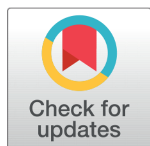
INDIAN JOURNAL OF SCIENCE AND TECHNOLOGY

RESEARCH ARTICLE



OPEN ACCESS

Received: 30-08-2022

Accepted: 07-10-2022

Published: 15-03-2023

Endophytic *Streptomyces rochei* BpR-2 GER (ON142045) from *Bryophyllum pinnatum* (Lam.) Oken with Antimicrobial and Antibiofilm Potential

Sujata Yadav[1]*, Geetika Wag[1], Ashwini Kumar Gupta[2]

1 Research Scholar, School of Studies in Life Science, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

2 Professor, School of Studies in Life Science, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

Citation: Yadav S, Wag G, Gupta AK (2023) Endophytic *Streptomyces rochei* BpR-2 GER (ON142045) from *Bryophyllum pinnatum* (Lam.) Oken with Antimicrobial and Antibiofilm Potential. Indian Journal of Science and Technology 16(11): 775-784. https://doi.org/10.17485/IJST/v16i11.1458

* **Corresponding author**.

yadavsujata1892@gmail.com

Funding: Council of Scientific and Industrial Research (CSIR) for the Junior Research fellowship

Competing Interests: None

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Published By Indian Society for Education and Environment (iSee)

ISSN
Print: 0974-6846
Electronic: 0974-5645

Abstract

Objectives: To explore the endophytic actinobacteria from the root tissue of *Bryophyllum pinnatum* (Lam.) Oken and its assessment for antimicrobial and antibiofilm activity against human pathogens. **Methods:** Surface sterilized root tissues of *B. pinnatum* have been seeded in different isolation media, to obtain colonies of endophytic actinobacteria. A polyphasic approach has been used to identify the isolate. Cross streak and disc diffusion methods have been used to perform the antimicrobial activity. However, the crystal violet assay assessed the antibiofilm activity, and its visualization was done under the light and fluorescent microscope. **Findings:** By polyphasic approach, Actinobacteria strain BpR-2 GER has been confirmed to be the member of genus *Streptomyces*, further through 16S rRNA gene sequence analysis, the strain has been identified as *Streptomyces rochei* that has 100% sequence similarity with *Streptomyces rochei* NRRL B-2410 (MUMD01000370). The isolate has exhibited extensive antagonistic interaction with Clinical and MTCC (Microbial Type Culture Collection) pathogens ranging from 9.75±0.71 to 19.38±1.30 mm. The highest zone of inhibition was recorded against Gram-positive bacteria *B. subtilis* (MTCC-441) of 19.38±1.30 mm and Gram-negative bacteria *Proteus mirabilis* (Clinical) of 16.25±0.71 mm. The strain has also exhibited significant antibiofilm potential against the MTCC-7443 (85.13±0.003 to 88.28±0.003%) and Clinical (85.13±0.003 to 88.28±0.003%) strain of *Staphylococcus aureus*. **Novelty:** This is the first report of *S. rochei* BpR-2 GER, a root endophyte of *B. pinnatum* with broad-spectrum antimicrobial activity and a significant antibiofilm property.

Keywords: Antimicrobial activity; Antibiofilm activity; Bryophyllum pinnatum; Endophytic Actinobacteria; Streptomyces rochei

1 Introduction

Continuous amplification of antimicrobial resistance (AMR) in pathogens has escalated a health threat worldwide. As a result, difficulty in the treatment of AMR -associated infections has become an alarming problem, accelerating the rate of morbidity and mortality[1]. Multidrug-resistant pathogens (MDR) possess extensive genetic and phenotypic mechanisms to withstand antibiotic treatment. The formation of biofilm is one of the phenotypic mechanisms that facilitates the prolonged survival of microorganisms against the action of antibiotics, thus enhancing the risk of disease transmission[2]. Further, misuse of drugs, failure of conventional antibiotics and reduced rate of more effective and novel antimicrobial discoveries have played a vital role in AMR development. Statistical analysis of the data in 2019 from 204 countries revealed that bacterial AMR has contributed to 1.27 million deaths. Alone, methicillin-resistant *Staphylococcus aureus* has caused 0.5 million deaths. At the same time, other clinically significant pathogens, such as *Escherichia coli, Klebsiella pneumonia, Streptococcus pneumonia, Acinetobacter baumannii,* and *Pseudomonas aeruginosa*, are responsible for 0.05 to 0.1 million deaths[3]. Considering bacterial AMR's effect on human health, there is an urgent need for new and effective antibacterial agents that can interfere with various AMR mechanisms.

One of the alternative strategies for combating AMR pathogens is the extensive exploration of pharmaceutically significant microorganisms, especially actinobacteria. This group of bacteria holds a prominent position as a potential reservoir of clinically diverse bioactive metabolites. Most commercial antibiotics have derived from soil actinobacteria, especially *Streptomyces sp*. Drugs with a broad range of pharmaceutical properties, including antimicrobial, antiviral, anti-inflammatory and antitumor obtained from this microorganism[4]. The frequency of getting novel and effective antimicrobials from soil *Streptomyces sp*. has reduced. Also, the study of endophytic actinobacterial species is still peripheral. Therefore, exploration of endophytic actinobacteria overcomes the problem of repetitive discovery of known bioactive metabolites. As an endophyte, these microbes incorporate into the metabolism of the host plant, consequently exerting beneficial effects on growth and survival through the induced production of secondary metabolites[5]. Thus, endophytic actinobacteria could be an excellent source of bioactive metabolites with unique biosynthetic potential. Investigation of endophytic actinobacteria from medicinal plants and their biological function is essential. Reports on the reconnaissance of these microorganisms from the medically valuable flora of Chhattisgarh are significantly lesser to date[6]. Hence, the present study aimed to isolate therapeutically potent root-endophytic actinobacteria of *B. pinnatum* (Lam.) Oken, and to investigate their antimicrobial and antibiofilm activity.

2 Methodology

2.1 Isolation of Endophytic Actinobacteria

B. pinnatum plants were collected from Mahaveer garden, G. E. Road, Raipur, Chhattisgarh. After surface sterilization, root tissues were either macerated using a mortar pestle or chopped into pieces and placed in four different isolation media. For 21 days of incubation, temperature was kept at 28±2 °C[7]. To avoid additional bacterial and fungal contamination, nalidixic acid and cycloheximide/ nystatin (50 mg/ l) were utilized, respectively. Actinomycetes isolation agar (ISP-1), Starch casein nitrate agar (SCNA), Tyrosine agar base (ISP-7) and Tap water yeast extract agar were used as isolation media.

2.2 Characterization of Endophytic Actinobacteria

The endophytic strain BpR-2 GER was identified using cultural, morphological, physiological, biochemical characterization and molecular techniques. Growth rate, aerial and substrate mycelium, colony texture and diffusible pigments were among the cultural traits that were noticed using nine different media: International Streptomyces Project (ISP-1 to ISP-7) media, Starch casein nitrate agar (SCNA), and Glucose soybean meal agar (GSMA). Using a light (LABOMED VISION 2000) and scanning electron microscope at a magnification of x1000 and x5000 respectively, morphological characteristics such as spore's surface, shape and chain type were observed[8].

For biochemical characterization, catalase test, starch hydrolysis, lipid hydrolysis, gelatin hydrolysis, lecithinase activity, proteolytic activity, xanthine degradation, hippurate hydrolysis, nitrate reduction, and H_2S production test were performed. In physiological characterization, growth at different temperatures 28, 37, 45, and 50 °C and at a varying concentration of NaCl (1-10% w/v) was observed. Susceptibility to different standard antibiotics viz., Azithromycin (0.015 mg), Chloramphenicol (0.01 mg), Ciprofloxacin (0.01 mg), Erythromycin (0.01 mg), and Tetracycline (0.01 mg) (Himedia, Mumbai, India) was tested. The ability to utilize different carbon sources viz., Cellulose, D-fructose, D-Glucose, I-Inositol, D-Mannitol, Rhamnose, Raffinose, D-Xylose, Sucrose and nitrogen sources viz., inorganic (NH_4Cl_2, NH_4NO_3, $(NH_4)_2SO_4$), organic (Meat extract, Yeast extract, Peptone, Tryptone, Asparagine, Glutamic acid, Histidine, Phenylalanine and Proline) were tested. Carbon and nitrogen sources at concentration of 1% (w/v) were incorporated into the basal mineral salt agar medium. After 14 days of incubation at 28±2

°C, the results were recorded [9].

Molecular identification was done by 16S rRNA sequencing. Actinobacterial genomic DNA was extracted using ZR Bacterial DNA MiniPrep kit (Make Zymo Research), amplified using the universal primers (27F (AGAGTTTGATCCTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT) and then visualized on a 1% (w/v) agarose gel. PCR amplified DNA was then purified using QIAquick Gel Extraction Kit (Make Qiagen) and used for sequencing. Obtained assembled sequence of 16S rRNA gene were analysed by Finch TV software version 1.4 and the similarity index was checked with BLAST tool in NCBI and EzBiocloud portal (http://www.ezbiocloud.net/). In MEGA7 software, the neighbor-joining method was used to deduce the evolutionary history of isolate.

2.3 In vitro screening for Antibacterial Activity

2.3.1 Test pathogens

MTCC pathogens: *Bacillus cereus*-430, *Bacillus subtilis*-441, *Staphylococcus aureus*-7443, *Staphylococcus epidermidis*-435, *Escherichia coli*-739, *Pseudomonas aeruginosa*-741, *Proteus mirabilis*-3310, *Salmonella enterica typhimurium*-98 were purchased from Microbial Type Culture Collection and Gene Bank, IMTECH Chandigarh and clinical strains of these pathogens were procured from Department of Microbiology, Jawaharlal Nehru Medical College, Raipur, Chhattisgarh.

2.3.2 Preliminary Screening

For the initial assessment of the antimicrobial potential of BpR-2 GER, the cross streak method was used [10]. Muller-Hinton agar plates were prepared and inoculated with a loop full of pure actinobacterial culture by a single streak on one side of the Petridish and incubated at 28±2 °C for 7 days. The purpose of doing this was to give the organism enough time to release bioactive metabolites, which were then diffused in the medium. A line of test pathogens 30 mm was drawn perpendicularly against the line of a fully developed actinobacterial culture. Actinobacteria and the test pathogens were streaked at a distance of 5 mm. Further, the plates were incubated at 37 °C for 24h. The inhibitory activity was measured as percent growth inhibition (GI %) using the following formula:

$$\mathrm{GI}(\%) = \frac{\text{Length of Inhibition}}{\text{Total length of streaking}} \times 100$$

2.3.3 Secondary Screening

Antimicrobial activity of crude filtrate of BpR-2 GER was further analyzed against test pathogens using the disc diffusion method [11]. Submerged fermentation was done in starch casein nitrate broth at 28±2 °C and 120 rpm for 14 days. Then sterile discs (6 mm diameter) loaded with crude filtrate (100 μl) were settled on Muller Hinton agar plates with target pathogens 1x10^8 CFU /ml). After incubation at 37 °C for 24h under static condition, the diameters of the clear zone formed around the disc were measured.

2.4 Screening for Inhibition of Biofilm formation

Biofilm inhibitory activity was assessed in a flat-bottom 96-well microtiter plate using a crystal violet staining assay with some modifications [8]. Overnight cultures (1x10^8 CFU/ ml) of *S. aureus* strains (MTCC- 7443 and Clinical) were inoculated in the TSB medium supplemented with 2% glucose to attain 1:10 dilution and treated with crude filtrate ranging10-50% (v/v). After incubation at 37 °C for 24h under static condition, the unattached cells were washed out from the wells with 100 μl phosphate-buffered saline (PBS) twice and the resulting bacterial biofilm was flooded with 0.4% (v/v) crystal violet dye for 30 min. Finally, plates were rinsed twice with deionized water after discarding excess dyes and air dried. The optical density of the resolubilized biofilm in 95% ethanol was recorded at 450 nm using a microplate reader (Meril EIAQuant Microplate Reader). Biofilm inhibitory activity was determined by using the following formula:

$$\text{Percent inhibition } = \frac{\text{Control } OD_{450} - \text{ Treated } OD_{450}}{\text{Control } OD_{450}} \times 100$$

2.5 Microscopic Visualization of Biofilm Inhibitory Activity

For microscopic visualization, glass pieces (1x1cm) were placed in polystyrene plate containing cultures of *S. aureus* strains and treated with crude filtrate (10%, v/v). After incubation for 24h at 37°C, glass pieces were washed with deionized water. The glass pieces were stained with two distinct dyes, crystal violet solution 0.4% (v/v) and ethidium bromide (EtBr) 0.01% (w/v) and left

for 20 min and one min, respectively [8,12]. The crystal violet stained glass pieces were visualized under the light microscope at x400, while ethidium bromide stained glass pieces were observed under the fluorescent microscope at x200 (Leica DM 1000).

2.6 Statistical analysis

Data were displayed as means ± standard deviation (SD) using the data analysis tool pack of Microsoft Office Excel 2007. The results were statistically compared with control using the t-test, where $p<0.0001$ was taken significantly different. All experiments were performed in replicates.

3 Results and Discussion

3.1 Isolation of Endophytic Actinobacteria

Gray colored colony of *Streptomyces rochei* BpR-2 GER was obtained as root endophyte of *B. pinnatum*. The surface sterilization process has helped to eradicate epiphytic microorganisms and to isolate pure colonies. *Streptomyces sp*. is a dominant genus of soil actinobacteria and has been frequently reported as an endophyte of various plants [13]. Recently, the strain of *Streptomyces sp.* having 95% sequence similarity with *S. rochei* has been reported as an endophyte of tomato plant (*Lycopersicon esculantum*). Presence of *Streptomyces sp.* as an endophyte could be a symbiotic association, since they produce bioactive compounds which exert beneficial functions for plant growth and protection in return the host plant allows the survival of these microorganisms [14].

3.2 Cultural and Morphological characterization

The cultural traits of *S. rochei* BpR-2 GER on different media revealed that the growth rate ranged from fast to moderate. Further, colony texture, the appearance of aerial and substrate mycelium and secreted diffusible pigment were observed, as described in Table 1. When cultural and morphological traits were compared to the non-endophytic strain of *S. rochei* AM8, some properties were found to be similar, such as growth rate on ISP-2 and SCNA and pigment production on ISP-2 and ISP-5. However, the growth of BpR-2 GER was good and fast on ISP-4 and ISP-5, while that of AM8 was moderate. On various ISP media, it was observed that BpR-2 GER and AM8 differed in terms of the color of the aerial and substrate mycelium [15]. Additionally, it was found that the BpR-2 GER exhibited a higher resemblance with the MSA 14 strain of *S. rochei* in terms of growth rate and morphological features on various ISP media [16]. In the present study, it was noticed that *Streptomyces sp.* differs in its morphological traits on various medium (Figure 1 A–B), which is consistent with past observations [8]. Changes in the isolate's phenotype show that variation in the composition of media influence the primary and secondary metabolism [14]. Light microscopic observation revealed that the strain is polysporous with branched mycelium. The spore chain was of spira (S) type, in which spores were arranged in open, loose and stretched spirals (Figure 1 C). From the scanning electron microscope (Figure 1 D), extended spirals were observed bearing globose spores with a smooth surface. Accordingly, the morphological traits of *S. rochei* BpR-2 GER examined under the scanning electron microscope are comparable with the *S. rochei* ANH identified by Hamdan et al. [17]. The cultural and morphological characteristics mentioned above imply that various strains of the same *Streptomyces* species may display distinct cultural characteristics.

Table 1. Cultural and Morphological characteristics of BpR-2 GER

Media	Growth	Aerial mycelium	Substrate mycelium	Colony texture	Diffusible pigment
ISP-1	Good, Moderate	Yellowish white	Light greenish yellow	Powdery	NDF
ISP-2	Good, Fast	Grayish brown	Deep yellowish brown	Matty	NDF
ISP-3	Good, Fast	Grayish olive	ND	Powdery	NDF
ISP-4	Good, Fast	Medium gray	Dark grayish yellow	Leathery	NDF
ISP-5	Good, Fast	Light gray	Light olive brown	Powdery	NDF
ISP-6	Good, Fast	Light gray	Brilliant yellow	Matty	NDF
ISP-7	Good, Fast	Grayish greenish yellow	Dark grayish olive	Powdery	NDF
SCNA	Good, Fast	Light grayish olive	Pale greenish yellow	Powdery	NDF
GSMA	Good , Moderate	White	Vivid yellow	Powdery	NDF

Not defined (ND), No diffusible pigment (NDF)

Fig 1. Colonial Morphology of BpR-2 GER in (A) ISP-3, (B) ISP-6, (C) Light microscopic image at x1000, (D) SEM micrograph at x5000

3.3 Physiological and Biochemical characterization

The optimum temperature for growth of *S. rochei* BpR-2 GER was 28 °C, although considerable growth at 37 and 45 °C showed the mesophilic and moderately thermophilic nature of the organism. At 50 °C, however, no growth was seen. The extent of development of the strain varied from good to poor in various NaCl concentrations, highlighting its broad range of tolerance and mild halophilic character[18]. Resistance in the BpR-2 GER strain against antibiotics suggests its ability to produce enzymes that render the drug inactive[19]. The growth and metabolism of cells depend on carbon and nitrogen[7]. The strain BpR-2 GER exhibited good growth when exposed to several carbon and nitrogen sources, but not when exposed to glutamic acid as a nitrogen source. According to biochemical characteristics of BpR-2 GER, it produces the following enzymes but not gelatinase and hydrogen sulfide: amylase, catalase, lipase, proteases, lecithinase, xanthine oxidase, hippurate, and nitrate reductase (Figure 2). Our findings are consistent with those of Hamdan et al., who described the pattern of antibiotic resistance, biochemical traits, and capacity to utilize different carbon sources of ANH, isolated from the Alexandrian Mediterranean Seacoast, Egypt[17].

Physiological characteristics

Temperature (°C)

28	37	45	50
+	+	+	-

NaCl concentration (% w/v)

1	2	3	4	5	6	7	8	9	10
Good	Good	Good	Moderate	Moderate	Poor	Poor	Poor	Poor	No growth

Susceptibility to standard antibiotics

Azithromycin	Chloramphenicol	Ciprofloxacin	Erythromycin	Tetracycline
Resistant	Resistant	Susceptible	Resistant	Resistant

Utilization of different Carbon sources

Cellulose	D-fructose	D-Glucose	I-Inositol	D-Mannitol	Rhamnose	Raffinose	D-Xylose	Sucrose
++	++	++	++	++	++	+	++	++

Utilization of different Nitrogen sources

Inorganic			Organic								
NH_4Cl_2	NH_4NO_3	$(NH_4)_2SO_4$	Meat extract	Yeast extract	Peptone	Tryptone	Asparagine	Glutamic acid	Histidine	Phenylalanin	Proline
+	+	+	+	+	++	+	++	-	+	+	++

Biochemical Characteristics

Catalase test	Starch hydrolysis	Lipid hydrolysis	Gelatin hydrolysis	Lecithinase activity	Proteolytic activity	Xanthine degradation	Hippurate hydrolysis	Nitrate reduction	H_2S Production
+	+	+	-	+	+	+	+	+	-

+ (positive), ++ (strongly-positive), - (negative)

Fig 2. Physiological and Biochemical characteristics of BpR-2 GER

3.4 Molecular Characterization and Identification

16S rRNA gene sequencing of BpR-2 GER has generated a sequence of 1422 base pairs. The strain showed 100% sequence similarity with *Streptomyces rochei* NRRL B-2410 (MUMD01000370). The phylogenetic tree has revealed the relationship of BpR-2 GER with other *Streptomyces* species (Figure 3). Further, this gene sequence was submitted to NCBI GenBank as "*Streptomyces rochei* BpR-2 GER" and allotted the accession number ON142045.

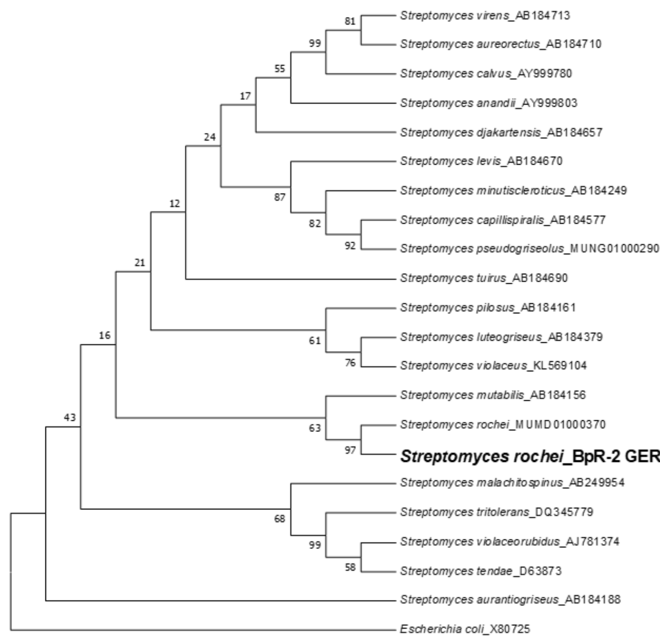

Fig 3. Phylogenetic tree of BpR-2 GER exhibiting relationship with other Streptomyces genera, based on the Neighbor-Joining method. Numerical values showed next to the branches are the percentages of replicate trees in the bootstrap test (500 replicates).

3.5 In vitro Screening for Antibacterial Activity

3.5.1 Primary Screening

The cross-streak method was used to assess the spectrum of antibacterial activity of BpR-2 GER against MTCC and the clinical strains of test pathogens. According to the results presented in Table 2, the BpR-2 GER possessed broad-spectrum antibacterial activity against both strains of pathogens that ranged from strong to weak. It was observed that BpR-2 GER strongly inhibited *B. cereus* (MTCC), *B. subtilis* (MTCC and Clinical), and *S. typhimurium* (MTCC), as shown in Figure 4 A-B. Gram-negative pathogens were moderately inhibited, however, no inhibition was observed against *P. aeruginosa* (MTCC and Clinical). Weak inhibition was noted against *S. aureus* (MTCC) and *S. epidermidis* (MTCC and Clinical), while no inhibition was observed against *S. aureus* (Clinical).

Table 2. Antibacterial activity of BpR-2 GER in primary screening

Test Pathogens	Antagonistic activity	
	MTCC	Clinical
Bacillus cereus	Strong	Moderate
Bacillus subtilis	Strong	Strong
Staphylococcus aureus	Weak	No inhibition
Staphylococcus epidermidis	Weak	Weak
Escherichia coli	Moderate	Moderate
Pseudomonas aeruginosa	No inhibition	No inhibition
Proteus mirabilis	Moderate	Moderate
Salmonella enterica typhimurium	Strong	

No inhibition (0%), Weak inhibition (<50%), Moderate inhibition (<75%), Strong inhibition (>75%)

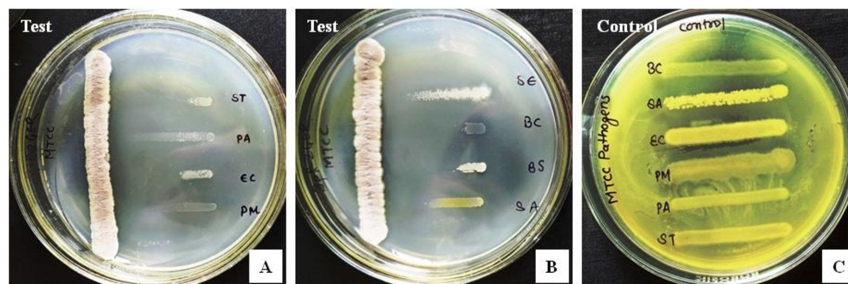

Fig 4. Antagonistic activity of BpR-2 GER against MTCC pathogens. (A-B) Test, (C) Control

3.5.2 Secondary Screening

Further, the crude filtrate of BpR-2 GER was analysed for antagonistic activity. The significant inhibitory activity against all the pathogenic bacterial strains was measured as the zone of inhibition (ZOI) (Figure 5). The maximum ZOI was observed against Gram-positive bacteria *B. subtilis* (MTCC-441) 19.38±1.30 and Gram-negative bacteria *Proteus mirabilis* (Clinical) 16.25±0.71 mm. However, the smallest ZOI was noted against *P. aeruginosa* (Clinical) 9.75±0.71 mm. The results were similar to the findings of an investigation done by Gohain et al., in which most of the endophytic isolates were found to be active against Gram-positive bacteria, while no activity was observed against *P. aeruginosa*[13]. The pattern of antimicrobial effect of a rare endophytic actinobacteria *A. cyanogriseus* SIR5 was found to be similar and in agreement with the present investigation[6].In Gram-negative bacteria, the production of hydrolytic enzymes, especially β-lactamase, and the presence of a lipopolysaccharide (LPS) layer on top of the peptidoglycan cell wall are responsible for reduced vulnerability to antibiotics[6,20]. Previously, the strain of *S. rochei* MSA14, isolated from uranium contaminated marine sediments, has been reported to exhibit prominent antibacterial property with wide spectral inhibitory zone ranging between 5±0.09 and 13±0.25 mm against test bacterial pathogens. Additionally, the endophytic strain of *S. rochei* was also reported as a biocontrol agent and plant growth promoter[21]. Bioactive metabolites such as borrelidin, Pyrrolidine-2, 4-Dione and a class of hydrolytic enzymes from *S. rochei* with significant pharmaceutical properties are well documented[15,22,23]. The overall result of the present in vitro antimicrobial screening and previous reports validates *S. rochei* BpR-2 GER as a potent producer of bioactive compounds with significant pharmaceutical properties.

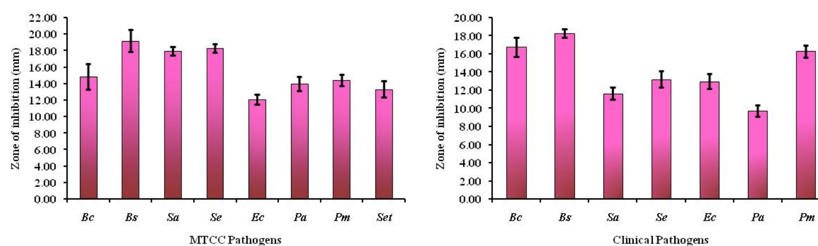

Fig 5. Secondary screening of antimicrobial activity of BpR-2 GER

3.6 Inhibition of Biofilm formation

Biofilm inhibitory activity of a crude filtrate of BpR-2 GER was evaluated against two strains of *Staphylococcus aureus*. In this assay, the cells embedded in the biofilm were observed by staining with crystal violet dye. This staining technique imparts the direct visualization of the biofilm forming ability of bacteria. Both strains of *S. aureus* were treated with crude filtrate in a dose-dependent manner and relative biofilm inhibition was measured quantitatively. The filtered broth of BpR-2 GER exhibited the remarkable antibiofilm property with the biofilm inhibitory effect ranging between 85.13±0.003 to 88.28±0.003% against *S. aureus* (MTCC-7443) and 82.78±0.005 to 85.83±0.002% against *S. aureus* (Clinical). It was seen that the inhibitory effect on both the strains of *S. aureus* rises along with the increase in the concentration of crude filtrate. Wells treated with 50% (v/v) crude filtrate had a maximal inhibitory effect of 88 and 86% against the MTCC-7443 and the clinical strains of *S. aureus*, respectively (Figure 6 A-B). Significant biofilm formation was observed in untreated wells while in treated wells reduced biofilm occurred,

which strongly advocated the antibiofilm potential of *S. rochei* BpR-2 GER. To the best of our knowledge, this is the first report on the antibiofilm potential of *S. rochei* and the present investigation revealed that BpR-2 GER has bioactive components with a damaging effect on the biofilm matrix. Hence, *S. rochei* BpR-2 GER could be a suitable choice to treat the infections caused by biofilm forming *S. aureus* strains. Previously, marine endophytic *Streptomyces sp.* with significant antibiofilm activity against methicillin-resistant strains of *S. aureus* and multidrug-resistant *P. aeruginosa* was reported[(24)]. Soil *Streptomyces sp.* GMR22 has been identified recently as a remarkable antibiofilm agent against a fungal pathogen *Candida albicans*[(25)].

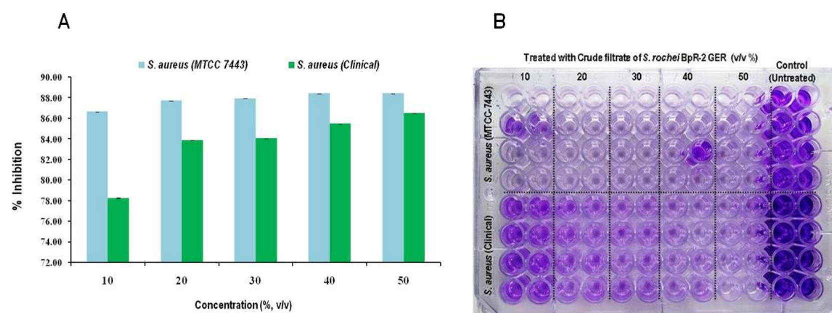

Fig 6. Effect of crude filtrate of BpR-2 GER against biofilm formation of *S. aureus* strains, % inhibition was statistically significant in all concentrations with reference to control (A). 96-well micro titer plate assay demonstrating antibiofilm activity of BpR-2 GER (B).

3.7 Microscopic Visualization of Biofilm Inhibitory Activity

Further, the effect of a crude filtrate of *S. rochei* BpR-2 GER on biofilm formation of *S. aureus* (MTCC and Clinical) strain was evaluated by light and fluorescent microscope using crystal violet and EtBr stain, respectively. The biofilm formed on a glass surface in the absence of crude filtrate exhibited the typical biofilm architecture with densely accumulated cells (Figure 7 A-B, E-F). However, a disrupted biofilm matrix with dispersed bacterial cells was seen when glass slides were treated with crude filtrate (Figure 7 C-D, G-H). It was noticed that fluorescent dye EtBr enhanced the visualization of typical biofilm architecture formed on the glass slide. In treated glass slides, the intensity of fluorescent light was reduced as compared to control, which indicates the damaging effect of crude filtrate on an extracellular matrix formed by the bacteria[(12)]. Findings of the present study provide insights on prominent biofilm inhibitory activity of the *S. rochei* BpR-2 GER against both the strains of *S. aureus*.

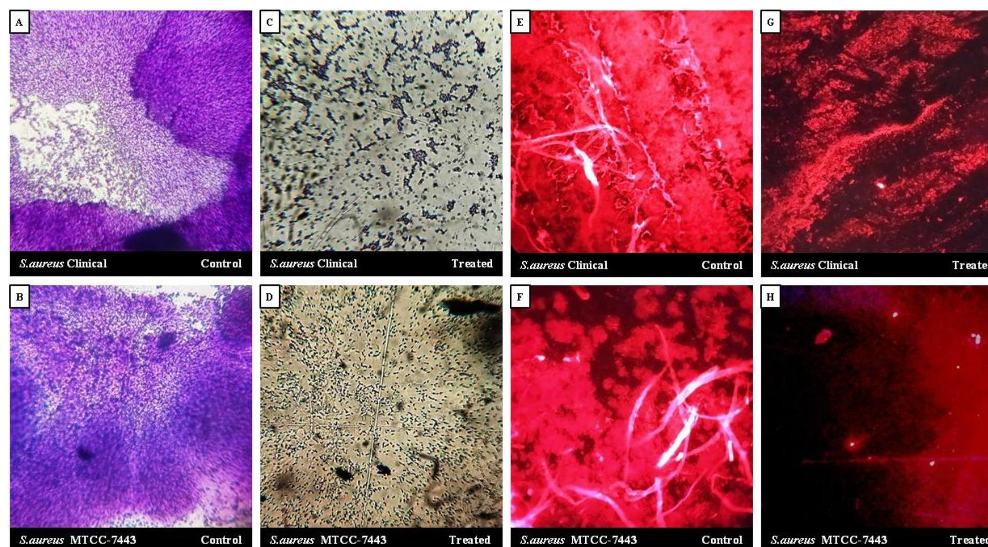

Fig 7. Antibiofilm activity of BpR-2 GER against *S. aureus* MTCC-7443 and Clinical strain under the (A-D) light microscope (x400) and (E-H) fluorescent microscope (x200).

4 Conclusion

To combat the elevated level of antimicrobial resistance, actinobacteria, particularly soil *Streptomyces sp*., have contributed a variety of antibiotics. However, extensive exploration is still required to isolate potent strains of *Streptomyces sp*. that harbour inside the tissue of diverse medicinal flora. To our knowledge, the ethnobotanically significant plant *Bryophyllum pinnatum* (Lam.) Oken has not been investigated earlier for the endophytic actinobacterial diversity in the Chhattisgarh region. Therefore, the current work aimed to isolate endophytic actinobacteria from the root tissue of *B. pinnatum*. As a result, *Streptomyces rochei* BpR-2 GER was obtained with significant antagonistic potential against MTCC and clinical human pathogens, exhibiting ZOI ranging from 9.75 ± 0.71 to 19.38 ± 1.30 mm. In addition, this work is the first to document the antibiofilm potential in the strain of *Streptomyces rochei*. The isolate has exhibited remarkable antibiofilm efficacy against MTCC and Clinical strains of *S. aureus*, having 88 and 86% biofilm inhibitory effect, respectively. Thus, the current investigation highlighted the therapeutic potential of *S. rochei* BpR-2 GER and suggests that it could be an alternative source for more effective and broad-spectrum bioactive metabolites to tackle antimicrobial resistance.

Acknowledgement

The authors acknowledge Head, SoS in Life Science, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, for providing the research facilities and the first author expresses high regard towards the Council of Scientific and Industrial Research (CSIR) for the Junior Research fellowship.

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