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Structural and Functional Dynamics of Secondary Metabolite from *Actinokineospora cibodasensis* against *Pseudomonas aeruginosa* Biofilm

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ABSTRACT

Biofilm formation has incredible detrimental effects and has brought huge issue to our daily life. *Pseudomonas aeruginosa* is recognized as an opportunistic pathogen and its one of the antibiotic resistance mechanisms includes biofilm formation. To survive against harsh environmental conditions the microorganisms form an extracellular polymeric substance matrix. Natural products, particularly, marine natural products have been established useful in providing new chemical entities to mark biofilm inhibition and dole out as a rich source of privileged frames for studying chemical as well as biological applications. In the present study, antibiofilm potential of bioextract from *Actinokineospora cibodasensis* (MW513387) is tested against *Pseudomonas aeruginosa* (ATCC 27853) biofilm. The analysis of the quorum sensing modulation was done using exopolysaccharide (EPS) quantification and Cytotoxicity assays were performed to check toxicity. Scanning electron microscopy (SEM) was also conducted to visualize the mitigation of biofilm using the biocontrol agent. All the assays conducted evidently indicated that the potential bioactive compound from Actinokineospora cibodasensis has greater inhibition effect against pathogenic biofilm. In the near-term years it is expected that these pharmaceutical compounds from natural source be translated into antibiofilm drugs.

Keywords: Biofilm, Antibiofilm drug, *Actinokineospora cibodasensis*, *Pseudomonas aeruginosa*, Exopolysaccharide, Antibiotic resistance and cytotoxicity.

INTRODUCTION

Comprehensive initiatives are in a mission for urgent action against antimicrobial resistance in view of the fact that it is a precarious and constantly escalating problem, leading to a swell in health care costs, reduced treatment options, and obviously a greater occurrence of mortality due to various bacterial or viral infections worldwide[1]. World Health Organization not only recommends for development of novel antimicrobial therapies but also advocate to lessen the contribution of humans to increasing antimicrobial confrontation (WHO, 2018). In some contexts, sluggish growth and adaptive stress response can be interconnected. For instance, in biological systems, organisms might exhibit

sluggish growth due to environmental stress. This stress may activate adaptive response to better survive in challenging conditions, and configuration of persisted cells throw in to the elevated levels of antibiotic resistance[2-3]. Specific techniques can be followed for biofilm quantification[4-6]. Redox assays utilizing tetrazolium salts where the tetrazolium reduction is proportional not only to cell count but also to check the metabolic activity makes it a powerful tool for assessing the viability and activity of cells. Even if there is large number of cells and metabolically inactive, they will show minimal reduction activity[7-8].

Comparing the results of tetrazolium reduction assays to microscopy counts is a clever way to gain insights into the proportion of active and inactive cells in different samples. Microscopy allows for the direct visualization of cells, which can provide valuable information about their morphology, structure, and state. By combining this visual data with the metabolic activity data from the tetrazolium assay, researchers can draw more comprehensive conclusions about the overall functionality of the cell population[9-10]. Routinely used assays are (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and methoxynitrosulfophenyl-tetrazolium carboxanilide (XTT) assay[11-15]. Here the bio extract of Actinokineospora cibodasensis targeted against the biofilm produced by the pathogen *Pseudomonas aeruginosa*. This investigation revealed that the potential anti-biofilm properties of the *Actinokineospora cibodasensis* bio extract, which may be of significant interest in areas related to antibiofilm agent.

This study aimed to identify the bioactive compound from active actinomycetes and evaluate the cytotoxic activity of their crude extracts, additionally, the extracts were assayed against *Pseudomonas aeruginosa* biofilm. The analysis of quorum sensing modulation was done using exopolysaccharide (EPS) quantification. Scanning electron microscopy (SEM) was also conducted to visualize the mitigation of biofilm.

MATERIALS AND METHODS

Microbial strain and Bioactive Compound identification by NMR spectroscopy

The microbial strain used in the study was extracted from marine water sample and is identified as *Actinokineospora cibodasensis*, and the gene sequence deposited in National Center for Biotechnology Information (NCBI) genbank, accession number obtained is MW513387. Evolutionary analyses were conducted in MEGA X[16]. The biofilm formed by *Pseudomonas aeruginosa* ATCC 27853 is used throughout the study for biofilm formation and inhibition and microbial culture purchased from American Type Culture Collection (ATCC).

Extraction of secondary metabolites was done by concentrating the crude extract of the *Actinokineospora cibodasensis*, (obtained by ethyl acetate and liquid-liquid extraction) and was observed that the extract of isolate posses' unique compounds showing bioactive properties. The predominant compound isolated showed structural similarities to streptomycin after analyzing the results of Fourier transform infrared (FTIR) and High-Performance Thin Layer Chromatography (HPTLC) techniques[16]. In the present study, the extract is also analyzed with Nuclear Magnetic Resonance (NMR) spectra for further confirmation of structure similarity to streptomycin. NMR spectroscopy was done at Central Laboratory for Instrumentation and Facilitation, Kerala University Trivandrum. This technique relies on the phenomenon of nuclear magnetic resonance to provide detailed information about the structure, dynamics, reaction state, and chemical environment of the molecule[17].

Assay for biofilm formation

The procedure is carried out to characterize the biofilm-forming capacity of bacteria. A 3 mL of cells from overnight culture was inoculated in 100 mL of nutrient broth (HiMedia, Mumbai) and biofilm was allowed to form in 96-well poly-styrene Microtiter plates at 258^{0}C for 24 hours. Growth of cell was measured by reading the absorbance (OD at 650 nm) of each well using a plate reader. Medium was discarded and individual wells were stained with 0.1% crystal violet (CV). Subsequently, the amount of cells attached was estimated by measuring the absorbance (OD at 650 nm) of CV dissolved in 0.5% Sodium Dodecyl Sulphate (SDS) by the plate reader. Then, the value of biofilm was normalized according to the amount of cells. This value (CV/growth) was termed 'relative biofilm', and for each strain, it was indicated as the ratio of its relative biofilm to that of wild type.

Biofilm inhibition assay

A bacterial suspension of *Pseudomonas aeruginosa* is prepared.100 µL of this bacterial suspension is added to each well of the 96-well plate. Then 100 µL of nutrient broth was added to each well, along with the bacterial suspension. This provides the necessary nutrients for bacterial growth and biofilm formation. Different concentrations of test samples (potentially antimicrobial agents or compounds being tested for biofilm inhibition) were added to the wells. This step is crucial for assessing the inhibitory effects on biofilm formation. Here a well with sample-free bacterial suspension serves as the positive control. This control helps to compare the biofilm inhibition effects of the test samples. Then the 96-well plate was incubated at 37°C for 2 days. This allows the bacteria to form biofilm under the influence of the test samples. After the incubation period, the nutrient broth carefully removed from each well to eliminate unattached cells. The wells were then washed three times with sterile distilled water to remove any remaining unattached cells. Then 100 µL of a 1% aqueous solution of crystal violet was added to each well. The plate then left for 30 min to allow for the staining of the biofilm. The crystal violet solution was carefully removed, and the wells were washed thoroughly to remove excess dye[18].

Ethanol Treatment was done after this by adding 95% ethanol to each well and incubated for 15 minutes. This step helps in solubilizing the crystal violet and extracting it from the biofilm. Finally, this was read at 570nm. By following the above steps, there formed liquid culture of *Pseudomonas aeruginosa* in nutrient broth. This culture can serve as a source of bacteria for further experiments or analyses, such as those related to biofilm formation, antimicrobial testing, or another microbiological studies[19-20].

$$\% \text{ Inhibition} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Modulation of quorum sensing by cell quantification assays

Quantification of Exopolysaccharide (EPS)

Extracellular polysaccharide (EPS) constitutes the basic unit of biofilm matrix. EPS in the formed biofilm was determined by phenol sulphuric acid method. In order to quantify the EPS on the surface of the culture plate, the test organism was grown individually in the Nutrient Broth (Hi Media, Mumbai) for 10 days in 24 well plates at 37^{0}C. Then the sample with concentration of 10 mg/mL added and incubated & continued at same conditions for another 3 days. It was then removed, washed in PBS and mixed with 0.5 mL 5% Phenol (in 0.1 N HCl) followed by 2.5 mL of concentrated sulphuric acid at normal temperature. The optical density was measured at 490nm and percentage of inhibition was calculated. A control without the extract and a blank without organism were also maintained throughout the study[21-22].

Cytotoxicity studies-MTT assay and statistical interpretation

After 24 h of incubation, 30 µL of reconstituted MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) solution was added to all the test and cell control wells.

The plate was gently shaken to ensure even distribution of the MTT solution. The plates then placed back in the incubator at 37°C for an additional 4 hours. During this time, viable cells with active mitochondria will convert the MTT into formazan crystals, giving a purple color. After the 4-hour incubation period, the supernatant from each well was collected. The collected supernatant then centrifuged at 6000 rpm for 10 minutes. The resulting pellets were then resuspended in 100 µL of MTT Solubilization Solution, which is a mixture of Dimethyl sulphoxide (DMSO). The wells were mixed gently by pipetting up and down to solubilize the formazan crystals. Followed by Second centrifugation, the content obtained was centrifuged at 6000 rpm for 5 min and the supernatants were collected.

The MTT substrate was prepared in a physiologically balanced solution, added to cells in culture and incubated for 4 hours. The quantity of formazan is measured by recording changes in absorbance. The absorbance values measured using a micro plate reader at a wavelength of 540 nm. This measurement reflects the amount of formazan produced, which is directly proportional to the metabolic activity of the cells[23].

Statistical Analysis

Statistical analysis is performed using SPSS software, version 3. This is likely used to analyze the data and determine if there are any significant differences between experimental groups. The references to[24], likely provide further insights into

the methodology and its application in your specific study. This MTT assay is a widely used technique for assessing cell viability and is particularly useful in studying the effects of treatments or substances on cell health and metabolism. The percentage of growth inhibition was calculated using the formula:

Percentage of viability = Mean OD of samples / OD of control X 100

Cell viability and Proliferation XTT assay and statistical evaluation

Methoxy nitro sulfophenyl-tetrazolium carboxanilide (XTT) assay was carried out in 72 h old bacterial biofilm of *Pseudomonas aeruginosa*. The biofilm was treated with samples of different concentrations such as 6.25, 12.5, 25, 50 and 100 µL respectively and incubated at room temp for about one day. After 24 h of incubation period, 50 µL of reconstituted XTT (detection Solution) was made to mixed with all test and control wells, the plate was gently shacked well, then incubated at 37°C in incubator for 2-5 hours. The wells were mixed gently to solubilize the formed formazan crystals. The content was centrifuged at 6000rpm for 5 mins supernatants were collected. The reading was taken at 450 nm. Statistical study was also conducted using Statistical Package for Social Sciences (SPSS) software version 325. Understanding the growth phase, metabolic activity, and potential interferences specific to the cell type and experimental conditions is crucial for obtaining accurate results. This information will help researchers to interpret their data effectively and draw reliable conclusions from their experiments.

The percentage of metabolic activity of cell was calculated using the formula:

In % = Mean OD of samples / OD of control X 100.

Scanning Electron Microscopy Analysis to observe the mitigation of biofilm

P. aeruginosa culture was incubated 24 h at 37°C. The inoculum was kept for biofilm formation on micro-slide/cover slip for 24-48 h at 37°C. After incubation, keep one slide as control for each organism. The required number of cover slips is treated with compound in different concentrations if necessary[26]. Modifications were made in the fixation procedure, Primary fixation was done with 2.5% gluteraldehyde for 1 h at room temperature. Washing was done with 0.1 M Sodium phosphate buffer (pH 7.3) for 3-5 times. Dehydration was done with 25% ethanol, 50% ethanol, 75% ethanol and 90% ethanol for 5 min each and final dehydration with 100 % ethanol for 5-10 min and repeated twice. The cover slips were dried and fixed, mounted on studs and coated with gold plasma and examined using SEM (JEOL Model JSM - 6390LV)[27].

RESULTS AND DISCUSSION

Secondary metabolite identification by NMR spectroscopy

Spectra are shown in Fig. 1 showing major similarity to structure of streptomycin and the comparison with standard compound with the sample extract is represented

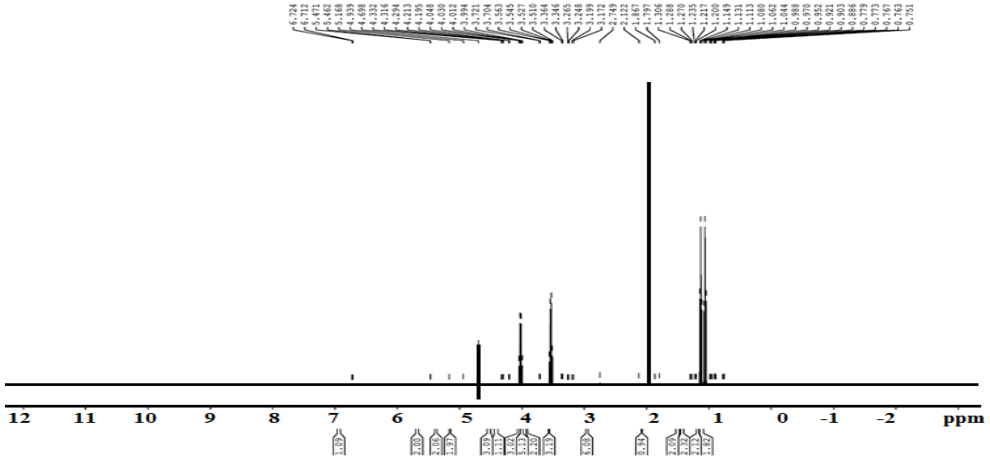

Fig. 1(a). NMR spectra of bioextract from *A. cibodasensis*. NMR spectra of the bioactive compound showed different peaks and chemical shifts indicating the presence of unique compounds

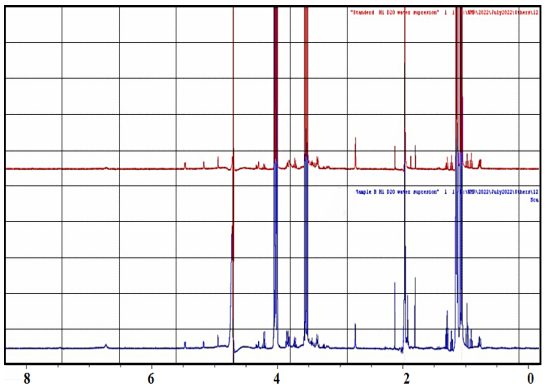

Fig. 1(b). NMR spectra show the comparison-sample and Streptomycin. The structural bonding and chemical shifts support the similarity of the drug to Streptomycin[28]

These below shifts are essential information for interpreting proton nuclear magnetic resonance (^{1}H NMR) spectra. Here's a summary of the key points mentioned: Aliphatic Hydrogen in C-H Bonds: Typically appear in the range of δ 1-2 ppm. Hydrogens near Double Bonds (C=C or C=O): Experience deshielding and exhibit chemical shifts downfield, around δ 2-2.5 ppm. Hydrogens near Electronegative Atoms (O-C-H): These Hydrogens are further deshielded, and their chemical shifts move even further downfield, to approximately δ 3-4 ppm. Hydrogens directly bonded to Double-Bonded Carbons: Found at approximately δ 4.5-6 ppm. Aromatic Hydrogens (H on Benzene Ring): Exhibit chemical shifts around δ 7 ppm. Hydrogens in OH (Alcohol) or NH (Amine) Groups: Show a relatively large range of chemical shifts, from δ 1-5 ppm.

These chemical shifts provide valuable information about the local chemical environment of protons within a molecule. By analyzing the positions and relative intensities of these peaks in a ^{1}H NMR spectrum, chemists can gain insights into the structure and composition of organic compounds. Understanding these general trends is crucial for interpreting NMR spectra, as it allows scientists to make educated determinations about the presence and arrangement of functional groups within a molecule.

Different peaks due to chemical shifts in the sample were noted at the ranges δ 0.9 -1.2 ppm corresponding to R-CH_3or alkyl, δ 1.5-2.5 ppm corresponding aromatic CR_2=CR-CHR_2, δ 3.3-4 ppm belongs to HR-O-CH_3, δ 3.5-4 ppm ranges for OH group, δ 4-4.5 ppm ranges for NH_2 group and δ 4-5 ppm ranges for double bonded carbon atoms. The streptomycin compound bearing the amino and hydroxyl groups with two benzene rings and one pentose ring shows great similarity to one of the major compounds presents in the bioextract as shown in the above figures coinciding their peaks. Comparison with streptomycin standard proves the hypothesis and thus the compound is confirmed to have structural identity with streptomycin[29-31].

Biofilm formation and inhibition assays

Assay for biofilm formation

The assay was conducted as per biofilm formation assay and the results are tabulated and estimated as follows:

Table 1: The relative biofilm value

Organism	OD at 650 nm	Relative Biofilm (Control value/growth)
Pseudomonas aeruginosa	0.305	0.3

*Control (Control Value) = 0.932

Biofilm inhibition assay

The assay was conducted and the results are tabulated and plotted as a graph as follows:

Table 2: Results of *Pseudomonas aeruginosa* ATCC 27853 biofilm inhibition assays, bioextract from sample organism (*Actinokineospora cibodasensis* MW513387)

S. No	Concentration of the sample (ppm)	OD(at 570 nm)	Percentage of biofilm inhibition
1	Control	0.305	
2	100	0.23	24.5
3	200	0.188	38.3
4	300	0.167	45.2
5	400	0.142	53.4
6	500	0.118	61.3

Conc. of the stock=1 mg/mL

The results clearly indicated a gradual rise in percentage of *P. aeruginosa* biofilm as the concentration of the biological extract used was increased in a regular pattern (Fig. 2). Microtiter assay was performed (Figure 3).

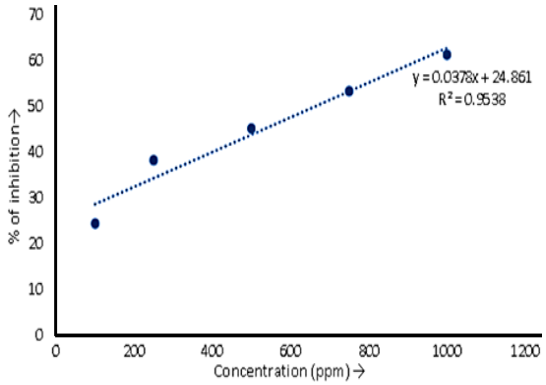

Fig. 2. Plot showing *P. aeruginosa* biofilm inhibition by the sample extract. The graph shows the regular reduction of biofilm formation as the concentration of sample is increased at regular intervals

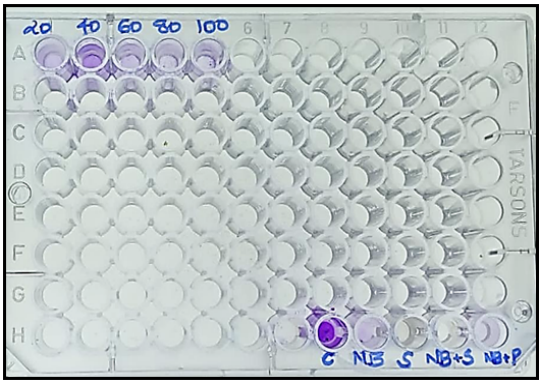

Fig. 3. Microtiter assay: The microtiter plate shows the periodical reduction of biofilm on addition of different concentrations of bioextract using crystal violet assay

Modulation of quorum sensing by cell quantification assays

Quantification of EPS

The EPS quantification was done as per discussed in 2.4.1. The calculations are tabulated as follows:

The standard graph for glucose was first estimated and tabulated in Table 3 and depicted in Fig. 4. Table 4 shows the assay of EPS quantification with absorbance values and amount of carbohydrates present.

EPS is a direct measure of biofilm quantification since the biofilm are always accompanied with EPS matrix for their survival. The study on EPS quantification by phenol sulphuric acid method accurately showed the biocontrol pathogenic biofilm on treatment with the bioextract in the present investigation. Standard glucose graph represents the concentrations of EPS on x axis on absorbance read at 490 nm on y axis 32. From this result, it was confirmed that the test isolate produced the EPS as a strong mat. Attenuation of biofilm requires the formation, evaluation and quantification of extra polymeric substances. This aids in the systematic analysis of biofilm growth and inhibition[33].

Table 3: Standard Glucose graph. The values are measured accordingly at regular intervals of various concentrations and the absorbance in the form of optical density measured at 490 nm is tabulated as given below

S. No	Concentration(µg/mL)	Absorbance(OD at 490nm)
1	200	0.1526
2	400	0.2869
3	600	0.3386
4	800	0.3904
5	1000	0.4810

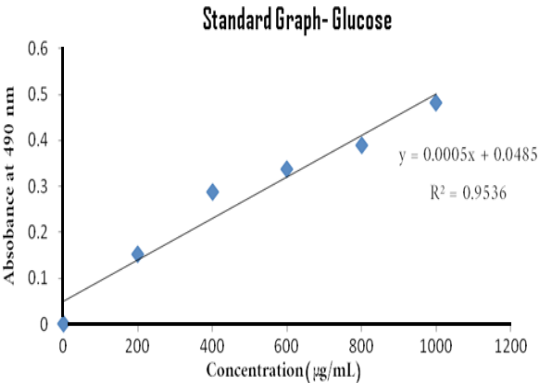

Fig. 4. Standard graph of Glucose for estimation of carbohydrates. The graph shows a periodical rise in absorbance with increase in concentration

Table 4: EPS quantification. The results shows a steep decrease in the amount of carbohydrates present in this, after the treatment with the sample compared with the control showing the role of biocontrol of *P. aeruginosa* biofilm on addition with streptomycin containing extract of *A. cibodasensis*

S. No	Sample code	Absorbance (OD at 490nm)	Amount of carbohydrates (mg/mg of sample)
1	Control	0.6173	1137.6
2	Sample	0.4221	747.2

*Concentration of Stock is 10 mg/mL DMSO (Dimethyl sulphoxide)

Anti bacterial assays including well and tube dilution methods, Minimum inhibitory concentration assays to find Sub MIC s were also conducted. Gene expression studies conducted to prove the down regulation of biofilm forming

genes of *Pseudomonas aeruginosa*[16]. Gene expression study showed that the biofilm forming genes namely, LasR and RhIR of *Pseudomonas aeruginosa* were drastically down regulated on treatment with extract from *Actinokineospora cibodasensis*. Expression fold change was found as -1.2038102 compared to control[16].

Cytotoxicity studies-MTT assay and statistical interpretation

The assay was conducted as per described in 2.4.2.1. The results are estimated and tabulated in Table 5. Fig. 5 shows the Microtiter assay and statistical interpretation conducted for the same.

Table 5: MTT assay. The results clearly indicate the percentage of viability reduces as the concentration of test sample increases with respect to control sample

Sample Concentration (µL)	OD value I	OD value II	OD value III	Average OD	Percentage of viability
Control	2.4516	2.7527	2.4102	2.5381	100
Sample code : Sample 1					
6.25	2.4685	2.3338	2.6027	2.4683	97.24
12.5	2.268	2.2541	2.3981	2.3067	90.88
25	1.9951	1.9874	1.8951	1.9592	77.19
50	0.7104	0.6824	0.5451	0.6459	25.44
100	0.3358	0.3427	0.2648	0.3144	12.38

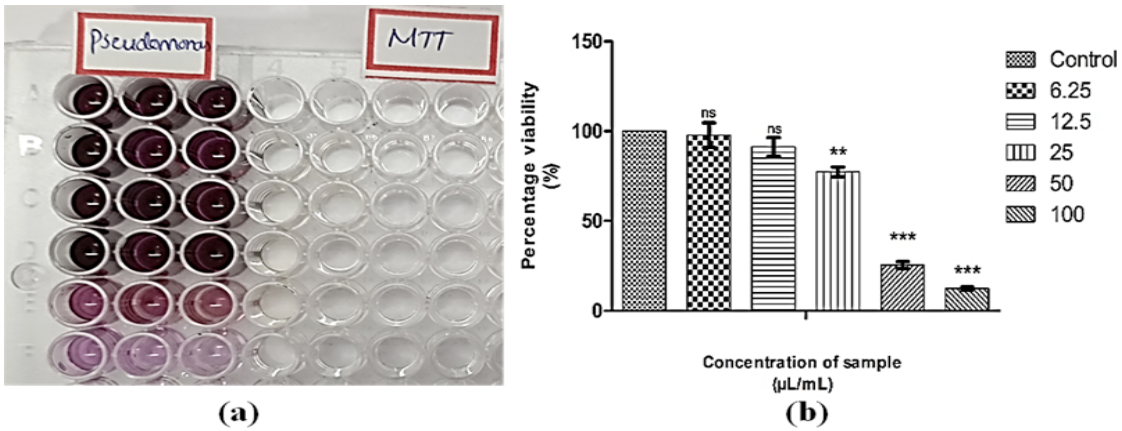

Fig. 5(a). Microtiter plate and (b) Statistical interpretation for MTT assay. Figure shows a reduction in crystal violet staining on addition of bioextract at different concentrations and the statistical analysis clearly indicate the same by SPSS software

MTT assay also strongly support the fact of biofilm reduction in *P. aeruginosa* on treatment with the bio compound extracted from *A. cibodasensis*.

Cell viability and Proliferation XTT assay and statistical evaluation

The assay was carried out as per 2.4.2.2 and the results are showed in Table 6 and Fig. 6 shows the statistical evaluations and Microtiter plate assay for the same.

The data from XTT assay also sustain the fact that the *A. cibodasensis* extract show a valid antibiofilm activity against pathogenic *P. aeruginosa*.

Table 6: XTT assay results. The tabulations and calculations clearly demonstrate the antibiofilm activity of the sample extract against *P. aeruginosa*

Organism: *Pseudomonas aeruginosa* ATCC 27853					
Concentration(µL)	OD I	OD II	OD III	Average	% of CMA
Control	0.5633	0.5399	0.5692	0.5574	100
Sample-1-Extract from Actinokineospora cibodasensis (Gen Bank No: MW513387)					
6.25	0.4752	0.4102	0.4031	0.4295	77.05
12.5	0.3512	0.3535	0.3031	0.3359	60.26
25	0.2701	0.2532	0.2676	0.2636	47.29
50	0.1675	0.1623	0.1541	0.1613	28.93
100	0.0615	0.0477	0.0581	0.0557	9.99

*CMA-Cellular modulating activity

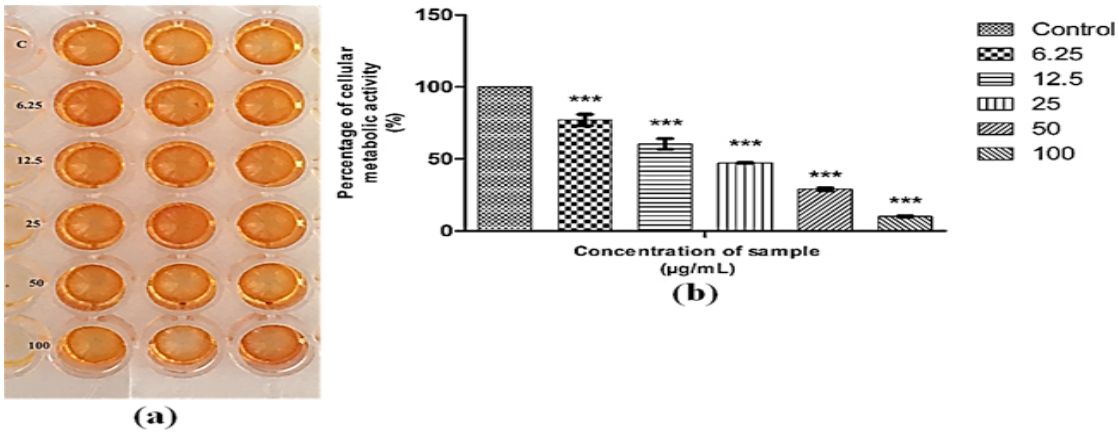

Fig. 6(a). Microtiter plate and (b) Statistical evaluation for XTT assay. The Microtiter plate shows the rapid reduction of cellular modulating activity of *P. aeruginosa* cells on addition of increasing concentrations of sample extract. The statistical experiment done by SPSS software supports the data

Scanning Electron Microscopy (SEM)

SEM was performed as detailed in section 2.5. The SEM images clearly point out the mitigation of *P. aeruginosa* biofilm by the extract from *A. cibodasensis* at two different magnifications which confirms the fact of its bioactive ability (Figure 7. (b), (d).

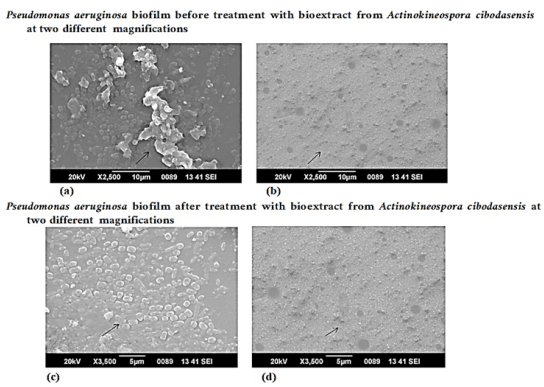

Fig. 7. SEM. The SEM image shows the biofilm inhibition by the bioextract at two modes of magnifications. Images (a) & (c) shows the matured biofilm formed by *Pseudomonas aeruginosa*. Image (b), (d) shows the biofilm inhibition with the bioextract from *Actinokineospora cibodasensis*

The whole study proves the biopotential of secondary metabolite extract from *Actinokineospora cibodasensis* (Gen Bank No: MW513387) against pathogenic *Pseudomonas aeruginosa* (ATCC 27853). NMR spectroscopy reveals that there are unique components in the extract showing various antimicrobial properties. The major component was recognized to be a compound very similar to streptomycin through various assays (Sreelakshmi *et al.,* 2023). Proton NMR studies also hold up the same data. Some other studies also report that the genera of the organism under study produce antibiotic similar compounds that show biofilm activity. For example, there was a recent report published about the some unique biocompounds isolated from the marine water bacterium Actinokineospora spheciospongiae sp[34]. Similar reports were published about various species of Actinokineospora about their antifungal and antibacterial effects[35-37]. This is found to be the first study reporting the presence of streptomycin like compound from a novel species of *Actinokineospora* spp.

The present investigation about biofilm inhibition was proved using Microtiter plate assay. Most of the reports are with regard to the antimicrobial activity of secondary metabolites from Actinokineospora isolated from wet ecosystems as already published[38-41]. This study reveals a new era of biological activity to the compounds produced from a marine microbe that can be used as a marine derived cheaply produced drug in the field of medicine against the dangerous diseases caused by pathogenic biofilm.

Several studies are conducted in the field of cytotoxicity with regard to bioactive components from *Actinokineospora* species. A study in 2018 demonstrated the identification of 2 alkaloid compounds with the radical scavenging activity from Actinokineospora strain[42]. Previous studies also performed cytotoxicity studies of *Actinokineospora* sp. EG49. Other papers are also published the similar data which are comparable to the reports we retrieved in the study[43-44]. The study revealed

the cytotoxic nature of the bioextract against the pathogenic biofilm.

Scanning electron microscopy is a technique that is used to visualize the cell growth in a cubic space. SEM images have helped in portrayal of various antimicrobial activities of actinomycetes in different aspects. SEM images for biocontrol activity of Actinokineospora had been conducted by several researchers as listed[45-47]. The present study focused on the antibiofilm effect of Actinokineospora cibodasensis which have been clearly displayed at various magnification of SEM.

Thus, the present study focused on a novel therapeutic agent from a new strain of *A. cibodasensis*, similar to the structure of antibiotic streptomycin that possessed immense biofilm activity against opportunistic pathogen *P. aeruginosa* which is a potent threat to various industries. Future prospective of the study may include the detailed structural elucidation that may reveal the real mechanism of therapeutic action of the potent bio compound in a broad aspect. Transmission electron microscopic and confocal microscopic techniques of biofilm reticence from different surfaces can also lead to a better visualization effect of the bio properties[48].

CONCLUSION

The present study demonstrated that the Streptomycin like compound produced from *Actinokineospora cibodasensis* (Gen Bank No: MW513387), which can be produced by fermentation using cheap nutrients as fermentation medium. *A. cibodasensis* is a novel strain that possess very much unique biological properties, of which antibiofilm activity against *Pseudomonas aeruginosa* (ATCC 27853) is the study of interest. The strain isolated in this study producing streptomycin resembling compound is the first ever report to the best of our knowledge along with its potency in antibiofilm activity on medical devices which can be utilized in therapeutic industry. There is no such scientific report on antibiotic producing *A. cibodasensis* from samples collected from coastal regions of Kerala district to the extent of author's awareness. The antibiofilm activity was confirmed using EPS quantification, SEM images also clearly visualized the mitigation of pathogenic biofilm. Thus, the bioextract from *A. cibodasensis* found to be very relevant pharmacological drug that showed great similarity to streptomycin with greater activity after structural elucidation using various techniques. This paves the way for the future studies with regard to the structural aspect of the novel compound and more into its gene level studies that can probably lead to an immense upheaval in various industries including the medical diligence.

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Conflict of interest

There is no conflict of interest

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