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EVALUATION OF ANTIBIOTIC PRODUCTION IN *MICROMONOSPORA* BY HPTLC

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ABSTRACT

A total of 5 different types of actinobacteria were isolated from the soil of Ganges river bed, Haridwar. All the isolated actinobacteria were characterized and identified based on the morphological and biochemical characteristics. The isolates were belonged to the genera *Micromonospora, Streptomyces* and *Nocardia*. Only *Micromonospora* sp. was selected for further studies. Antibacterial potential was determined by both cross streak method and disc diffusion method. The result of the screening revealed that the crude extract was effective against *E.coli* and *S. aureus*. It was further analyzed by high performance thin layer chromatography. Rf value in the TLC chromatogram characterized that, the metabolite comes under aminoglycoside group very much related to gentamicin.

KEYWORDS: *Micromonospora, E. coli, S. aureus, Bacillus*, antibiotic production, HPTLC.

INTRODUCTION

Among the various groups of microorganisms present in the soil, actinobacteria are prolific producers (Lechevalier 1970, Waksman 1961). They are Gram-positive, rod shaped to filamentous, aerobic and generally non-motile in vegetative phase having high G+C (>55%) content in their DNA. The majority of actinobacteria are free living, saprophytic bacteria widely distributed in soil, water and colonizing plants. The actinobacteria are noteworthy as antibiotic producers, making three quarters of all known products and other class of biologically active secondary metabolites (Okami and Hotta 1988). In the late 60s', after the discovery of gentamicin, originated from *Micromonospora*, the study of non streptomycete actinobacteria received increasing attention. The strains of this genus produced many kinds of antibiotics such as micromonosporin, megalomicin, mutamicin, fortimicin, sagamicin,

verdamicin, dapiramicin, clostomicin, mycinamicin, dynemicin, macquarimicin, holomicin, quinolidomicins, arisostatins A and B, anthraquinones, and so on. As a result of this significant property, the genus has been subjected to extensive study through out the world. In the present study, isolation and characterization as well as inhibitory effects of *Micromonospora* sp. tested against multiple antibiotic resistant bacteria.

MATERIALS AND METHODS

Sampling

The soil samples were collected in sterile plastic bags from the Ganges river bed, Haridwar.

Screening of actinobacteria from soil

One gram of dried soil was taken in 9 ml of distilled water, agitated vigorously for 5-10 minutes and appropriate ten fold serial dilutions (1:10) were prepared. Finally, dispensed 0.1 ml of diluted soil suspensions in sterile petri plates containing glycerol yeast extract agar medium supplemented with ketocip (50 µg/ml of medium). Plates were incubated at 30˚C for 7-14 days or more because actinobacteria are the slow growers. Various biochemical tests were performed for the identification and characterization of isolated actinobacteria cultures viz- gram staining, catalase test, starch hydrolysis and IMViC test according to methods given in *Bergey's manual of determinative bacteriology*.

Antibacterial potential of *Micromonospora* sp.

Test pathogens

Three pathogenic bacteria *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* were used throughout the study obtained from dept. of microbiology, Girls Campus, Gurukula Kangri Vishwavidyalaya, Haridwar.

Primary screening

A cross- streak method was used for determining antibacterial activity (Lakshmanaperumalsamy 1978). Single streak of *Micromonospora* culture was made on surface of the solidified nutrient agar and incubated at 28°C. After observing a good growth of *Micromonospora* culture, the test organisms were streaked at 90º angle to the original streak of actinobacteria culture and incubated at 28°C. Incubation distance was measured after 24-48 hrs.

Extraction of crude antibiotic metabolite

Glycerol yeast extract broth was inoculated with *Micromonospora* culture and incubated in shaker at 28˚C/120 rpm for 5-7 days. Fermented broth was filtered and subjected for solvent extraction method to recover the crude antibiotic metabolite in pure form. Ethyl acetate was added to the filtrate in the ratio of 1:1(v/v) and shaken vigorously for 1 hour for complete extraction. Ethyl acetate phase contains antibiotic substances, separated from aqueous phase. It was evaporated to dryness in water bath at 80°-90°C and the residue obtained was used to determine antimicrobial activity (Liu *et al.*, 1986).

Determination of antibacterial activity

Antibacterial activity was determined by disc diffusion method (Moshi *et al*., 2006). Test organisms (0.5 McFarland turbidity standards) were swabbed on mueller- hinton agar plates. Sterilized 5 mm-Whatman No. 1 filter paper discs were used in this method. The discs were soaked into the dissolved crude extracts for a minimum of two hours. Gentamicin disc was used as standard. By use of sterile forceps, both discs were placed equidistantly onto each of the inoculated plates. The plates were incubated at 37°C for 18-24 hours and examined. Antimicrobial activities were determined by measuring the diameters of zones of inhibition in mm.

HPTLC analysis

The crude extract was carried out for HPTLC analysis (Hubicka *et al*., 2009). It was performed on 10 × 10 cm aluminum backed plate coated with silica gel. 5 µl of the ethyl acetate fraction and reference antibiotic (gentamicin) were applied on the plate as bands by sample applicator equipped with a 100-µL Hamilton syringe. A small amount of methanol: ammonia: chloroform (3:2:1), as mobile phase was used. Chromatogram was scanned at 254 and 366 nm with a Camag TLC scanner ш.

RESULTS AND DISCUSSION

Identification of actinobacteria

The natural habitat of most actinobacteria is the soil but they can also be found in aquatic environment. Five actinobacteria were isolated from the soil samples. All the isolates were Gram-positive, yellowish white to gray in color having single or long chain of spores. They were characterized as Streptomyces, *Micromonospora* and Nocardia sp (Table 1). *Micromonospora* sp. was screened for antibacterial potential.

Antibacterial activity

Many actinobacteria produce secondary metabolites in the stationary phase. Determination of antibacterial potential against selective pathogen is essential for proper therapy. Primary screening was used to determine the range of microorganisms that are sensitive to the antibiotic and secondary screening was crucial to select the isolates for further studies. *Micromonospora* sp. showed antibacterial activity against pathogenic E.coli, *Bacillus* and Staphylococcus aureus in primary screening. Extract showed highest antibacterial activity against *E. coli* (38 mm) followed by *S. aureus* (32 mm) and nil activity against *Bacillus*. Gentamicin was used as standard control which showed 30 mm diameter zone of inhibition for *E. coli* and 11 mm for *S. aureus*. (Fig: 1).

HPTLC analysis

When chromatogram was visualized under UV rays, extract showed five spots. Retention factor of the moved spots were: 0·58, 0·76, 0·81, 0·97 and 0·85. (Table: 2, Fig: 2, 3). According to the HPTLC analysis, the Rf value of one compound is similar to the reference antibiotic.

Table 1: Morphological and biochemical characterization of actinobacteria:

Characteristics	**Streptomyces sp**	**Nocardia sp_1**	**Nocardia sp_2**	**Nocardia sp_3**	***Micromonospora* sp**
Nature and color of colony	Yellow colony embedded in agar	Hairy, pinkish white	Smooth, white colony	Powdery, creamish-white colony	Powdery, white to gray colony
Type of spore	Long chain of spore	Long chain of spore	Long chain of spore	Long chain of spore	Monosporo-phore
Gram staining	+	+	+	+	+
Catalase	+	+	+	+	+
Starch hydrolysis	-	-	-	-	Weekly +
Indole	-	-	-	-	-
Methyl red	+	+	+	+	+
VP	-	-	-	-	-
Citrate utilization	+	+	+	+	+

(+) Positive, (-) Negative

Table 2: Rf values of metabolites

S. No.	Substance Name	Rf Value
1.	**E**	**0.97**
2.	D	0.85
3.	C	0.81
4.	B	0.76
5.	A	0.58

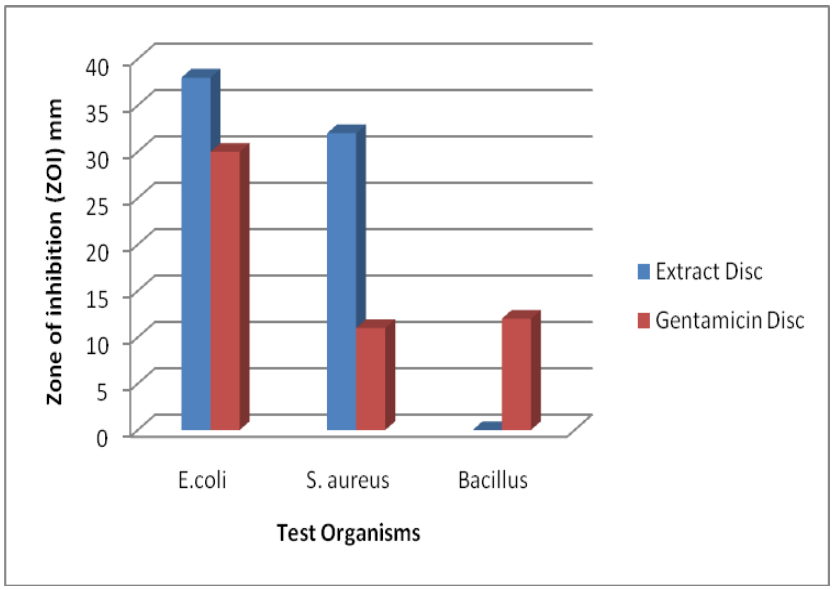
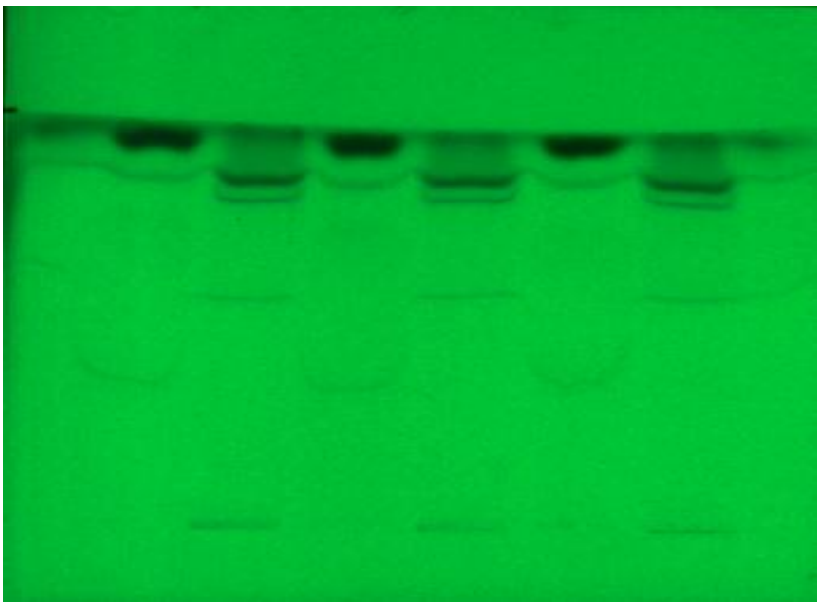


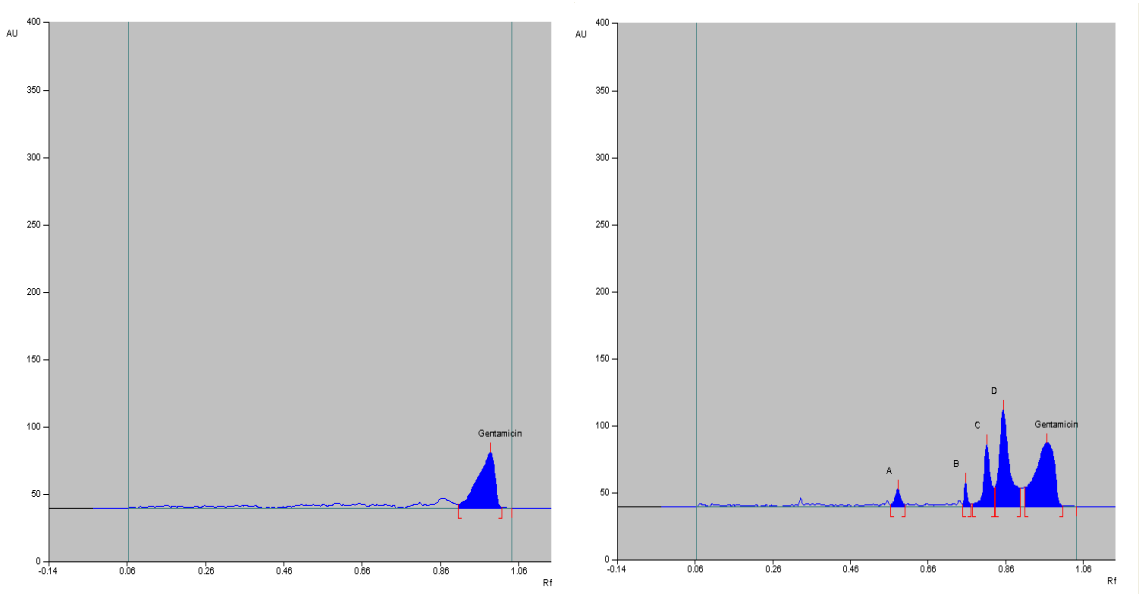
Fig 1: Antibacterial activity of crude extract and gentamicin



R1 T1 R2 T2 R3 T3

R=Reference, T= Test sample

Fig 2: TLC plate at 254 nm



Reference antibiotic **Ethyl acetate fraction**

Fig 3: Chromatogram of reference and crude extract

CONCLUSION

Antibacterial metabolite extracted by ethyl acetate was more effective and gave zone of inhibitions of 32-38 mm against the used pathogenic strains which were even more than the zone of inhibition shown by the reference antibiotic. The result in the present investigation is not only comparable even better in some cases. Thus, in this study we found that *Micromonospora* is a potential source of new antibacterial compounds and it can be used for the treatment of various bacterial infections.

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