carried out concerning purification characterization and identification of active secondary metabolites.

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