Original Research Article

Antifungal and Antibacterial activity of Actinomycetes isolated from various soil samples of *Arachis hypogea* L. and *Gossypium herbaceum* L.

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 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 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. 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. Actinomycetes are gram-positive bacteria with high guanine and cytosine content of over 55% [3] in their DNA, which have been recognized as sources of several secondary metabolites, antibiotics, and bioactive compounds that affect microbial growth. [4] Actinomycetes have filamentous nature, branching pattern, and conidia formation, which are similar to those of fungi. For this reason, they are also known as ray fungi. [5] Actinomycetes produce branching mycelium which may be of two types, viz., substrate mycelium and aerial mycelium. Streptomyces is the dominant of all actinomycetes. [6]

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 [7]. Actinomycetes are a capable source of many biologically active compounds, [8,9,10,11] which have various clinical effects and significant applications in human medicine. [12]. It has been estimated that about one-third of the thousands of naturally occurring antibiotics have been obtained from actinomycetes. [13].

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. [14]

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 ^{0} 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, 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.

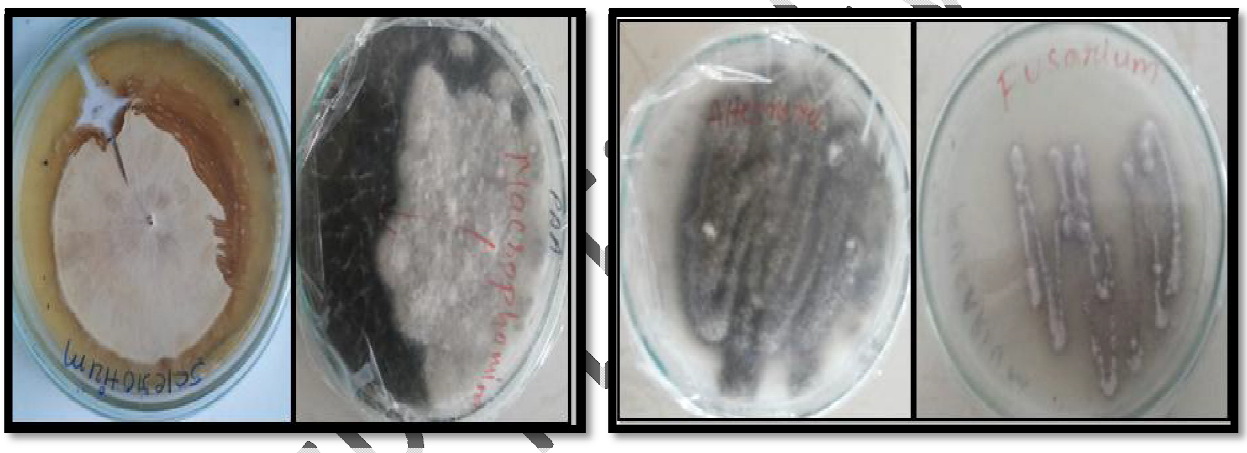

Figure 1: Antifungal activity Screening

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. Result & 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

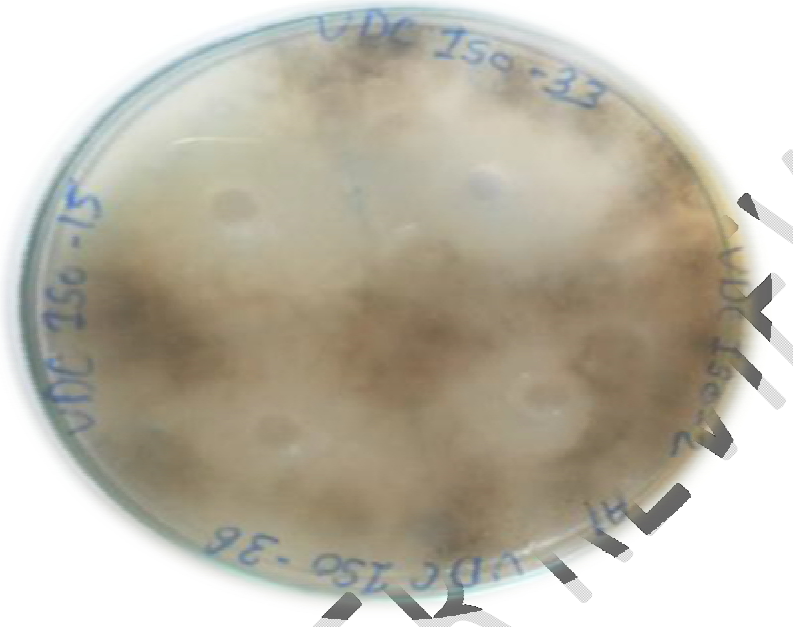

Figure 2: Zone of inhibition of antifungal activity

3.2 Antibacterial Activity

A large majority of antibiotics have been isolated in the numerous tested programs for new chemotherapic agents against various bacteria [15]. Two-thirds of the naturally available antibiotics were produced from actinomycetes [16].

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

Table 2: Results of Antibacterial Activity of Actinomycetes

Sr no	Isolate name	*P. aeruginus* (zones in	*S. typhi* (zones in cm)	*B. cereus*(zones in cm)	*S. aureus* (zones in cm)	*B. megaterium* (zones in	*Shigella* (zones in cm)

		cm)								*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	-	-	-	-	-	-

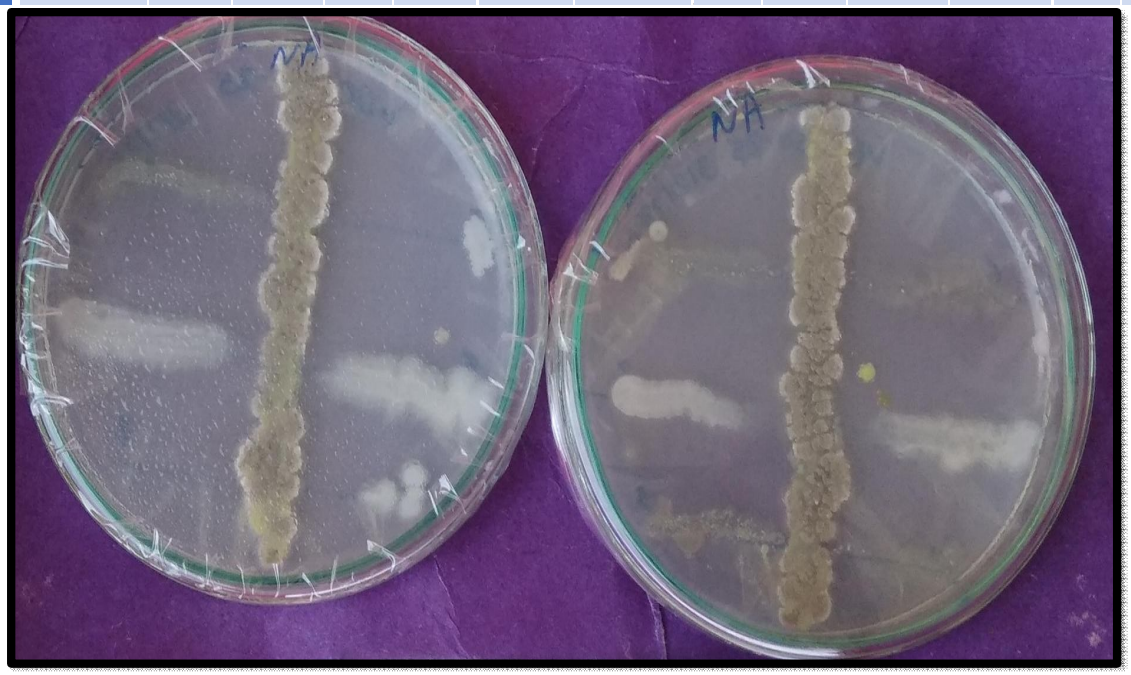

Figure 3: Line of inhibition of antibacterial activity

3.2.1 Data Analysis:

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.

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