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Biocontrol Potential of Actinomycetes Against Xanthomonas-Induced Oily Spot in Pomegranate

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ABSTRACT

Punica granatum (Pomegranate) faces significant challenges due to diseases such as oily spot caused by the bacterium Xanthomonas axonopodis pv. punicae, which severely affects fruits quality and yield. Actinomycetes are known for their versatility in production of antagonistic compounds. This study aimed at isolation and characterization of antagonistic actinomycetes from natural sources for controlling oily spot disease in pomegranates. Soil samples were collected from diseased orchards and other natural habitats like the sites where spoiled and waste pomegranates and peels are dumped. Serial dilution technique and Glycerol Aspargine agar and Actinomycete agar were used for isolation and screening of the actinomycetes against the pathogen. The pathogen of oil spots was isolated from alcohol disinfected spots and using Potato dextrose agar, Starch agar. Promising strains of actinomycetes demonstrated significant inhibition of the pathogen. The isolates were characterized based on their colony morphology, pigmentation, spore pattern and biochemical properties. The findings suggest that certain actinomycetes (Streptomyces spp. and Micromonospora spp.) have potential as biocontrol agents offering an eco- friendly alternative to chemical treatments for managing oily spot disease in pomegranates. Further study is in progress to optimize the use of these strains in sustainable agricultural practices.

Keywords: *antagonistic actinomycetes, Xanthomonas axonopodis pv. punicae, oily spot disease, biocontrol, pomegranate, etc*

1. INTRODUCTION

Pomegranate (Punica granatum), a fruit cultivated extensively in tropical and subtropical regions, is renowned for its sweet and acidic taste, as well as its numerous medicinal properties [1]. This fruit holds significant economic value, contributing to the livelihoods of many farmers and playing a crucial role in the agricultural economy of several countries [2]. However, the production of pomegranates faces severe challenges due to various diseases, with oily spot disease,caused by the bacterium *Xanthomonas axonopodis pv. punicae*, being particularly detrimental [3].

Oily spot disease is characterized by the appearance of black oily spots on the fruit and leaves, which not only affects the aesthetic quality of the fruit but also leads to substantial reductions in yield and market value [4]. The disease can cause premature leaf drop, fruit cracking, and in severecases, complete crop loss [5]. This has significant economic implications for farmers, as the affected fruits are often deemed unmarketable [6].

Traditional chemical treatments for controlling oily spot disease have been widely used; however, they have faced considerable criticism [7]. These treatments often have a negative impact on the environment, contributing to soil and water pollution [8]. Additionally, the overuse of chemical pesticides has led to the development of resistance in pathogens, rendering these treatments less effective over time [9]. There are also growing concerns about the safety of these chemicals for human health, as residues can remain on the fruit and enter the food chain [10].

In response to these challenges, there is a growing interest in exploring biological control methodsas a more sustainable and environmentally friendly alternative. Among the various biological agents, actinomycetes have emerged as promising candidates. Actinomycetes are a group of Gram-positive bacteria known for their ability to produce a wide range of secondary metabolites, including antibiotics, enzymes, and other bioactive compounds [11]. These metabolites have beenshown to inhibit the growth of various plant pathogens, making actinomycetes a potential tool formanaging oily spot disease in pomegranates [12].

This study aims to isolate and characterize actinomycetes from natural sources, such as soil and plant material, to evaluate

their antagonistic activity against *Xanthomonas axonopodis pv. punicae*.By identifying effective strains of actinomycetes, this research seeks to provide a sustainable and eco-friendly solution for pomegranate farmers, ultimately enhancing the productivity and profitability of pomegranate cultivation

2. METHODOLOGY

Sample Collection

Soil samples were collected from two main sources: diseased pomegranate orchards, particularly from areas showing visible signs of oily spot infection, and natural habitats such as dumping sites for spoiled pomegranates and peels. Samples were taken at a depth of 10 cm and transported to the laboratory in sterile polythene bags for further processing [6].

Isolation of Actinomycetes

The soil samples were subjected to serial dilution, followed by inoculation onto Glycerol Asparagine agar and Actinomycetes isolation agar. Plates were incubated at 30°C for 4-7 days. After incubation, actinomycetes colonies exhibiting distinct morphological characteristics were picked and streaked on fresh media for purification [11]. The isolates were coded and stored in glycerol stocks for further studies.

Isolation of the Pathogen

The oily spot pathogen, *Xanthomonas axonopodis pv. punicae*, was isolated from the diseased pomegranate fruits. The fruits were disinfected with 70% ethanol, and the affected areas were excised and placed onto Potato Dextrose Agar (PDA) and Starch Agar. After incubation at 28°C for 48 hours, colonies resembling *Xanthomonas* showing typical yellow-coloured mucoid colonies were further purified. The isolates were further tested for their ability to produce xanthomonad pigment, which is confirmatory test for the genus *Xanthomonas*. The confirmed isolates were further subjected to biochemical and morphological characterization [13].

Pathogenicity test

Healthy pomegranate fruits were collected for pathogenicity tests. The fruits were surface sterilized, punctured and sprayed with a bacterial cell suspension, with a control group sprayed with sterile saline. Disease symptoms were monitored and after their appearance, the pathogens were reisolated and compered with the original isolates based on morphology and biochemical characteristics. The identification of the isolate was confirmed using morphological, biochemical data and comparison with Bergey's Manual of Systematic Bacteriology [13].

Screening for Antagonistic Activity

The actinomycetes isolates were screened for their antagonistic activity against *Xanthomonas spp*. using the perpendicular streak method. Actinomycetes were streaked across the center of the agar plates, while the pathogen was streaked perpendicular to the actinomycetes streaks. The plates were incubated at 28°C for 48 hrs, and zones of inhibition were recorded [12].

Characterization of Actinomycetes

Actinomycetes isolates showing promising antagonistic activity were characterized based on colony morphology, pigmentation, spore pattern, and biochemical properties. Morphological observation was performed using the slide culture technique, while biochemical tests such as catalase, oxidase, and starch hydrolysis were conducted to identify the actinomycetes [11].

3. RESULTS AND DISCUSSION

Isolation and characterization of the isolates:

Two isolates of pathogens were obtained from infected pomegranate fruits. These displayed typical yellow mucoid colonies on PDA and Starch agar plates [Table 1]. A total of six actinomycetes isolates were obtained from the collected soil samples. All the isolates exhibited significant inhibitory effects against the isolates from the infected fruit with inhibition zones ranging from 15 to 25 mm [Table 4]. These findings corroborate earlier studies that highlight the potential of actinomycetes in controlling plant pathogens. Biochemical characterization confirmed its identity,

with positive catalase and starch hydrolysis reactions [Table 3], consistent with previously reported characteristics of the oily spot pathogen.

Table 1. Results of colony and morphological characterization of isolates obtained from diseased fruits samples:

Isolates	Xan 1	Xan 2

Colony characters on Potato dextrose agar and Starch agar after 24 hrs at 28^0C		
Size	1mm	1mm
Shape	Circular	Circular
Colour	Yellow	Yellow
Margin	Entire	Entire
Elevation	Raised	Raised
Pigment	Yellowish	Yellowish
Opacity	Opaque	Opaque
Consistency	Moist	Moist
Odour	Soury	Soury
Morphological and staining properties		
Gram nature	Gram -negative	Gram -negative
Morphology	Rod shaped	Rod shaped
Capsule	Present	Present
Motility	Motile	Motile

Table 2. Results of biochemical characterization of isolates obtained from diseased fruits samples:

Isolates	**Xan 1**	**Xan 2**
Biochemical Tests		
Hugh and Leifson test	Aerobic oxidative	Aerobic oxidative
Catalase production	+	+
Oxidase production	_	_
Amylase production	+	+
Caseinase production	+	+
Nitrate reductase production	_	_
Gelatinase production	_	_

Phenylalanine deaminase production	_	_
H2S production	_	_
Indole production	_	_
Methyl Red test	+	+
Voges-Proskauer test	_	_
Citrate utilization test	_	_
Glucose fermentation	+	+
Lactose fermentation	_	_
Sucrose fermentation	+	+
Dextrose fermentation	+	+
Mannitol fermentation	_	_

Table 3. Results of colony and morphological characterization of actinomycetes isolates obtained from soil samples:

Isolates	**A1**	**A2**	**A3**	**A4**	**A5**
Colony characters of the isolates on Actinomycetes Isolation agar and Glycerol Asparagine agar					
Size	2mm	1mm	3mm	1mm	2mm
Shape	Circular	Circular	Circular	Circular	Circular
Colour	white	White	White	White	Yellowish white
Margin	Entire	Entire	Entire	Entire	Entire
Elevation	Concave	Concave	Concave	Concave	concave
Pigment	Brown	Non-pigment	Brown	Brown	Non-pigment
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque
Consistency	Dry	Dry	Dry	Dry	Dry
Odour	Earthy smell	Earthy smell	Earthy smell	Earthy smell	Earthy smell
Morphological characteristics					
Gram nature	Gram positive	Gram positive	Gram positive	Gram positive	Gram positive
Motility	Non motile	Non motile	Non motile	Non motile	Non motile

Table 4. Results of antagonistic activity of *Actinomycetes* isolates against pathogenic *Xanthomonas* isolates:

Isolate	A1	A2	A3	A4	A5
Xan 1	1.8 cm	1.1 cm	0.8 cm	1.9 cm	1.4 cm
Xan 2	1.7 cm	1.3 cm	1.0 cm	1.8 cm	1.1 cm

4. DISCUSSION

The results of this study demonstrate the potential of *Actinomycetes spp.* as biocontrol agents against *Xanthomonas spp.*, the pathogen of the oily spot disease. Among the six actinomycetes isolates, showing inhibition zones ranging from 0.8 to 1.9 cm. These findings support existing literature on the plant pathogens through the production of secondary metabolites, including antibiotics, making them suitable candidates for developing eco-friendly treatments for oily spot disease [12].

The biochemical and morphological characterization of both the pathogenic and actinomycetes isolates further confirmed their identities. The *Xanthomonas* isolates were consistent with previous reports, displaying yellowish, mucoid colonies and testing positive for catalase and amylase production [3, 13]. The actinomycetes isolates also exhibited characteristics typical of their genus, such as earthy odor and dry colony morphology. Their Gram- positive nature and ability to inhibit the pathogen underline their potential as biocontrol agents [11]. The results of this study offer a promising basis for future research aimed at field applications and formulation of biocontrol products [7, 8].

5. SUMMARY AND CONCLUSION

This study successfully isolated and characterized antagonistic actinomycetes from natural sources, including diseased pomegranate orchards. The findings confirm that certain actinomycetes exhibit strong antagonistic activities against *Xanthomonas spp.* the causative agent of oily spot disease in pomegranates. Their ability to inhibit the pathogen through the production of secondary metabolites highlights the potential for these microorganisms to be developed into

biocontrol agents.

In conclusion actinomycetes represent a viable, environmentally sustainable alternative to chemical pesticides for managing oily spot disease. Further research is needed to optimize the application of these biocontrol agents under field conditions and to better understand the mechanisms through which they suppress pathogens. This study offers a promising avenue for improving pomegranate cultivation practices while reducing the reliance on synthetic chemical treatments

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