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Isolation and partial characterization of actinomycetes with antimicrobial activity against multidrug resistant bacteria

Smriti Singh¹, Pramod Kumar², N Gopalan², Bhuvnesh Shrivastava³, RC Kuhad³, Hotam Singh Chaudhary^{1*}¹ Department of Biotechnology, Madhav Institute of Technology & Science, Gwalior-474005, India² Defense Research and Development Establishment, Gwalior-474002, India³ Lignocellulose Biotechnology Laboratory, Department of Microbiology, University of Delhi, N Delhi, India

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ABSTRACT

Objective: To isolate strains of Actinomycetes from different locations of Gwalior to evaluate its antimicrobial activity against multidrug resistant pathogenic strains. **Method:** Soil samples collected from different niche habitats of Gwalior were serially diluted and plated on selective media. Potential colonies were further purified and stored in agar slants and glycerol stocks. Isolates were biochemically characterized and purified isolates were test against pathogenic microorganisms for screening. Isolates with antagonistic properties were inoculated in production media and secondary metabolites or antimicrobial products were extracted. **Result:** The seven actinomycetes strains showing maximum antibacterial activity were isolated further characterized based on their colony characteristics and biochemical analyses. The isolates were screened for their secondary metabolites activity on three human pathogenic bacteria are *Escherichia coli* (*E. coli*), Methicillin-Resistant *Staphylococcus aureus* (*S. aureus*) and Vancomycin-Resistant *Enterococci* (VRE). **Discussion:** The strain MITS 1005 was found to be more active against the test bacteria.

1. Introduction

Actinobacteria, high guanine and cytosine ($\geq 55\%$) containing bacteria is one of the dominant phyla of the bacteria found on almost natural substrates^[1,2]. They play an important role in decomposition of organic materials and carbon cycle. The taxonomy of the actinomycetes has been subject to unending controversy because of its filamentous, branching growth which resembles with a fungal type of morphology^[3].

Actinomycetes represent a high proportion of the soil microbial biomass and have the capacity to produce a wide variety of antibiotics and extracellular enzymes^[4,5]. Most of the known natural antibiotics are produced from actinomycetes^[5]. Moreover, these are important source for novel antibiotics and hence having a high pharmacological and commercial interest including control of infectious diseases^[6–8]. Medical or economic significant Actinobacteria mainly lies in

subclass Actinobacteridae, order Actinomycetales. The order Actinomycetales is composed of approximately 80 genera, nearly all from terrestrial soils, where they live primarily as saprophytes.

Actinomycetes are important sources of new bioactive compounds such as antibiotics and enzymes^[6,9,10] which have diverse clinical effects and are active against many pathogenic organisms Actinomycetes and their bioactive compound show antibacterial and anti microbial against various pathogens and multi drug resistant pathogens e.g. Vancomycin-Resistant *Enterococci*, Methicillin-Resistant *Staphylococcus aureus* (*S. aureus*), *Shigella dysenteriae* (*S. dysenteriae*), *Klebsiella* sp. and *Pseudomonas aeruginosa* (*P. aeruginosa*) etc^[11–14]. The need for new, safe and effective antimicrobial agent is the major challenge to the pharmaceutical industry now a days, especially with the obvious increase in opportunistic infections in the immune compromised host via and multiple drug resistant strains^[10].

Among all the known microbes, members of the actinomycetes genus especially *Streptomyces* species have been recognized as prolific producer of useful

*Corresponding author: Hotam Singh Chaudhary, Assistant Professor, Department of Biotechnology, Madhav Institute of Technology & Science, Gwalior – 474005, India.

Tel: (0751) 2409352

E-mail: hotamsingh@gmail.com

bioactive metabolite with broad spectrum of activities which has antibacterial, antifungal, antibiotic, antiphlastic, antitumor, antiviral, insecticide, herbicide, immunomodulators, antithrombotic agents[15]. Thus screening and isolation of promising strains of actinomycetes with potential antibiotics is a thrust area of search since many years. Streptomycetes are widely used in industries due to their ability to produce numerous chemical compounds including antibiotics, enzymes and anti-tumor agents[7,16].

Actinomycetes are ecological diverse group of bacteria, constitute the major microbial population in soil producing active secondary metabolites. As there is geographic variation in Indian soil type and their contents, hence it is quite likely that the distribution of antibiotic producing actinomycetes is also variable. Therefore, exploration of unexplored ecosystems for actinomycetes is necessary for the identification of novel antibacterial metabolites. The objective of present study was isolation of actinomycetes from different soil samples (Gwalior) for screening of antibacterial compounds against multidrug resistant bacteria of clinically relevance.

2. Materials and methods

2.1. Soil sampling

Soil samples were collected from different niche habitats of Gwalior (Hostel zone of MITS, Cancer hospital territory, IITM ground, Kuldeep nursery, Near MITS drainage, forest areas of Sanjeevani nursery). The samples can be collected by inserting a polyvinyl corer (previously sterilized with alcohol) into the sediments. The corer is sterilized with alcohol before sampling at each station.

The central portion of the top 2 cm sediment sample can be taken out with the help of a sterile spatula. This sample can be transferred to a sterile polythene bag and transported immediately to the laboratory. The soil samples were air-dried for one week at RT, crushed in mortar and pestle to make fine particles, sieved and used for actinomycetes isolation[12].

2.2. Isolation and microbiological analysis

One gram of soil sample was 10 fold serially diluted sterile distilled water and plated on nutrient agar (NA, pH 7.2), casein starch agar (CSA, pH 7.2), glycerol asparagine agar and yeast extract glycerol agar[4]. The inoculated plates were incubated for 1 week at 28 °C. The suspected colonies for actinomycetes were selectively isolated and transferred to actinomycetes isolation agar medium with the help of loop inoculum method[17]. The

different Coloration of aerial and substratum mycelia and diffusible pigments at bottom of inoculated plates were observed[4]. The seven strains of actinomycetes were selected on the basis of mycelium coloration and diffusible pigments.

The morphology of the filaments or mycelium was determined by light microscopy. The gram's stain was used to determine positive and negative reaction of actinomycetes with the help of Nikon photo micrographic unit at the magnification of 100 ×.

2.3. Biochemical characterization

The seven isolates of the actinomycetes were used for biochemical studies. The various biochemical tests (Catalase test, Casein hydrolysis, Starch hydrolysis, Indole test, Triple Sugar Iron (TSI) agar) were performed for the identification of the potent isolates. All the cultures were incubated at 28 °C for 24–48 h.

2.4. Extraction of secondary metabolites

Actinomycetes isolates were inoculated for submerged fermentation. It was carried out in 100 mL starch casein agar medium pH 7.2 in a 250 mL capacity conical flask under sterile conditions. Flasks were lodged on the flask shaker at a speed of 110 rpm at room temperature for one week. After one week fermentation, the medium was found to change in turbidity. The culture was harvested and centrifuged to remove cells and debris and the resultant broth was added with equal volume of ethyl acetate to extract secondary metabolites[13].

2.5. Screening for antibacterial activity

The secondary metabolites were screened for antibacterial activity against multidrug resistant bacteria (Methicillin-Resistant *Staphylococcus aureus*, Vancomycin-Resistant *Enterococci*, *Escherichia coli* ATCC 25922) [5,9,15].

The antibiotic sensitivity of test strains was determined by the standard Disc diffusion method against a number of antibiotics. The potency of antibiotics per disc was as follows, clindamycin (10 µg/disc), methicillin (15 µg/disc), erythromycin (15 µg/disc), linezolid (15 µg/disc), pristinamycin (15 µg/disc), vancomycin (30 µg/disc), gentamicin (30 µg/disc), ciprofloxacin (30 µg/disc) (Table 1). All antibiotic discs were purchased from the Hi-Media Pvt. Ltd. (Bombay, India). The antibacterial activity was performed according to CLSI, USA guidelines on Mueller Hinton Agar well medium using diffusion method[18,19].

3. Result

Table 1.

Antibiotic susceptibility pattern of multidrug resistant bacteria for MRSA and VRE show resistance to methicillin, erythromycin, clindamycin, prestniamicin and gentamicin and sensitive to vancomycin, linezolid and ciprofloxacin respectively.

Strain	Methicillin	Vancomycin	Erythromycin	Clindamycin	Prestniamicin	Gentamicin	Linezolid	Ciprofloxacin
MRSA	R	S	R	R	R	R	S	S
VRE	R	R	R	R	R	R	S	S

R: resistant, S: sensitive.

Table 2.

Only isolate MITS1005 out of seven isolates show maximum antibacterial activities against pathogenic bacteria *Escheria coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, Methicillin resistant–*Staphylococcus aureus* and Vancomycin resistant–*Enterococci*.

Bacteria	Zone of inhibition (diameter in mm)						
	MITS1001	MITS1002	MITS1003	MITS1004	MITS1005	MITS1006	MITS1007
<i>E. coli</i> ATCC 25922	–	–	–	–	14	–	–
<i>S. aureus</i> ATCC 25923	–	–	–	–	14	–	–
MRSA	–	–	–	–	14	–	–
VRE	–	–	–	–	13	–	–



Figure 1. Mycelium and diffusible pigment.

Actinomycetes have been intensively studied in several underexplored environments, niche and extreme habitats in various parts of the world (including India) in the last few years. Yet there is no report regarding isolation of Actinomycetes from Gwalior city (India). Therefore, the soil samples were collected from different parts of the Gwalior city and made an attempt to isolate Actinomycetes strains. The seven actinomycetes strains were isolated from different area of soil samples based on the colony morphology, mycelium coloration and pigment diffusion (Figure 1).

The biochemical properties such as Catalase, Casein hydrolysis, Starch hydrolysis, Indole activity and Triple Sugar Iron (TSI) Agar of actinomycetes isolates were studied. All the isolates showed the positive test with catalase, starch and casein utilization. It was observed that none of the isolates showed indole

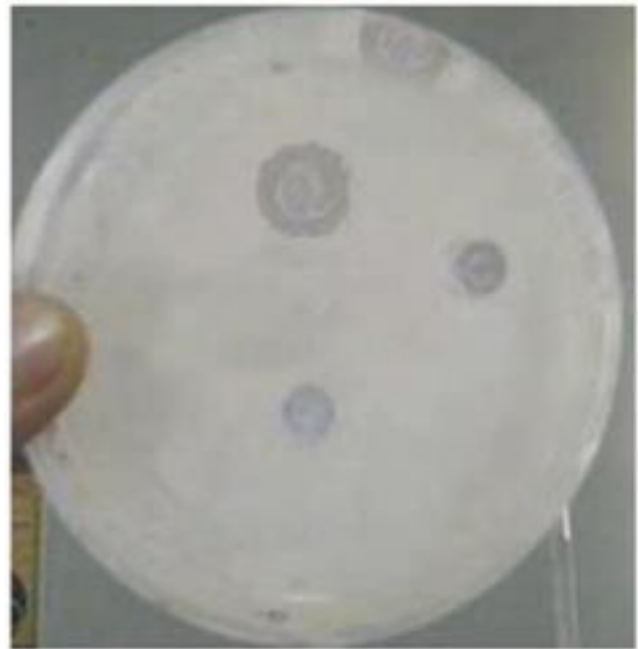


Figure 2. Antibacterial activity of actinomycetes MITS isolate against VRE.

and TSI utilization. The isolates were screened for their inhibitory activity against the human pathogenic bacteria *E. coli*, *S. epidermis* and MDR (VRE) (Table 1) (Figure 2).

4. Discussion

The result showed that all the isolates were able to inhibit the extracellular growth of filaments in the test organism. The isolates were not able to inhibit intracellular growth of mycelium. The reason might be the non-reaching of the isolate extract to intracellular cell of the test organism and non-denaturation of the bacterial cell wall by the isolate's extract. Although, the maximum potential to inhibit the extracellular mycelium growth of the test bacteria were found in isolate 5 with the maximum zone of inhibition of 14 mm.

Mycelium color and diffusible pigment of actinomycetes isolates MITS 1001 produce black mycelium and no pigment, MITS 1002 produce black mycelium and pigment, MITS 1003 produce black mycelium and no pigment, MITS 1004 red and pink pigment, MITS 1005 produce dark brown mycelium and yellow pigment, MITS 1006 produce white gray mycelium and no pigment, and MITS 1007 produce brown and yellow pigment.

All seven actinomycetes isolates show positive results for catalase, Starch Utilization and Casein Utilization and showed negative results for Indole and Triple Sugar Iron biochemical tests.

It was found that the isolate 5 had broad spectrum antimicrobial activity as it suggested its potential against Gram positive and Gram negative bacteria and Vancomycin-Resistant *Enterococci*.

The Gwalior city is rich in biodiversity of flora, fauna and also for microbial diversity. Therefore, it is suggested for intensive studies on the actinobacterial diversity of the region to establish the rich actinomycetes diversity and could put an important input into pharmaceutical industries.

Conflict of interest statement

We declare that we have no conflict of interest.

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