*Indian J. Applied & Pure Bio.* Vol. 40(2), 1294-1301 (2025). **ISSN: 0970-2091**
**A Web of Science Journal**

# Actinomycetes as potent Antibacterial agents and Biofilm inhibitors

**Shivani Patil[1] and Tessy Anu Thomas[*]**

Department of Microbiology, Krupanidhi Degree College
Bangalore - 560035 (India)[1,*]
Email: patil.shivani1001@gmail.com
Address of Corresponding author Dr. Tessy Anu Thomas[*]
Department of Microbiology Krupanidhi Degree College,
Bangalore. 560035 (India)
Email: tesaby08@gmail.com*
Orcid id: 0000-0002-1388-4525

## Abstract

Oral health influences the general quality of life and poor oral health is linked to chronic conditions and systemic diseases which continues to be a major health problem worldwide. Dental caries and periodontitis are the two most common oral diseases and are closely related with the oral microbial flora that includes bacteria such as *Streptococcus mutans* and other anaerobic bacteria like *Porphyromonas* and *Bacteroides spp* which are also potent biofilm producers. On the verge of increased incidence of antibiotic resistance, the aim of the study was to evaluate the potency of actinomycetes as potent antibacterial agents and biofilm inhibitors. In context to this, actinomycetes strains were isolated from soil samples from various parts of India and identified in the generic level by following the Dichotomous key followed by assessing the antimicrobial potency against *Streptococcus mutans* and *Streptococcus oralis* by Kirby Bauer method. The strains were also assessed for their biofilm inhibiting potency by Crystal violet method. Among all the strains tested, the actinomycetes isolated from Gujarat exhibited potent antimicrobial and antibiofilm activity which subsequently resulted to be identified as *Streptomyces roseofulvus* through 16S rRNA using Sanger sequencing with 98.96% and by PCR amplification.

**Key words :** *Streptocoocus mutans, Streptococcus oralis, Streptomyces roseofulvus,* periodontitis, biofilm, 16S rRNA.

---

*Associate Professor,

Dental caries, one of the most widespread chronic conditions globally, is primarily driven by the activity of acid-producing bacteria, notably *Streptococcus mutans*[14]. These bacteria break down dietary sugars to produce acids, which result in the demineralization of tooth enamel and the formation of cavities. A key factor in the persistence and adherence of these cavity-causing bacteria is the formation of dental plaque, a biofilm that coats the tooth surface. The biofilm creates a protective environment, enhancing bacterial survival and increasing their resistance to antimicrobial agents and the host's immune system. This biofilm structure poses a significant barrier to effective treatment of oral infections by limiting antimicrobial penetration and reducing their effectiveness. This resilience underscores the necessity for new therapeutic approaches, such as developing agents that can either disrupt biofilm formation or improve the penetration of current antimicrobials[10].

Actinomycetes are a group of Gram-positive bacteria, recognized for their filamentous growth, resembling fungi, and for having high G+C content in their DNA, typically between 60% and 78% [4]. These microorganisms are primarily found in soil but also inhabit a variety of environments, including marine ecosystems. The genus *Streptomyces* is the most extensively studied among actinomycetes due to its prolific production of secondary metabolites with crucial biomedical applications[6]. Historically, actinomycetes played a pivotal role in the discovery of antibiotics, particularly during the golden age of antibiotic discovery (1950–1970), when several antibiotics such as streptomycin, vancomycin, and tetracycline were commercialized to treat infectious diseases[12].

*Streptomyces*, the dominant genus within actinomycetes, is responsible for producing over two-thirds of all known antibiotics[13]. In addition to antibiotics, soil actinomycetes produce antifungal, antiviral, antitumor, and immunosuppressive agents[7]. The biosynthetic capacity of actinomycetes is vast, with more than 22,000 bioactive compounds identified, many of which are utilized in medicine and agriculture[3]. Their ability to synthesize enzymes like proteases and pectinases further underscores their importance in biotechnology.

This particular study was carried out to isolate and identify the actinomycetes strains from various soil samples across India and to assess their antibacterial and antibiofilm potency so as to provide a platform for the discovery of novel therapeutic agent.

*Isolation and Identification of Actinomycetes strains :*

For this study, soil samples were procured from different-different states (Gujrat-Rajkot, Maharashtra-Baramati & Jalgaon, Madhya-Pradesh-Burhanpur, Karnataka-Bangalore, Kerala-Alleppey & Kannur, Tamil-Nadu-Hosur), followed by isolation of Actinomycetes strains by serial dilution from $10^{-1}$ to $10^{-6}$ dilution by spread plate method on Actinomycetes Isolation Agar plates and incubated at 28°C for 72 hrs [11]. Morphological identification at Genus level was carried out based on colony morphology, pigmentation, and spore formation followed by Gram staining and

biochemical tests including catalase, IMVIC, starch hydrolysis, TSI [13].

*Antimicrobial activity of the Actinomycete strains :*

Petri plates containing 20 ml MHA agar were seeded using cotton swabs with the 24-hour (old) culture of the microbial strains of *Streptococcus mutans* and *Streptococcus oralis*. Wells of 6mm were punch on the plates using cork borer and test samples (Actinomycete strains) were added into each well. The plates were then incubated at 37° C for 24 h. The anti-microbial activity was assessed by measuring the diameter of the inhibition zone formed around the wells[5].

*Antibiofilm activity by Crystal Violet assay method :*

Actinomycete strains was dissolved in LB and biofilm quantification was performed following the method of Alva *et al.*,[1]. A 100 µl sample of the diluted culture of *S. mutans* and *S. oralis* strains were placed in a microtiter plate and incubated for 24 hours at 37°C. The attached cells were washed three times with PBS at pH 7.4. Then, 125 µl of 0.1% freshly prepared crystal violet solution was added to the dried pellet and incubated for 10 minutes. After staining and washing, 200 µl of 30% acetic acid was added to the pellet and incubated for 15 minutes to dissolve the stain. A 100 µl aliquot was transferred to a new plate, and the optical density was measured at 600 nm using an ELISA reader (Biorad, USA). The reduction in biofilm formation in the presence of plant extracts was calculated as percent inhibition using the formula: [(OD of control–OD of treated) / OD of control] x 100.

*Taxonomic Identification of Bacterial Strain by Sequencing :*

The DNA extraction of the selected strain was carried out using the Xploregen gDNA Extraction Buffer™ as per the manufacture protocol. The PCR amplification and cycling conditions are tabulated in Table-1.

*Isolation and Identification of Actinomycetes :*

Morphological Characterization: Actinomycetes were successfully isolated from soil samples collected from various regions, including Gujarat (Rajkot), Maharashtra (Baramati & Jalgaon), Madhya Pradesh (Burhanpur), Karnataka (Bangalore), and Kerala (Alleppey & Kannur), Tamil Nadu (Hosur). Following incubation on Actinomycete Isolation Agar (AIA) at 28°C for 72 hours, distinct colonies were observed. These colonies exhibited characteristics typical of Actinomycetes, including filamentous growth, earthy odour, and pigmentation ranging from white to pale yellow. The genus level identification was

Table-1. PCR and Amplification Conditions

| **PCR Conditions** | **Initial Denaturation** | **Denaturation** | **Hybridization** | **Elongation** |
|---|---|---|---|---|
| 25 cycles | 96°C for 5 min | 96°C for 30 sec | 50°C for 30 sec | 60°C for 1.30 min |
| **Cycling conditions** | | | | |
| **Initial Denaturation** | **Denaturation** | **Annealing** | **Extension** | **Final Extension** |
| 3 minutes at 94°C | 1 minutes at 94°C | 1 minutes at 94°C | 2 minutes at 94°C | 7 minutes at 94°C |

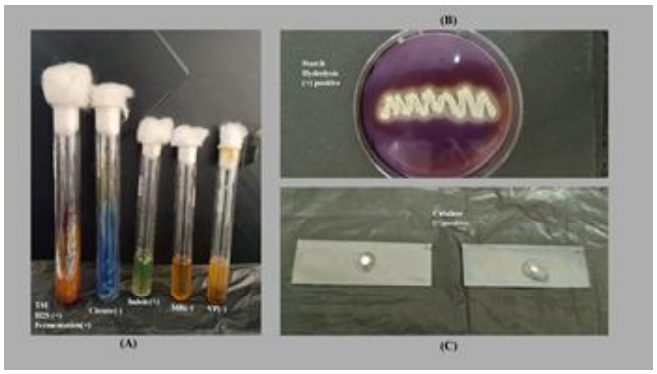

Fig 1. Biochemical tests: **(A)** IMVIC tests showing positive results, **(B)** Starch hydrolysis test indicating a positive result, **(C)** Catalase test confirming positive activity.

Table-2. Biochemical characteristics and Genus Identification of Actinomycetes strains

| Sample / Tests | Indole | Methyl red | Vogus Pros-kauer | Cata-lase | TSI | | Citrate | Starch hydro-lysis | Gram Charac-ter | Genus |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | H2S | Ferme-ntation | | | | |
| MH-1 | -ve | +ve | -ve | +ve | –ve | -ve | +ve | +ve | +ve | Streptomyces |
| MH-2 | -ve | +ve | -ve | +ve | -ve | +ve | +ve | +ve | +ve | Streptomyces |
| GJ-3 | -ve | +ve | -ve | +ve | -ve | +ve | +ve | +ve | +ve | Streptomyces |
| GJ-4 | -ve | +ve | -ve | +ve | -ve | +ve | +ve | +ve | +ve | Streptomyces |
| MP-5 | -ve | -ve | -ve | +ve | -ve | -ve | +ve | +ve | +ve | Nocardia |
| MP-6 | -ve | -ve | -ve | +ve | -ve | -ve | +ve | +ve | +ve | Nocardia |
| MH-7 | -ve | -ve | -ve | +ve | -ve | -ve | +ve | +ve | +ve | Nocardia |
| MH-8 | -ve | -ve | -ve | +ve | -ve | -ve | +ve | +ve | +ve | Nocardia |
| TN-9 | +ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | +ve | Actinomyces |
| TN-10 | +ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | +ve | Actinomyces |
| All-11 | +ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | +ve | Actinomyces |
| All-12 | +ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | +ve | Actinomyces |
| Kanr-13 | -ve | +ve | -ve | +ve | -ve | -ve | +ve | +ve | +ve | Streptomyces |
| Kanr-14 | -ve | +ve | -ve | +ve | -ve | -ve | +ve | +ve | +ve | Streptomyces |
| Kr 15 | -ve | +ve | -ve | -ve | -ve | +ve | +ve | +ve | +ve | Streptomyces |
| AP-16 | +ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | +ve | Actinomyces |
| AP-17 | +ve | +ve | -ve | -ve | -ve | -ve | -ve | -ve | +ve | Actinomyces |

proceeded by the Gram staining and Biochemical tests as stated in Table 2 and Figure 1.

*Antibacterial Activity of Actinomycetes:*

The antimicrobial activity of the isolated Actinomycetes strains was evaluated using the well diffusion method against *Streptococcus oralis* (MTCC-2696) and *Streptococcus mutans* (MTCC 890).

The results indicated significant antimicrobial activity of the Actinomycetes isolates, as evidenced by the clear zones of inhibition around the wells on Mueller-Hinton Agar (MHA) plates. The diameter of the inhibition zones ranged between 12 mm to

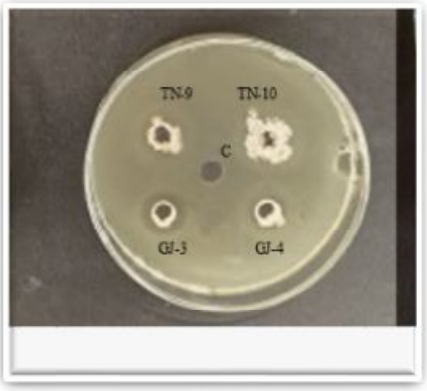


Fig. 2 Antimicrobial activity of actinomycetes (A) *Streptococcus oralis (B) streptococcus mutans* showing zone of inhibition.

20 mm, demonstrating the potent antimicrobial properties of the isolates. The highest inhibition zone was observed against *S. mutans* 16 mm indicating a strong antagonistic effect by GJ-3 Actinomycetes strains which was isolated from the Gujrat (Rajkot) soil sample (Figure 2).

*Antibiofilm Activity of Actinomycetes :*

*Crystal Violet Assay :*

The antibiofilm activity of the Actinomycetes isolates was assessed using the crystal violet assay. The ability of the isolates to inhibit biofilm formation by *S. oralis* and *S. mutans* was determined by measuring the optical density at 595 nm (OD595) after staining with crystal violet. The highest biofilm inhibition activity against *Streptococcus mutans* was exhibited by GJ-3 (57.73%), MH-8 (50.31%), and MH-7 (49.55%). For *Streptococcus oralis*, the most effective strains were GJ-3 (61.29%), MH-8 (61.03%), and All-11 (59.28%) (Figure 3).

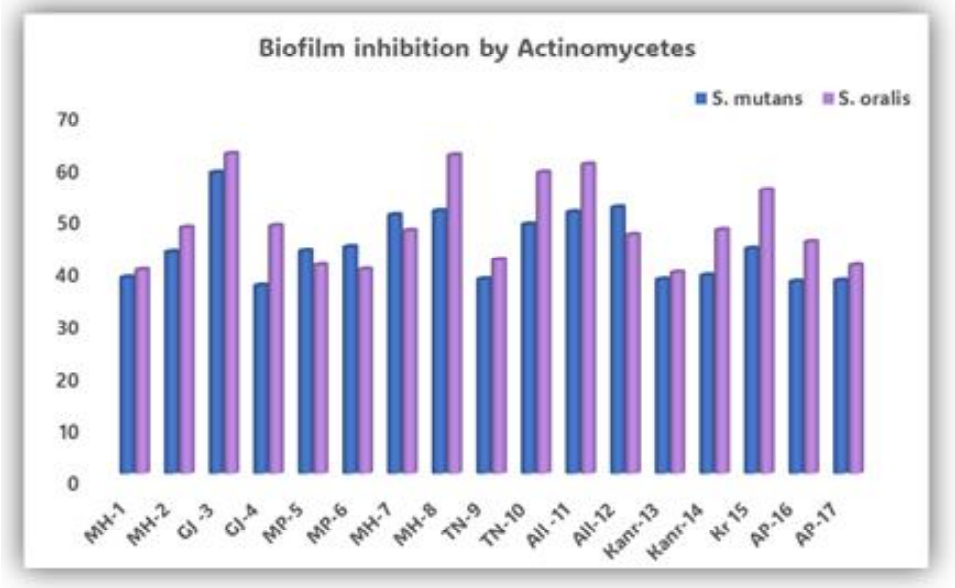


Fig 3. Percentage Inhibition of Biofilm Formation by Actinomycetes against *S. oralis* and *S. mutans*

*Nucleotide Sequencing :*

*DNA Quantification*

The quantification of DNA of GJ-3 was estimated to be 165 ng/µl.

*PCR amplification :*

The following were the primers used for the amplification process and Figure 4 illustrates the separation of the amplified bands in comparison with the control. The primer details are provided in Table-1.

Table-3. Primer Details

| Sr. no | Oligo name | Sequence ((5 'à 3') | Temp. (0°C) | GC-Content |
|---|---|---|---|---|
| **1** | ACT Forword | ATGTGCAAGGCCGGTTTCGC | 56 | 60.0% |
| **2** | ACT Reverse | TACGAGTCCTTCTGGCCCAT | 54 | 55.42% |

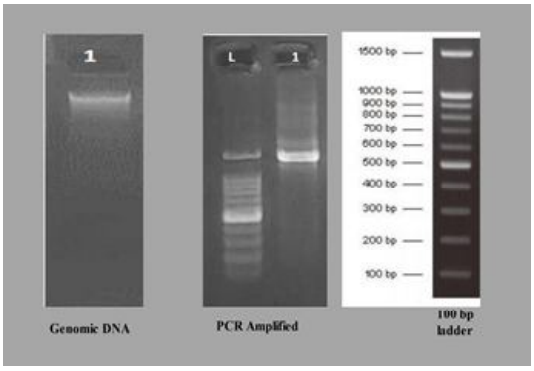


Fig. 4. Gel Electrophoresis of the GJ-3 strain in comparison with the control

*Phylogenetic Tree & Blast Data :*

The results obtained from the phylogenetic tree following sequence mapping resulted in the identification of GJ-3 strain as *Streptomyces roseofulvus* strain with 98.96% similarity as shown in Figure 5.

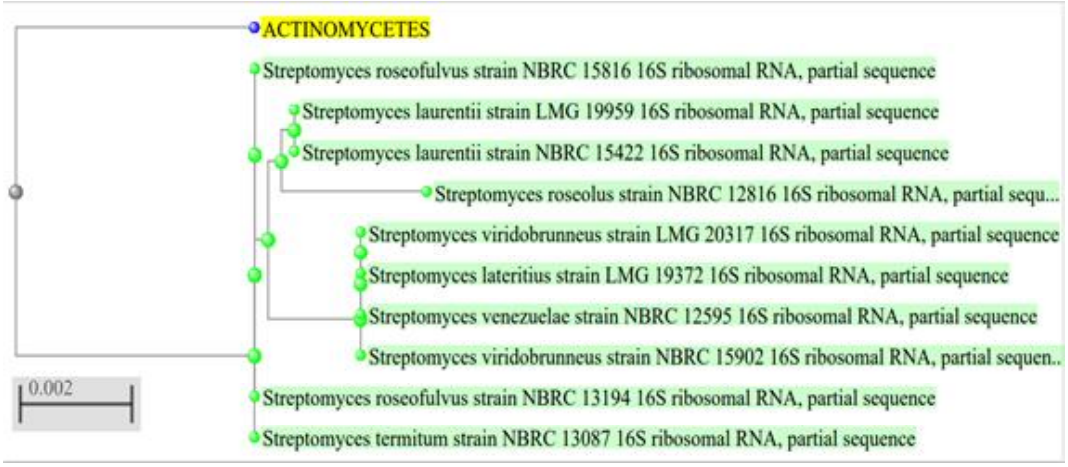


Fig. 5. Phylogenetic tree of GJ-3

*Microbial Identification :*

The Microbe was found to be *Streptomyces roseofulvus* strain NBRC 15816 16S ribosomal RNA with Sequence ID: NR_112579.1 The next closest homologue was found to be *Streptomyces roseofulvus* strain NBRC 13194 16S ribosomal RNA with Sequence ID: NR_041120.1

*Streptomyces roseofulvus* is a species of actinomycetes recognized for producing secondary metabolites with potent antimicrobial and antifungal properties. The industrial and pharmaceutical significance of *Streptomyces roseofulvus* has been well-documented since its classification This species is known for producing bioactive compounds, especially antibiotics. Previous studies on *S. roseofulvus* have shown its potential in producing compounds like fungichromin, which has strong antifungal properties[9].

The antimicrobial activity of GJ-3 strain aligns with this pharmaceutical potential, as observed significant antibacterial effects against *S. oralis* and *S. mutans*.

These antibacterial activities of isolated actinomycetes correlate with previous studies that demonstrate the broad-spectrum antimicrobial potential of *Streptomyces spp* which highlight that *S. roseofulvus* and other actinomycetes can produce bioactive metabolites effective against oral pathogens, ie. *S. mutans*[8]. The 16 mm inhibition zone we observed is consistent with the potent antibacterial activity, which was found in earlier work on actinomycetes, which will show the relevance of the GJ-3 strain in biomedical applications. The antibiofilm properties of the GJ-3 strain, particularly against oral pathogens, are in line with earlier studies where it is *demonstrated Streptomyces spp.* as effective agents against biofilm formation; so, the ability of *S. roseofulvus* strains, including GJ-3, to disrupt biofilm formation represents a significant therapeutic avenue.

*Ethics Approval and Consent to Participate:*

"This article does not contain any studies with human participants or animals performed by any of the authors."

**Competing Interests**

Shivani Patil and Tessy Anu Thomas "declare that they have no conflict of interest."

**Funding**

There are no funding bodies in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

**Authors Contributions**

The first author, Shivani Patil has carried out the present study under the supervision and guidance of the corresponding author, Dr. Tessy Anu Thomas.

References :

1. Alva PP, S Suresh, MP Gururaj, and R. Premanath (2019). *European Journal of Integrative Medicine.* Sep 1; *30:* 100931.
2. Bérdy, J. (2012). *The Journal of antibiotics*, *65*(8): 385-395.
3. Blánquez, A., A.S. Ball, and González-Pérez, J. (2017). *PLoS One, 12*(11): e0187649
4. Chaudhary, H.S., J. Yadav, A.R. Shrivastava,

S. Singh, A. K. Singh, and N. Gopalan, (2013). *Journal of advanced pharmaceutical technology & research, 4*(2): 118-123.

5. Cunha, M. B., A. F. Jorge, M. J. Nunes, J. R. Sousa, M. J. Lança, M. Gomes da Silva, and S.P. Gaudêncio, (2024). *Marine Drugs*, *23*(1): 1.
6. Demain, A.L., and S. Sanchez, (2009). *Journal of Antibiotics, 62*(1): 5-16.
7. Fitzgerald, J. (2012). *Engineering of Biosynthetic Pathways to the Type II Polyketides Frenolicin B and A-74528*. Stanford University.
8. Hopwood, D. A. (2007). *Natural Product Reports, 24*(6): 1280-1296.
9. Jiang, Q., J. Liu, L. Chen, N. Gan, and D. Yang, (2019). *Frontiers in cellular and infection microbiology, 8:* 442.
10. Kumar, N., R.K. Singh, S.K. Mishra, A. K. Singh, and U. C. Pachauri, (2010). *International Journal of Microbiology Research, 2*(2): 12.
11. Mahajan, G.B. and L. Balachandran, (2012). *Frontiers in Bioscience, E4:* 240-253.
12. Sharma, M., P. Dangi, and M. Choudhary, (2014). *International Journal of Current Microbiology and Applied Sciences*, *3*(2): 801-832.
13. Ye, D., Y. Liu, J. Li, J. Zhou, J. Cao, Y. Wu, ... and Q. Ma, (2024). *Critical Reviews in Microbiology*, 1-12.