Microbiology Research Journal International

Volume 33, Issue 1, Page 17-23, 2023; Article no.MRJI.97207
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Isolation of Actinomycetes from *Arachis hypogaea* L. and *Gossypium herbaceum* L. for Screening Antibacterial and Antifungal Activities

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Authors' contributions

This work was carried out in collaboration among all authors. Author JSM designed the research and performed the research. Author KNO wrote the paper. Author BAJ reviewed and modified the paper. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2023/v33i11359

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/97207

Original Research Article

Received: 02/01/2023
Accepted: 04/03/2023
Published: 10/03/2023

ABSTRACT

The principal objective of the present study was to check the antimicrobial activity of Actinomycetes isolated from soil samples collected from the fields of *Arachis hypogea* L. and *Gossipium herbaceum* L. against different plant pathogenic strains. Various soil samples were isolated from fields located near the Junagadh district, Gujarat, India. Isolation was followed by a serial dilution process which was later plated on Actinomycete Isolation Agar (AIA) media. Potential colonies were subjected to screening, purification, and storage in glycerol stock. Morphological and Biochemical characterization of the isolates was performed. Isolated candidates were subjected to extraction for the production of the antimicrobial compound. The antimicrobial activity of the purified extract of isolates was tested. Total 30 actinomycete isolates were evaluated for antagonistic activity against pathogenic microorganisms.
Isolates C-25, C-15, and G-1 showed the best results in the decreasing order of their potency

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against fungal pathogens, and C-5, C-25, C-14, and C-13 showed the best results in decreasing order of potency against bacterial pathogens. 3 isolates inhibited all 4 test fungi. 10 isolates inhibited 3 test fungi**.** 11 isolates inhibited 2 test fungi. 6 isolates did not inhibit any test fungi. 4 isolates show potent inhibition. 15 inhibited *Macrophomina*. C-10 showed a 1 cm inhibition zone & G-1 showed a 0.8 cm zone of inhibition. 12 isolates gave 0.2-0.6 cm zone and 15 isolates gave negative results against *Macrophomina*. C-10 showed a very potent zone of inhibition of 0.7 cm. 9 isolates showed a 0.1-0.5 cm zone of inhibition. 20 isolates did not show inhibition against *Fusarium*. 1 isolate C-11(a) gave the 1cm potent zone of inhibition. 15 isolates gave the 0.7-0.2cm inhibition of the growth. 14 isolates gave negative results against *Alternaria* fungus. From these results, it was concluded that isolates had antibacterial and antifungal activities and could be used in the development of new antibiotics for pharmaceutical or agricultural purposes.

Keywords: Antifungal activity; antibacterial activity; actinomycetes; Arachis hypogea L.; Gossipium herbaceum L.

1. INTRODUCTION

Microbial diversity is a major frontier and future source for the biotechnology sector [1]. Microorganisms produced natural products that are a good source of antibiotics, including actinomycetes [2]. Actinomycetes are one of the most unique groups of filamentous bacteria and are well known for their metabolic versatility. Several studies showed actinomycetes have a vast range of biomedical applications such as antibacterial and antifungal activities [3]. Actinomycetes are gram-positive bacteria with high guanine and cytosine content of over 55% in their DNA [4]. They are responsible for the production of over 20,000 natural products extensively used in the pharmaceutical and agrochemical industries [5]. Actinomycetes have the ability to produce a wide range of secondary metabolites (e.g., antibiotics and extracellular enzymes) [6] which can inhibit the growth of several fungal and bacterial pathogens [7].

Novel antibiotics are currently in demand due to the increasing amount of antibiotic resistance. In 2050, it is predicted that death due to antibiotic resistance will reach 10 million people [8,9]. The isolation of actinomycetes from various and unique sources is an essential step to achieving that goal [10]. Actinomycetes are a group of microbes widely distributed across the world's natural ecosystems and are especially valuable for their organic cycling role [11]. Soil microorganisms provide an excellent resource for the isolation and identification of therapeutically important products. A huge number of actinomycetes have been isolated and screened from the soil in the past several decades, accounting for 75%-85% of relevant secondary metabolites available commercially [12].

The resistance problem demands to discover new antibacterial agents effective against resistant pathogenic bacteria and fungi. So, we need to screen more and more actinomycetes from different habitats for antimicrobial activity in the hope of getting some new actinomycetes strains that produce antibiotics, which have not been discovered yet and are active against drug-resistant pathogens [13].

2. METHODOLOGY

2.1 Soil sampling and Pretreatment

Soil samples from the rhizosphere of Cotton & Ground nut crops were collected from 15-20 cm depth. Soil samples were Sun-dried, crushed in a mortar and pestle & sieved through a 2mm sieve. These samples were placed in sterile poly bags, sealed tightly, and transported immediately to the laboratory.

2.2 Isolation of Actinomycetes

Samples were given moist heat treatment at 60 º C in a 100 ml flask. Samples were serially diluted from 10^{-1} to 10^{-11}. 10^{-1}, 10^{-3}, 10^{-5}, and 10^{-7} were spread on an Actinomycete isolation Agar medium using the spread plate method. 30 isolates were selected for the study of antibacterial and antifungal activity.

2.3 Screening for Antifungal activity (Agar Well Diffusion Method)

All 30 actinomycete isolates were activated by inoculation in 50 ml of sterile Sabouraud Dextrose broth in a 100 ml flask & Incubated at 28°C for 5 days under shaking condition at 150 rpm. 4 test phytopathogenic fungi *Alterneria,*

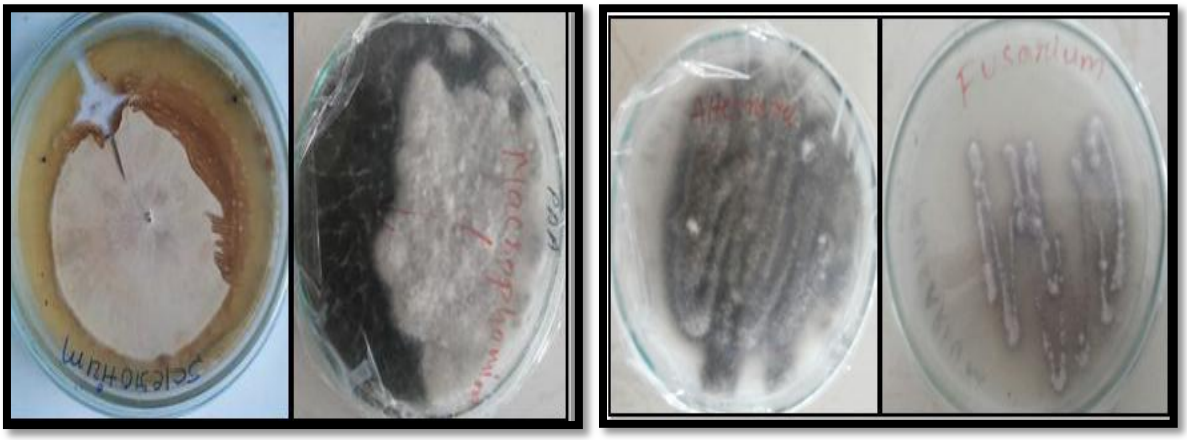

Fig. 1. Antifungal activity screening

Fusarium, Macrophomina & *Sclerotium* were obtained from Agriculture University, Junagadh. Test fungi were activated by spreading on potato dextrose agar medium & were incubated at 30 °C for 3 days. For Antifungal activity, all 4 test fungi were spread on Sabouraud's medium. SDA broth containing active Actinomycetes was centrifuged at 10000 rpm to obtain culture filtrate. 4 Wells of 6 mm diameter were made in 4 corners of Sabouraud's agar plates with the help of a sterile cup-borer. 70 µl of test culture filtrates were inoculated into the wells. Plates were incubated at 30 °C in an upright position for 48-72 hrs. The Appearance of the zone of inhibition shows positive results.

2.4 Screening for Antibacterial activity (Cross Streak Method)

30 Actinomycete isolates were streaked as a single line on a sterile Nutrient agar medium & incubated at 30 °C for 3 days. The test bacterial cultures *(Staphylococcus aureus, Salmonella typhi, Shigella, Bacillus megaterium, Bacillus cereus,* and *Pseudomonas aeruginosa*) were obtained from CCSIT college, Junagadh. Test bacterial cultures were cross-streaked perpendicular to the Actinomycete isolates & incubated at 37°C for 24-48 hrs. The Line of inhibition shows a positive result.

3. RESULTS AND DISCUSSION

3.1 Antifungal Activity

The degree of antifungal activity varied greatly among the Actinomycetes.

Antifungal activity of all 30 isolated Actinomycetes against 4 test fungi are represented in Table 1.

3.1.1 Data analysis

3 isolates i.e. G-1, C-15, C-25 inhibited all 4 test fungi. 10 isolates i.e. C-2, C-10, C-12, C-17, C-21, G-2, G-3, G-7, G-13 & C-29 inhibited 3 test fungi. 11 isolates inhibited 2 test fungi. 6 isolates did not inhibit any test fungi. 4 isolates C-10, C-11a, C-25, G-1 & C-27 show potent inhibition.

3.1.2 Antifungal activity against *Macrophomina*

15 isolates inhibited *Macrophomina*. C-10 showed a 1 cm inhibition zone & G-1 showed a 0.8 cm zone of inhibition. 12 isolates gave 0.2-0.6cm zone and 15 isolates gave negative results against *Macrophomina*.

3.1.3 Antifungal activity against *Fusarium*

C-10 showed a very potent zone of inhibition of ion 0.7cm. 9 isolates showed a 0.1-0.5 cm zone of inhibition. 20 isolates did not show inhibition against *Fusarium*.

3.1.4 Antifungal activity against *Alternaria*

1 isolate C-11(a) gave the 1cm potent zone of inhibition. 15 isolates gave the 0.7-0.2cm inhibition of the growth. 14 isolates gave negative results against *Alternaria* fungi.

3.1.5 Antifungal activity against *Sclerotium*

Most isolates suppressed the growth of Sclerotium & Clear plate was observed with slight growth.

Sclerotium was found as most sensitive against actinomycetes.

Table 1. Results of antifungal activity of actinomycetes

Sr. No.	Isolate name	Test fungi (Zone of inhibition) in cm			
		Macrophomina	***Fusarium***	***Alternaria***	***Sclerotium***
1.	C-2	0.4	0.5	-	No Growth
2.	C-5	-	-	0.7	No Growth
3.	C-6	-	-	-	-
4.	C-8	-	-	0.3	No Growth
5.	C-10	1	0.7	-	No Growth
6.	C-11 a	-	-	1	0.4
7.	C-12	0.3	-	0.4	No Growth
8.	C-13	0.5	-	-	0.3
9.	C-14	-	-	0.5	No Growth
10.	C-15	0.4	0.4	0.6	No Growth
11.	C-17	0.5	-	0.4	No Growth
12.	C-20	-	0.5	-	No Growth
13.	C-21	0.4	-	0.4	No Growth
14.	C-24	-	0.5	-	No Growth
15.	C-25	0.4	0.2	0.6	No Growth
16.	C-27	0.7	-	-	0.5
17.	C-29	0.4	-	0.5	No Growth
18.	GC-2	-	-	0.3	No Growth
19.	GC-3	-	-	-	-
20.	G-1	0.8	0.3	0.5	No Growth
21.	G-2	0.2	-	0.4	No Growth
22.	G-3	0.4	0.3	-	No Growth
23.	G-4	-	0.1	-	No Growth
24.	G-5	-	-	-	-
25.	G-6	-	-	-	-
26.	G-7	0.4	-	0.6	No Growth
27.	G-8	-	-	0.3	No Growth
28.	G-9	-	-	-	-
29.	G-10	-	-	-	-
30.	G-13	0.4	-	0.4	No Growth

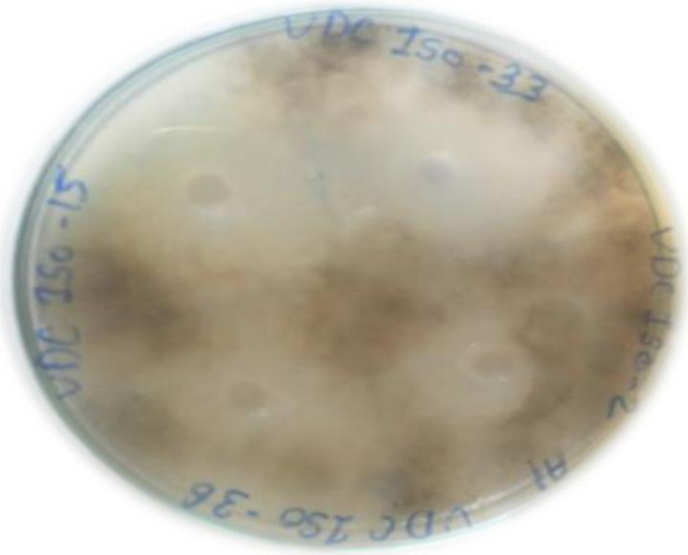

Fig. 2. Zone of inhibition of antifungal activity against *Macrophomina*

Table 2. Results of antibacterial activity of actinomycetes

Sr. no	Isolate name	*P. aeruginus* (zones in cm)		*S. typhi* (zones in cm)		*B. cereus* (zones in cm)		*S. aureus* (zones in cm)		*B. megaterium* (zones in cm)		*Shigella* (zones in cm)	
1	C-2	-	-	-	-	-	-	-	-	-	-	-	-
2	C-5	0.1	0.15	0.4	0.38	0.2	0.21	0.3	0.29	0.8	0.9	0.4	0.41
3	C-6	-	-	-	-	-	-	-	-	-	-	-	-
4	C-8	-	-	-	-	-	-	-	-	-	-	-	-
5	C-10	-	-	-	-	-	-	-	-	-	-	-	-
6	C-11	-	-	-	-	-	-	-	-	-	-	-	-
7	C-12	-	-	-	-	-	-	-	-	-	-	-	-
8	C-13	0.5	0.7	0.3	0.4	0.6	0.3	0.2	0.6	0.4	0.4	0.7	0.6
9	C-14	0.5	1	0.8	1	1	0.9	0.8	0.9	1.3	1.2	1.5	1.4
10	C-15	-		-		0.2	0.1	0.4	0.5	-		-	
11	C-17	-	-	-	-	-	-	-	-	-	-	-	-
12	C-20	-	-	-	-	-	-	-	-	-	-	-	-
13	C-21	-	-	-	-	-	-	-	-	-	-	-	-
14	C-24	-	-	-	-	-	-	0.2	0.1	-	-	-	-
15	C-25	2.5	1.5	0.6	0.8	2.5	2.1	2.5	2.4	0.7	0.6	3	3
16	C-27	-	-	-	-	-	-	-	-	-	-	-	-
17	C-29	-	-	-	-	-	-	-	-	-	-	-	-
18	GC-2	0.9	1	-	-	0.8	2.5	1.4	2.4	-	-	0.7	0.6
19	GC-3	0.2	0.3	-	-	3	0.28	3	0.29	-	-	0.3	0.3
20	G-1	-	-	-	-	-	-	-	-	-	-	-	-
21	G-2	-	-	-	-	-	-	-	-	-	-	-	-
22	G-3	-	-	-	-	-	-	-	-	-	-	-	-
23	G-4	-	-	-	-	-	-	-	-	-	-	-	-
24	G-5	-	-	-	-	-	-	-	-	-	-	-	-
25	G-6	-	-	1.9	1.8	2	0.19	1.6	1.5	1.6	1.5	1.5	1.4
26	G-7	0.3	0.2	-	-	-	-	-	-	0.5	0.3	0.2	0.1
27	G-8	-	-	-	-	-	-	-	-	-	-	-	-
28	G-9	-	-	-	-	-	-	-	-	-	-	-	-
29	G-10	-	-	-	-	-	-	-	-	-	-	-	-
30	G-13	-	-	-	-	1.7	1.7	-	-	-	-	-	-

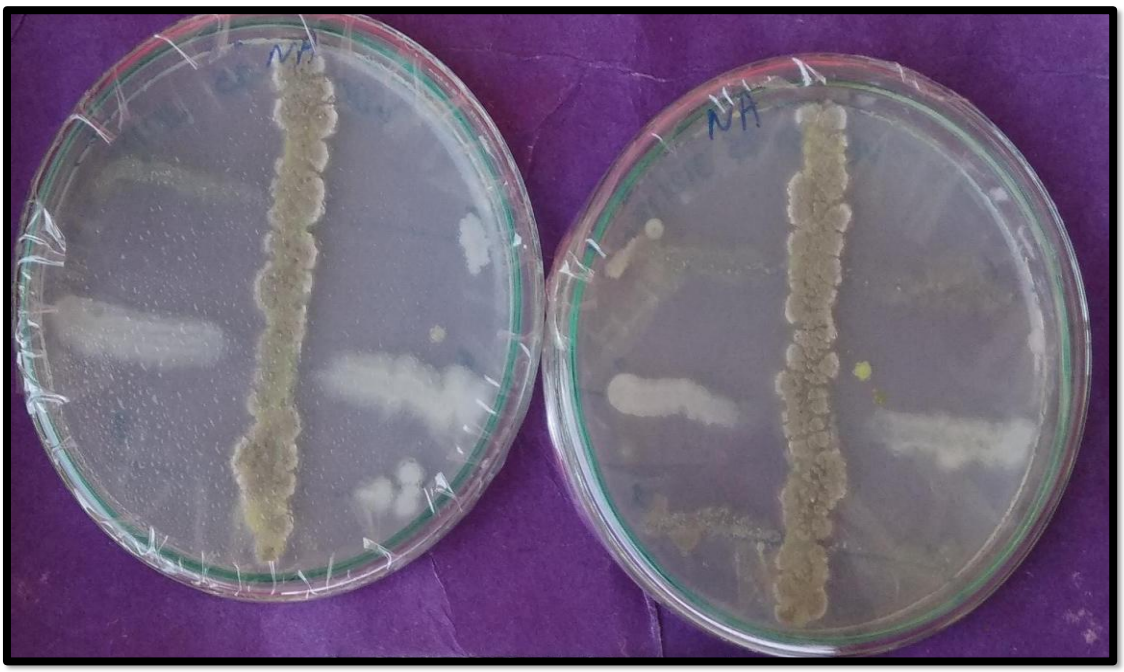

Fig. 3. Line of inhibition of antibacterial activity

3.2 Antibacterial Activity

A promising source of antibiotics is actinomycetes [14,15], with its largest group are the sources of most antibiotics currently used and other types of bioactive compounds, including the antibiotics discovered after the 2000s [16,10].

Antibacterial activity of all 30 isolated Actinomycetes against 6 test bacteria are represented in in Table 2.

3.2.1 Discussion

7 isolates were found to inhibit the growth of *P. aeruginosa. M*aximum inhibition of 2.5 cm by C-25. Minimum inhibition of 0.1 cm by C-5.

5 isolates inhibited the growth of S. *typhi.* Maximum inhibition of 1.9 cm by G- 6. Minimum inhibition of 0.3 cm by C-3.

9 isolates inhibited *B. aureus.* Maximum inhibition of 3 cm by GC- 3. Minimum inhibition of 0.2 cm by C-5 and C-15.

9 isolates inhibited *s. aureus.* Maximum inhibition of 3 cm by GC-3. Minimum inhibition of 0.2 cm by C-13.

6 isolates inhibited *B. megaterium.* Maximum inhibition of 1.6 cm by G-6. Minimum inhibition of 0.1 cm by C-5.

8 isolates inhibited the growth of *Shigella. M*aximum inhibition of 3 cm by C-25. Minimum inhibition of 0.1 cm by G-7. 4 isolates inhibited all 6 test bacteria. C-5, C-13, C-14 & C-2.

4. CONCLUSION

3 best isolates C-25, G-1 & C-15 showed the best results in decreasing the order of their potency for antifungal activity. 4 best isolates C-5, C-25, C-14 & C-13 showed the best results in decreasing the order of their potency for antibacterial activity. 3 best isolates C-25, C-13 & C-5 was selected for future work. Thus the results of the present study conclude that Actinomycetes isolated from a soil sample from the Rhizosphere of Cotton and Ground nut showed antifungal & antibacterial activity.

ACKNOWLEDGEMENT

We are thankful to Girish Bhai Ardeshna(Girishbapuji) who provided complete access to utilize his fields for collecting soil and also helped to choose the best soil samples and imparting fieldwork knowledge, we are also thankful to the lab assistants for their valuable assistance provided during the antifungal and antibacterial practical conducted in vitro. We also thank our colleagues for guiding us during the time of fieldwork and soil survey.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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