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ANTIFUNGAL ACTIVITY OF ACTINOMYCETES AGAINST PHYTOPATHOGENS OF *GLYCINE MAX*.OF CHAMBAL REGION (M.P.) INDIA

Charu Singh*, Ramendra Singh Parmar, Pragya Jadon, Gaurav Bhadauriya and Ajay Kumar

Dept. of Life Sciences, ITM University, Gwalior (M.P.)

Corresponding author email*: c_mbjadon@yahoo.co.in

ABSTRACT

Soybeans are a particularly important legume crop because of their high protein contents. Soybean has many benefits for human and animal nutrition. Fungal phytopathogens cause serious problem worldwide in agriculture. The objective of the present study was to isolate antifungal metabolites from actinomycetes isolated from different

regions of Madhya Pradesh. Total 80 strains of actinomycetes isolated from the soils of different habitats of Chambal region, Madhya Pradesh, were evaluated for their ability to inhibit plant pathogens Macrophomina phaseolina, Fusarium oxysporum, Collectotrichum truncatum, Rhizoctonia solani in vitro. Entire isolates were screened for their antifungal activity by agar overlay method against phytopathogenic fungi. After screening, out of these only one actinomycetae ACITM-1 showed antifunagal activity against Macrophomina phaseolina and Collectotrichum truncatum

Keywords: Antifungal activity; Habitat; Phytopathogens; Soybean.

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1. INTRODUCTION

Actinomycetes are prokayotes which have a hyphal (hence fungal-like) morphology. Most of the actinomycetes described are soil microorganisms and are active in the decomposition of plant tissue, and thereby in the recycling of carbon and nitrogen [1]. Actinomycetes are diverse group of Gram positive bacteria that usually grow by filament formation. They belong to the order Actinomycetales (Superkingdom: Bacteria, Phylum: Firmicutes, Class: Actinobacteria, Subclass: Actinobacteridae) [2]. They are free living, saprophytic bacteria and a major source for the production of antibiotics [3], widely distributed in natural and manmade environments and play an important role in the degradation of organic matter [4], [5]. Microorganisms are virtually unlimited sources of novel compounds with many medicinal and agricultural applications. Actinomycetes, among them, hold a prominent position due to their ability to produce numerous different metabolites such as antibiotics, enzymes and inhibitors [6]. *Actinomycetes* are known to be greatest sources of bioactive metabolites which has antibiotic antiparasitic, antitumor, insecticide, herbicide, alkaloid, enzyme inhibitor, immunoactive peptide, antithrombotic agent, and so forth [7].

Fungal phytopathogens pose serious problems worldwide in the cultivation of economically important plants. Fungal phytopathogens cause problems worldwide in agriculture and food industry especially in the subtropical and tropical regions. In addition, many also produce mycotoxins, which are harmful to humans and livestock. Biological control has been described as a nonhazardous strategy to reduce crop damage caused by plant pathogens when compared to the exclusive use of the chemical control of plant diseases. Thereby, reducing the use of agrochemicals and also maintains the crop productivity without damage to the ecosystem. Several rhizospheric bacteria and actinomycetes are used as biological control agent. They have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. Therefore, actinomycetes hold a prominent position due to their diversity and proven ability to produce new compounds.

2. MATERIALS AND METHODS

2.1 Collection of soil samples- soil samples were collected from different areas of Gwalior such as garden area, agricultural field, playground area, poultry farms and stored in sterile plastic bags, labeled in the field and stored at 4°C until use. Soil samples were carefully taken with spatula down to a 10 cm depth into the soil. All the samples were dried at room temperature before isolation.

2.2 Isolation of actinomycetes- Isolation of actinomycetes was performed by serial dilution technique on different media such as starch casein agar, soil extract agar, ISP medium, Nutrient agar, glycerol aspargine agar, starch agar, Yeast extract malt extract agar, actinomycetes isolation agar media and incubated for 6-7 days at 30°C [8]. The isolated actinomycetes culture were further purified on respective fresh media and stored in BOD incubator.

2.3 Isolation of test phytopathogens

The fungal pathogens *Rhizoctonia solani*, *Fusarium solani*, *Macrophomina paseolina* and *Collectotrichum truncatum* were isolated originally from different naturally diseased plant leaves collected from different soybean agricultural fields in Gwalior. The isolated fungi were grown on potato dextrose agar (PDA) plates and incubated at 28°C for 4 to 6 days. Detected isolates were then transferred into slant of PDA and kept at 4°C for further studies. Pure cultures of the isolated fungi were identified according to the cultural properties, morphological and microscopical characteristics of each fungus [9], [10], [11].

2.4 Screening for antifungal activity of Actinomycetes

Among 80 isolated actinomycetes only one actinomycete (ACITM-1) was selected on the basis of screening. All isolates were screened for their *in vitro* antifungal activity against the tested pathogenic fungi. Screening was done on the basis of primary and secondary method. Primary screening was done by the cross streak plate method [12]. In this method firstly prepared the potato dextrose agar media and poured into the plate then transferred the fungi at the corner of the plate and put actinomycetes at other corner of plate then incubated it for 4 days at 37ºC [13], [14].

Secondary screening was done by the well diffusion method. In this method prepared two different types of media i.e. starch casein broth and potato dextrose agar then inoculated ACITM-1 in two flask of starch casein broth. Flasks were incubated for 5 days in orbital shaker then prepared the potato dextrose agar media and made two wells in each plate using well cutter and each well was loaded with 150 µl of starch casein broth in the wells and put the *Macrophomina phaseolina* and *Collectotrichum truncatum* in each plate. Plates were incubated for 6-7 days at 37 ºC. A dead zone was formed around the well [15].

2.5 Cultural, morphological, physiological and biochemical characteristics of actinomycetae (ACITM-1)

Cultural characteristics such as color of aerial mycelium, color of substrate mycelium and pigmentation of the selected actinomycetes isolates were recorded on starch agar medium and starch casein agar medium.

2.6 Gram Staining

Smear was prepared by spreading the broth culture on a glass slide followed by heat drying. The smear was covered with crystal violet for 30-60 s and washed off with water. The smear was covered with Gram's iodine for 30-60 s, decolorized with alcohol, and washed with water. Finally the smear was stained with safranin counter stain for 45 seconds. After washing and drying, the slides were viewed under microscope. Spore morphology was observed by simple staining and mycelial morphology was done by slide culture method followed by Sudan black stain [16], [17].

2.7 Biochemical Characterization

After preliminary studies, the isolate which was found to be positive was selected for biochemical studies. Biochemical tests generally used were gelatin hydrolysis, starch hydrolysis, acid production from different sugars, hydrogen sulfide production test, motility test, citrate utilization test, indole test, methyl red test, Voges-Proskauer test, and catalase test [18], [19], [20].

3. RESULTS

Isolation and screening of actinomycetes

Out of 80 species of actinomyecetes isolated from different soil samples of different regions only one actinomycetes ACITM-1 were found which produced secondary metabolite against phytopathogenic fungi. Different ISP media were used for isolation of actinomycetes but maximum & fast growth of actinomycetes was found on Starch Casein Agar (SCA) media.

3.1 Cultural, morphological, physiological and biochemical characteristics of Isolates

Media used	growth	Aerial mycelium	Substrate mycelium
Yeast extract malt agar	Good	Grey	White
Starch agar	Good	Grey	White
Starch casein agar	Excellent	Grey	White
Soil extract agar	Good	Grey	White
ISP medium	Good	Grey	White
Actinomycetes isolation agar	Good	Grey	White
Glycerol asparagines agar	Good	Grey	White

Table 1- Cultural characteristics of the actinomycetes ACITM-1 on different ISP media

3.2 Morphological and Biochemical characterization:

Based on morphological and biochemical characteristics, isolate ACITM-1 was to be closely related to be genus *Streptomyces.* This gram positive organism has of grey aerial mycelia and white substrate mycelium. When observed under the scanning electron microscope (SEM) appeared rod like structures (chains of cells) and often branched to form a network of filaments (mycelium), smooth surface of the spore (5μm in length) developed on the terminal of the aerial mycelium.

CHARACTERISTICS	**ACITM-1**
Morphological characteristics	
Hyphae	present
Mycelium	present
Staining	Gram's +ve
Spore chain	Spiral
Spore mass colour	White
Substrate mycelium colour	Yellow

Table 2- Morphological characteristics of ACITM-1

Biochemical characteristics	**ACITM-1**
Citrate utilization test	+ve
Hydrogen sulfide production test	-ve
Catalase production test	+ve
Nitrate reduction test	+ve
Gelation hydrolysis test	-ve
Casein hydrolysis test	+ve
Starch hydrolysis test	-ve
Carbohydrate tests	
Glucose	AG
Xylose	G
Fructose	AG
Arabinose	AG
Sucrose	G
Ribose	G
Galactose	G
Maltose	AG
Rhaminose	G
Raffinose	G

*AG– Acid Gas and G- Gas

Table 3- Biochemical characteristics of ACITM-1

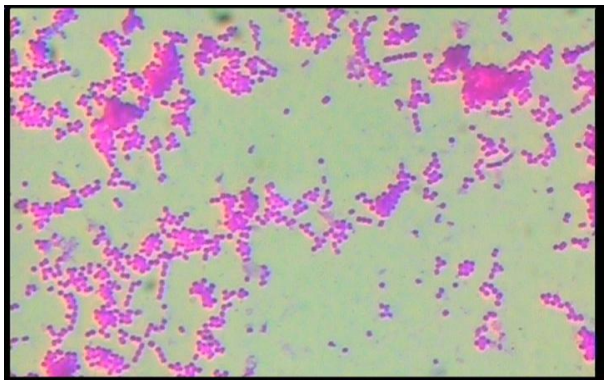

Figure1- Microscopic view of actinomycetes under 100x resolution

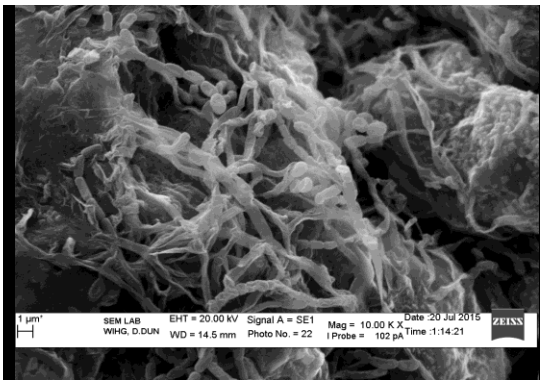

Figure 2- Scanning electron microscopy of actinomycetes

3.3 Determination of the Antifungal Activity

The antifungal activities of the Actinomycetes isolate against test fungal pathogens were showed in (Table 4). This study showed that the ACITM-1 isolate has good antifungal activity against *Macrophomina phaseolina* than *Collectotrichum truncatum.*

Table 4- Antifungal activity of actinomycetes of ACITM-1

Name of phytopathogens	Zone of inhibition (mm.)
Macrophomina phaseolina	22
Collectotrichum truncatum	16

4. CONCLUSIONS

The result of present study was that actinomycetes showed significant antagonistic activities against some important phytopathogenic fungi of soybean and reported first time from soil of chambal region. It is suggested that further studies on actinomycetes present in the chambal region's soil could provide novel species as well as novel antibiotics. It has been observed that the isolated actinomycete has inhibited the growth of two test phytopathogenic fungi. It is expected that the current attempt of isolation, characterization and the study on soil actinomycetes will be useful for identification of new antibiotics effective against challenging pathogens.

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