

Inhibition of Bacterial Biofilms by Streptomyces Derived Crude Extract

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ABSTRACT

One of the ability of bacteria is to form biofilms. Generally, bacteria prefer to live in communities in biofilms because by doing so they can acquire many advantages such as bacteria within biofilms show resistance to antimicrobial agents also they can overcome the host immune defense mechanism. The increasing rate of antibiotic resistance by the biofilm forming microorganisms have hampered the treatment options and highlighted the need for antibiofilm agents. Members of the Actinomycetes family are known to produce active antibiofilm compounds. This study was designed to find a broad spectrum, novel, non-toxic antibiofilm agent from *Streptomyces* against various biofilm forming bacteria like *Klebsiella*, *Pseudomonas*, *Staphylococcus* and *Enterobacter*. Ethyl acetate extracts of two *Streptomyces* species *Streptomyces viridochromogenes* strain NBRC 3113 and *Streptomyces levis* strain NRRL B-16370 were used to conduct the experiments. Results of *in vitro* biofilm formation assay have revealed that, extracts obtained from *Streptomyces* species strongly inhibit the biofilms of all the four bacteria under study on polystyrene surface. The extracts are dose dependent and do not affect the growth of bacterial species. Physical characterization showed that one of the extract is heat resistant and other is heat sensitive. Chemical characterization revealed that the bioactive component(s) of both the extracts are non-proteinaceous in nature. There was significant loss in the hydrophobicity of biofilm bacteria in presence of the extract. All together the results suggest that the extracts can be the good starting material for developing a novel antibiofilm agent since it inhibits the biofilms of different bacterial species belonging to different families and thereby prevent chronic infections caused by such pathogenic strains.

Keywords: Biofilm; Antibiofilm; Streptomyces; Crude extract

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INTRODUCTION

In nature bacteria prefer to live in communities with other bacteria in order to compete with the resources and space. Many of the bacteria have the ability to sense the environment around them and behave in a way that they could resist in different environmental niches [1]. One of the ability of most of the bacteria is to form biofilms. Generally, bacteria prefer to live in communities in biofilms as they confer many advantages by adapting such lifestyle. A biofilm is an aggregation of micro-organisms, in which the bacterial cells grow attached to a surface and are embedded in a self-produced extra polymeric substance. It is also known as slime layer, which covers the bacteria in such a way that it acquires resistant to not only antimicrobial agents but also overcome the host immune defense mechanism. Development of biofilms results in structural and phenotypic changes such as colonization of host tissues, expression or enhancement of virulence traits, protection from stress, sequestering of nutrients, enhanced cell to cell communications and survival in harsh conditions [2].

Biofilms can be formed on biotic and abiotic surfaces. They can be formed on, household surfaces in bath and kitchen, in toilet, sinks and pipelines. From medical point of view, they can be formed on implants and medical devices such as mechanical heart valves, catheters, pacemakers, surgical pins, prosthetic joints.

Also development of biofilms can occur on contact lenses, on tooth surface as well as on various tissue surfaces [3].

Biofilm may consist of a single species or multi species community. Single species biofilms are mostly involved in variety of chronic infections. Therefore, study of single species biofilms is of great interest in research [4].

Biofilm formation occurs in a series of steps. The first step involves initial attachment of planktonic bacteria to a solid surface, followed by production of extra polymeric substance, resulting in proliferation of attached cells and accumulation of more cells forming multilayer cell clusters which are called as micro colonies. This forms the early stage of biofilm architecture. The micro colonies further proliferate and form a mature biofilm. In the Last step the single cells disperse from the matrix [4,5]. The matrix covers the surface and allows the cells to resist against different environmental conditions. The matrix acts as a stabilizing scaffold for the three dimensional biofilm Nutrients get trapped in the matrix for metabolic utilizations by the resident bacteria and with the aid of hydrophilic polysaccharides, water is efficiently retained through H-bond interactions. Enzymes secreted by the bacteria modify EPS composition in response to changes in nutrient availability, resulting in changes in biofilm architecture to the specific environment. The structural components of the matrix are responsible for a highly hydrated, robust structure with high tensile strength that keeps bacteria in close proximity, enabling

intimate cell-to-cell interactions and DNA exchange, and also protects the biomass from desiccation, predation, oxidizing molecules, radiation, and antimicrobials [6]. The resistant nature of biofilms can also be attributed to the presence of environmental gradients within the biomass, which give rise to community with subpopulations of bacteria showing differential gene expression in response to local nutrient and oxygen availability [7].

The composition of extra cellular matrix varies among different bacterial species. It consists of exopolysaccharides, which is an essential component of almost all bacteria that provide a matrix for initial attachment of cells. Beta-1,6- linked Nacetyl-D-glucosamine is the most common component of many bacterial biofilm [8]. Besides these proteins, Pili, flagella and other adhesive fibres and extra cellular DNA are also present in the biofilm.

Biofilms show negative impact on industry, marine transportation and medicine. The increasing rate of antibiotic resistance, has become a threat to public health. They are involved in variety of human infections such as endocarditis, osteomyelitis, chronic otitis media, gastrointestinal ulcers, urinary tract infection, chronic lung infections like cystic fibrosis patients and periodontitis. The infection which is most widely reported is catheter associated urinary tract infection. Many of the chronic infections like diabetic, ulcers result from bacteria growing in biofilms.

Both gram positive and gram negative bacteria are capable of forming biofilms.

Klebsiella pneumoniae is one of the most important pathogen causing infections such as pneumonia, sepsis and urinary tract infection [9]. Besides *K. pneumoniae* is the major cause of infections in catheterized patients and therefore it is considered as top eighth nosocomial pathogen [10]. It is characterized by the virulence factors such as capsule, type 1 and type 3 pili, KPF-28 fimbriae and lipopolysaccharides. Among these capsule allows it to overcome phagocytosis and cause infection [10].

Staphylococcus aureus is an opportunistic pathogen that forms biofilms on medical devices and contact lenses. This organism is involved in nosocomial and chronic biofilm-associated infections. It forms biofilm by initial attachment of surface proteins to host proteins, which is followed by exopolysaccharides expression that allows aggregates of bacterial cells.

Pseudomonas aeruginosa is a pathogen involved in both acute and chronic infections. It can form biofilm on tissues; it is a major problem in burn wounds, chronic wounds, surface growth on implanted biomaterials, and hospital surfaces.

Recently it has been reported that *Enterobacter cancerogenus* cause infections in adults like wound infections and septicemia. It was also reported that this organism forms biofilm on the neonatal enteral feeding tubes. Therefore, neonatal feeding tubes can be considered as potential source infections in neonates.

Thus, due to the increasing resistance towards antimicrobial agents, research on how bacteria form biofilms and how these biofilms can be inhibited by various nontoxic compounds without affecting the growth of organisms is under focus.

The *Actinomycetes* are aerobic gram positive bacteria, with a high GC content, able to form branching filaments or hyphae. They resemble fungi in their morphology. They belong to the phylum Actinobacteria, they are found in both terrestrial and

aquatic ecosystems, mostly in soil. Around 23,000 biologically active secondary metabolites are known to be produced by micro-organisms of which approximately 45% are produced by actinomycetes. Among *Actinomycetes* majority of bioactive compounds are produced by *Streptomyces* species. *Streptomyces* have therefore become a hotspot for pharmaceutical industry. Members of this group are producers of antimicrobial, antiparasitic, antichlamydial, antioxidants, immunomodulatory, anticancer and antibiofilm agents.

Recently some reports have shown that, actinomycetes produce certain active antibiofilm agents for example, *Streptomyces albus* have shown to inhibit the biofilm of *Vibrio harveyi*, and *Streptomyces akiyosheinsis* inhibits the biofilm of *Staphylococcus aureus* [1].

Present study aimed to explore the potential of *Streptomyces viridochromogenes strain NBRC 3113* and *Streptomyces levis strain NRRL B-16370* to produce certain broad spectrum antibiofilm agents [11-18].

MATERIALS AND METHODS

Test bacterial isolates

Pseudomonas aeruginosa and *Staphylococcus aureus* were obtained from hospital. *Klebsiella pneumoniae* and *Enterobacter cancerogenus* were isolated from environmental sample. These four organisms were examined for their biofilm forming ability.

Screening bacterial isolates for their biofilm forming ability

Congo red assay (CRA): Congo red agar (Brain heart infusion broth-37 gm, sucrose-5 gm, Congo red dye-0.8 g, and agar-15 g per litre of distilled water) was used for selection of biofilm forming organisms. Congo red agar plates were inoculated with different organisms as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterobacter cancerogenus* and incubated for 24 hrs 37°C. After incubation colour of colonies were observed. Black colour colonies indicate organisms are biofilm producing.

Biofilm formation on glass surface: Sterile glass test tubes containing 2 ml of nutrient broth were inoculated with each bacterial suspension. Tubes were incubated with for 24 hrs at 37°C. Planktonic bacteria were discarded and tubes were washed with PBS and allowed to dry. Tubes were then stained with 0.3% of crystal violet. After 10 mins excess stain was discarded and tubes were washed, dried. Tubes were observed for their ability to form a biofilm ring onto the surface of glass.

Capsule staining: Capsule staining was performed using standard protocol.

Isolation of actinomycetes from different sources

Samples for *Streptomyces* cultivation were collected from different sources like from soil, chick slaughter wastes; a swab from hand was taken. These 3 samples were inoculated in each 100 ml flask containing Bennet's broth medium (Yeast extract 1 g/L, Beef extract 1 g/L Casein enzyme hydrolysate 2 g/L, Dextrose 10 g/L pH 7.3). Enrichment of actinomycetes was carried for 5 days in a rotary shaker (150 rpm) at 28°C. Isolation of *Streptomyces* was then carried out by spread plate method. For this purpose, sterile Bennet's agar Petri plates were prepared. 0.1 ml of enrichment

broth was spreader with a sterile spreader on several plates. The plates were incubated for 7 days at 28°C. Plates which showed zone of inhibition around a colony and which appeared chalky white in colour were subculture on new sterile Casein starch agar plates.

Microscopic examination

Microscopic examination of actinomycetes was done using standard slide culture method.

16S rRNA sequence determination

The isolated actinomycetes were sent for genetic identification by 16S rRNA technique to IISER Pune.

Extract preparation

Single colony of each isolated actinomycetes culture were inoculated in 150 ml Bennet's broth medium in 250 ml of conical flask and the strains were allowed to grow for 10 days at 28°C on a rotary shaker at 150 rpm. After centrifugation, the filtrates as well as the supernatants were treated with twice the volume of ethyl acetate separately. Bennet's broth medium served as control for the process and was treated in the similar manner with ethyl acetate and it was used as medium control for the assay. The extract was generated by evaporating the solvent for 48 hrs at room temperature. The dried extracts were dissolved in DMSO (final concentration 3.75% on the cells) and used for the anti-biofilm bioassay.

Effect of actinomycetes crude extract on pathogenic biofilms

To study the effect of crude extract, biofilms of pathogenic bacteria were developed. For this bacterial suspensions were made (OD₆₀₀= 0.05). 100 ml of bacterial suspension was inoculated in 96 well flat bottom polystyrene plates containing 200 ml of nutrient broth. The plate was incubated for 48 hrs at 37°C. Planktonic bacteria (free cells) were discarded by inverting the plate. 100 ml of crude extract was added to the existing biofilms and plate was incubated for 24 hours at 37°C. Plate was washed with sterile phosphate buffered saline twice, and was heat fixed at 65°C for 1 hr. 100 micro litre of 0.3% crystal violet was added to each well. After 5 mins plate was washed with distilled water and dried. 200 ml of acetic acid was added to each well to dissolve the content in the well and OD 492 readings were compared with the control. Biofilms which were not treated with extract served as controls with Bennett's medium as extract control.

Determination of biofilm inhibitory concentration (BIC 50)

Biofilms were formed in 96 well microtitre plates. The biofilm was treated with 80, 160, 240, 320, 400 µg/ml concentration of crude extract for 24 hrs 37°C. After treatment biofilm was washed with PBS and stained with 0.3% crystal violet for 5 mins. After staining plate was washed 2-3 times with water and OD was measured at 492 nm. Depending on OD biofilm inhibitory concentration was determined.

Growth measurement

Effect of actinomycetes crude extraction the growth of different biofilm forming bacteria was studied by growth curve analysis. The growth curve of these bacteria was determined upto 24 hrs. The crude extract at 50% BIC₅₀ and highest tested concentration was added to a conical flask containing bacteria of an OD 0.1 at 600 nm. These flasks were incubated at 37°C, 200 rpm. Nutrient broth without bacteria serves as a negative control. Growth medium with crude extract and bacteria serve as the control. The reading was observed continuously up to 24 hrs at 2 hrs of interval. Experiment was carried out with four independent cultures.

Physical and chemical treatment to the extract

To know the physical and chemical characters of the extract, they were subjected to heat and enzymatic treatment. The extracts were heated at 70°C and 100°C for 30 mins and cooled on ice. For enzymatic treatment, the extracts were treated with proteinase k (20 mg/ml). This mixer was incubated for 1 hour at 37°C. Extracts without enzyme served as control. Both the treated extracts were examined for their antibiofilm activity by crystal violet assay.

Cell surface hydrophobicity assay [Microbial adhesion to hydrocarbons (MATH)]

In 2.5 ml of phosphate buffer saline bacterial cells from different cultures were suspended in different tubes. 0.5 ml of xylene was added to the tubes. This mixture was incubated at 44°C for 10 mins. After this samples were vortex for 60 sec and were further incubated for 1 hr. The hydrocarbon and aqueous layer of the mixture were allowed to separate. Optical density of the aqueous phase was measured at 600 nm. The decrease in the OD of aqueous layer was the measure of cell surface hydrophobicity.

RESULTS

Selection of biofilm forming organisms

Congo red assay: Organisms showed black colour colonies on

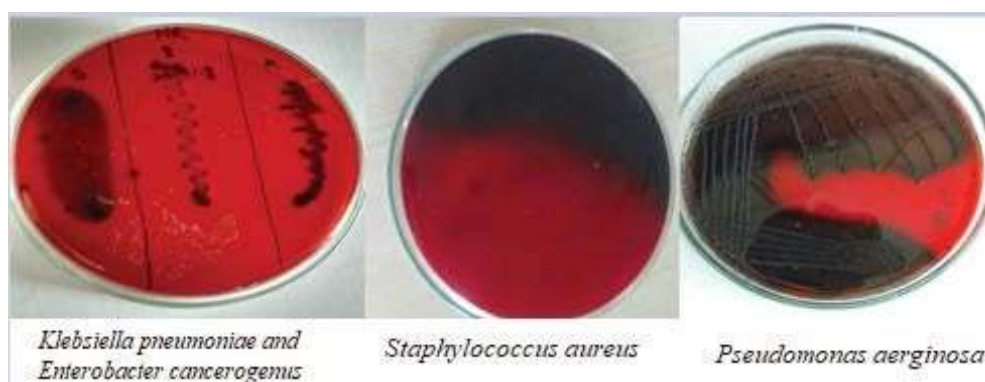


Figure 1: Organisms on congo red agar plate.

Congo red agar plate which confirmed that they are the biofilm producers (Figure 1).

Tube assay: Biofilm formation on glass surface by test bacterial isolates (Figure 2).

Capsule staining: 4 different organisms were shown (Figure 3).

Isolation of actinomycetes

The characters of strains are tabulated in Table 1 and shown in Figure 4.

Microscopy examination

The isolates were examined using slide culture technique under 40X and 100X. The microscopic examination showed filamentous aerial hyphae (Figure 5). The RNA Sequence determination has been shown in Figures 6 and 7. The Effect of crude extracts on

bacterial biofilms tested at highest concentration is shown in Figure 8.

Table 1: Characteristics of *Streptomyces viridochromogenes* and *Streptomyces levis*.

Characters	<i>Streptomyces viridochromogenes</i> strain NBRC 3113	<i>Streptomyces levis</i> strain NRRL B-16370 strain NRRL B-16370
Color	Bluish green	Off white
Size	1 mm	0.5 mm
Elevation	Flat	Flat
Margin	Irregular	Irregular
Consistency	Dry	Dry
Opacity	Opaque	Opaque
Gram staining	Gram positive	Gram positive



Figure 2: Biofilm formation on glass surface by test bacterial isolates.

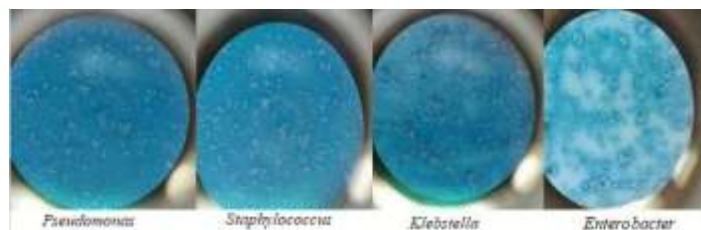


Figure 3: Capsule staining of different organisms.



Figure 4: Isolation of actinomycetes.



Figure 5: Microscopy examination results under 40X and 100X.

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>NR_112482.1 Streptomyces viridochromogenes strain NBRC 3113 strain NBRC
3113 16S ribosomal RNA gene, partial sequence
ACGAACGCTGGCGGCGTGCCTAACACATGCAAGTCGAACGATGAACCACTTCGGTGGGGATTAGTGGCGA
ACGGGTGAGTAACACGTGGGCAATCTGCCCTGCACTCTGGGACAAGCCCTGGAAACGGGGTCTAATACCG
GATACTGATCTACTTGGGCATCCAAGTGGTTCGAAAAGCTCCGGCGGTGCAGGATGAGCCCGCGGCCTATC
AGCTTGTGGTGAGGTAANGGCTCACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACA
CTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGAAAAGC
CTGATGCAGCGACGCCCGGTGAGGGATGACGGCCCTTCGGGTGTAAACCTCTTTCAGCAGGGAAGAAGCG
AAAGTGACGGTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCCGGTAATACGTAGGGCGC
AAGCGTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGC GGCTTGTCAAGTCCGTTGTGAAAAGCCCGG
GGCTTAACCCCGGTCTGCAGTCGATACGGCCAGCTAGAGTTCGGTAGGGGAGATCGGAATTCCTGGTG
TAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGC GAAGGCGGATCTCTGGGCCGATAC TGACG
CTGAGGAGCGAAAGCGTGGGAGCGAACAGGATTAGATAACCCTGGTAGTCCACGCCGTAACCGTGGGCA
CTAGGTGTGGGCAACATTCACGTTGTCCGTGCCGAGCTAACGCATTAAGTGCCCCGCTGGGGAGTAC
GGCCGCAAGGCTAAAAC TCAAAGGAATTGACGGGGCCCGCACAAGCGCGGAGCATGTGGCTTAATTCG
ACGCAACGCGAAGAACC TTACCAAGGCTTGACATACACCGGAAAGCATTAGAGATAGTGCCCCCTTGTG
GTCGGTGTACAGGTGGTGCATGGCTGTCTCAGCTCGTGTCTGTGAGATGTTGGGTTAAGTCCCGCAACGA
GCGCAACCCCTGTCCCGTGTGCGCAAGGCCCTGTGGTGTCTGGGACTCACGGGAGACCCCGGGGTC
AACTCGGAGGAAGGTGGGGACGACGTCAAATCATCATGCCCTTATGTCTTGGGCTGCACACGTGCTACA
ATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAAAGC CGGTCTCAGTTCGGATT
GGGCTGTGCAACTCGACCCCATGAAGTCCGAGTCCGTAGTAATCGCAGATCAGCATTGCTGCGGTGAATA
CGTTCGCCGGCCCTTGTACACACCGCCCGT CACGTACGAAAAGTCGGTAACACC CGAAGCCGGTGGCCCAA
CCCCCTGTGGGAGGGAGCTGTGCAAGGTGGGACTGGCGATTGGGACGAAGTCGTAACAAGGTAGCCGTAC
CGGAAGG
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Figure 6: 16S rRNA sequence of *Streptomyces viridochromogenes* strain NBRC 3113 strain.

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>NR_115778.1 Streptomyces levis strain NRRL B-16370 strain NRRL B-16370
16S_ribosomal RNA gene, partial sequence
GATCCTGGCTCAGGCTGGATCACCCTCCTTTCTGAGTTTGATCCTGGCTCAGGCTGGATCACCTCCTTTCT
GAGTTTGATTCTGGCTCAGGACGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAACCACT
TCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTTGGGCATCCTTGATGGTGTAAGCTCCGGCGGTGCAG
GATGAGCCCGCGGCCTATCAGCTTGTGGTGAGGTAATGGCTCACCAAGGCGACGAGGGTAGCCGGCCT
GAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCAGACTCCTACGGGAGGCAGCAGTGGGGAATA
TTGCACAATGGGCGAAAGCCTGATGCAGCGACGCCGCTGAGGGATGACGGCCTTCGGGTTGTAAACCTC
TTTCAGCAGGGAAGAAGCGAAAGTGACGGTACCTGCAGAAGAAGCGCCGGTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGGCGCAGCGTGTTCGGAAATTATGGGCGTAAAGAGCTCGTAGGCCGGCTTGTAC
GTCGGTTGTGAAAGCCCGGGCTTAACCCCGGGTCTGCAGT CGATACGGGCAGGCTAGAGTTCCGTAGGG
GAGATCGGAATTCCTGGTGTAGCGGTGAAATGCCAGATAT CAGGAGGAACACCGGTGGCGAAGGCGGAT
CTCTGGGCGGATACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCCTGGTAGTCC
ACGCCGTAACCGGTGGGCACTAGGTGTGGGCAACATTCACAGTGTGTCCGTGCCGCAGCTAACGCATTAAG
TGCCCCGCTGGGGAGTACGGCCGCAAGGCTAAACTCAAAGGAATTGACGGGGCCCGCACAAAGCGGCG
GAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCCTTACCAAGGCTTGACATACCCGGAAAACCCCTGG
AGACAGGTCGCCCTTGTGGTGGTGTACAGGTGGTGCATGGCTGTCTCGTCAGCTCGTGTCTGTGAGATGTT
GGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCCCGTGTGCGCAGCAGGCCCTTGTGGTGTCTGGGACTC
ACGGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAGTCAAGTCATCATGCCCTTATGTCTT
GGGCTGCACACGCTGCTACAATGGCCGGTACAATGAGCTGCGATACCGCGAGGTGGAGCGAATCTCAAAA
GCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTCTGCTAGTAATCGCAGATC
AGCATTGCTGCGGTGAATACGTTCCCGGCCCTTGTACACACCGCCCGTCACGTACGAAAGTCCGTAACA
CCCGAAGCCGGTGGCCCAACCCTTGTGGGAGGGAGCTGTCGAAGGTGGGACTGGCGATTGGGACGAAGT
CGTAACAAGGTAGCCGTACCGGAAGGTGC
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Figure 7: 16S rRNA sequence of Streptomyces levis strain NRRL B-16370 strain.

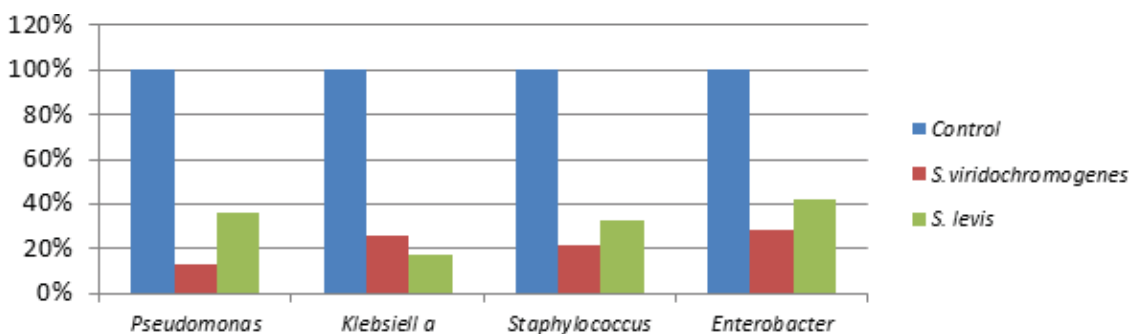


Figure 8: Effect of crude extracts on bacterial biofilms at highest tested concentration of 400 ug/ml.

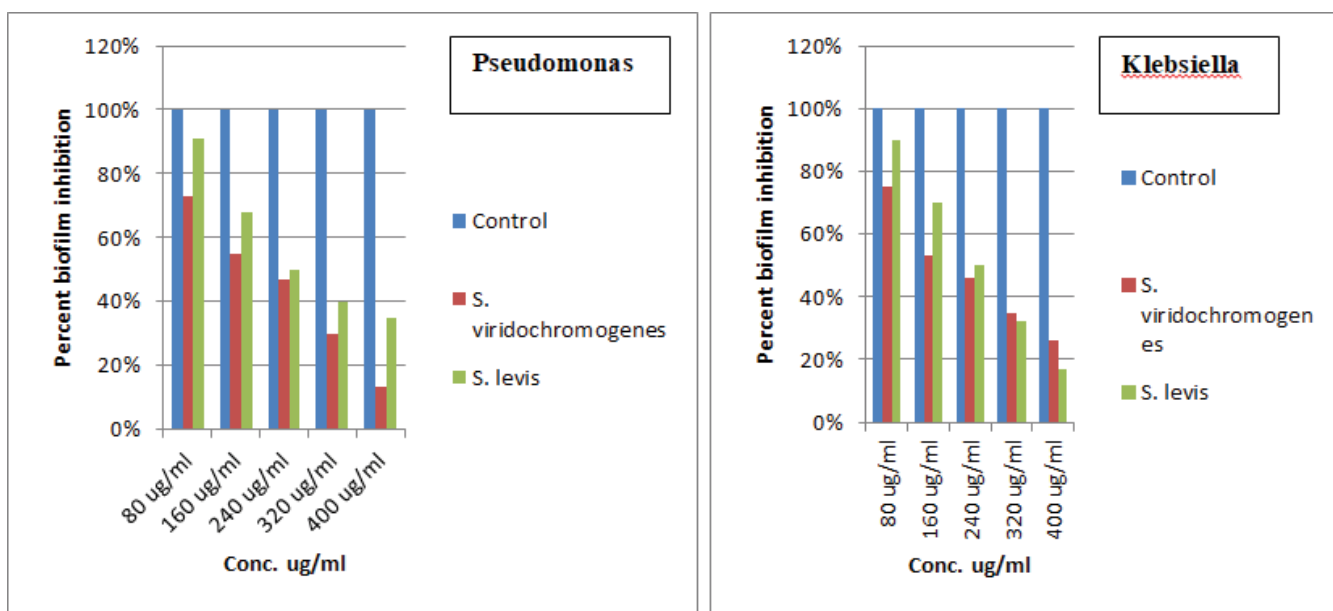


Figure 9: Biofilm inhibitory concentration of Pseudomonas and Klesbiella.

The biofilm inhibitory concentration has been determined in Figures 9 and 10.

Physical and chemical characterization of active compound in the extracts

Physical treatment (Heat): Streptomyces viridochromogenes

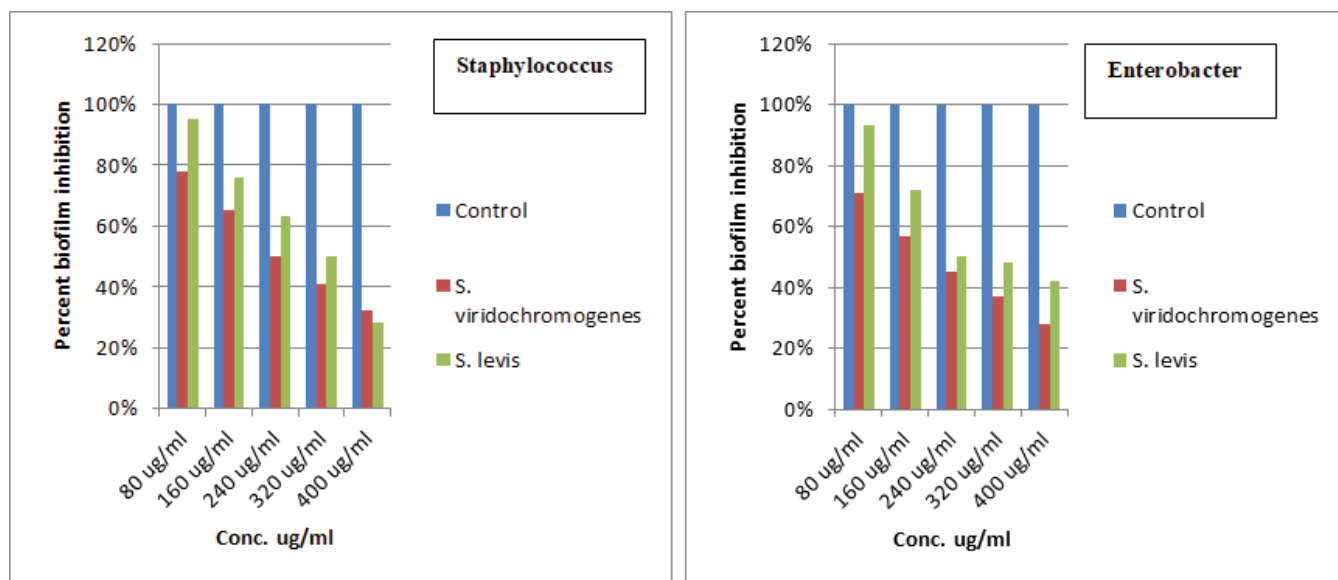


Figure 10: Biofilm inhibitory concentration of *Staphylococcus* and *Enterobacter*.

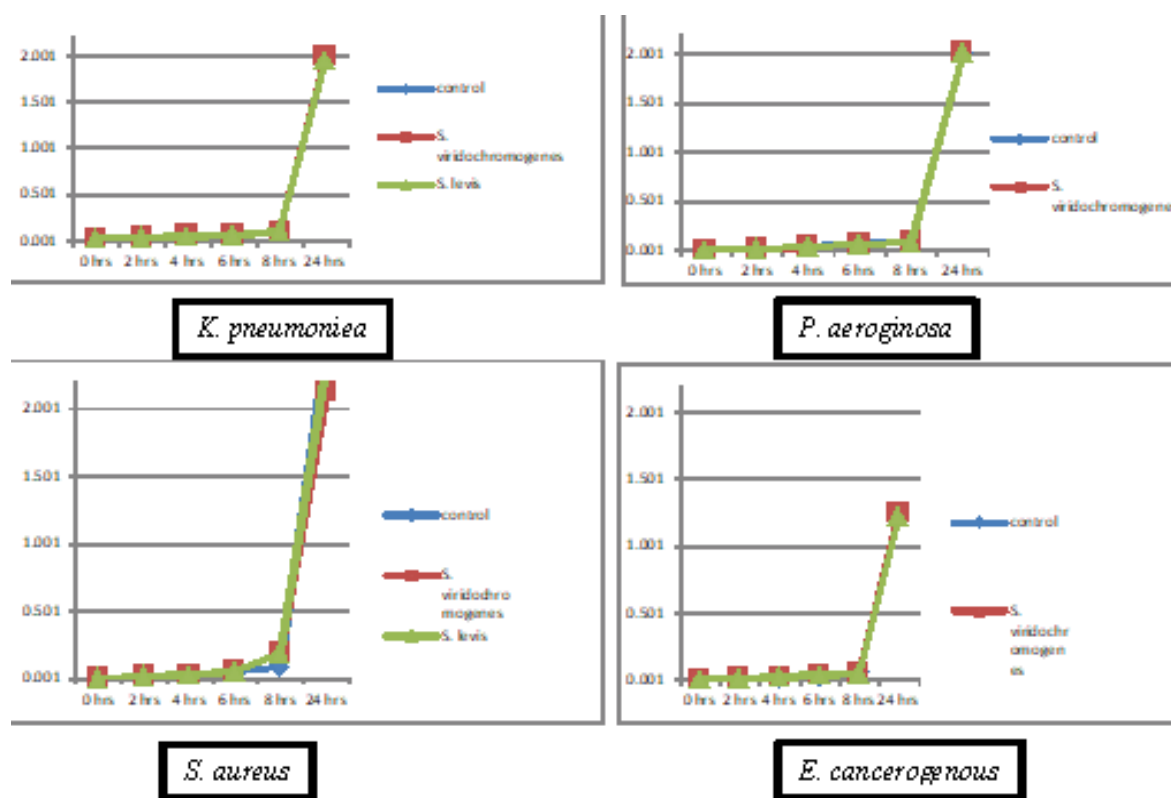


Figure 11: Growth curve of the species.

strain NBRC 3113 is heat resistant and *Streptomyces levis* strain NRRL B-16370 is heat labile.

Chemical treatment (proteinase K): Both extracts are non proteinaceous.

The Growth Curve of the organisms is shown in **Figure 11**.

Hydrophobicity

Surface hydrophobicity plays an important role in most of the biofilm forming organisms. It helps to adhere to the hydrophobic surfaces. For this reason, cell surface hydrophobicity assay was performed to determine the mechanism of inhibitory effect of the extracts. The assay results showed that in presence of extracts the cells become less hydrophobic (**Figure 12**).

DISCUSSION

Since biofilm formation nowadays has become a major problem, the need for developing new antibiofilm agents is under focus. Previous studies reported that marine *Streptomyces* extracts inhibit biofilm formation of *Staphylococcus aureus* but it was not effective on *Pseudomonas species* [1]. The aim of the present work was to isolate *Streptomyces* from different sources like chicken slaughter waste and screen for its antibiofilm activity. These Broad spectrum *Streptomyces* extracts not only inhibited biofilm of *Staphylococcus* but of *Pseudomonas Klebsiella* and *Enterobacter spp.* Previous studies presented antibiofilm activity of various *Streptomyces species* like *Streptomyces sp.* MCO25 and *Streptomyces sp* SBT343 extracts inhibit *Staphylococcus*

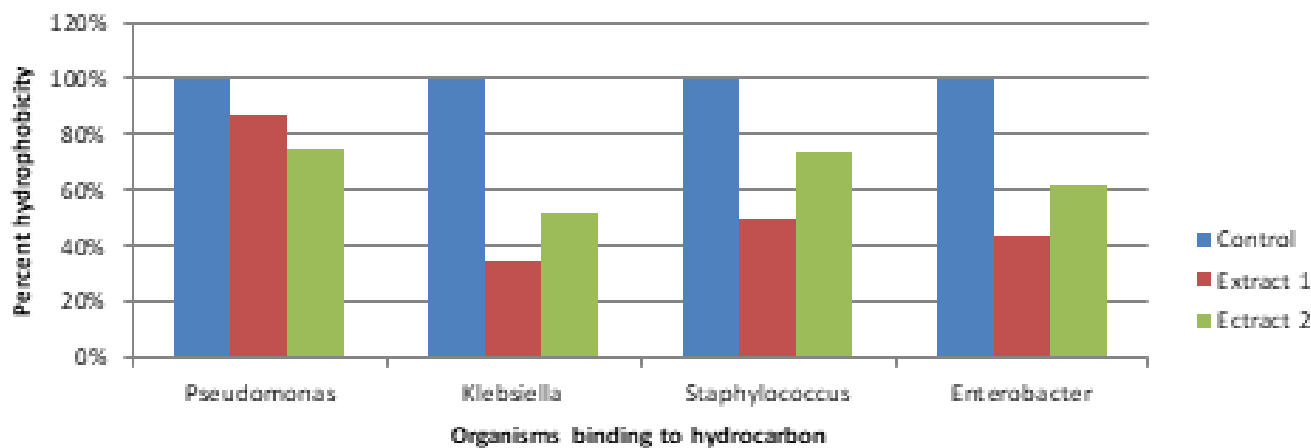


Figure 12: Hydrophobicity assay of the organisms.

aureus biofilms [1,15]. Current study presented the potential of *Streptomyces viridochromogenes* strain NBRC 3113 and *Streptomyces levis* strain NRRL B-16370 extracts to inhibit biofilms of bacteria for the very first time.

CONCLUSION

From the present study it can be noted that, apart from marine environment, there are large number of bioactive compounds present in different sources which are still unexplored. The two extracts derived from *Streptomyces viridochromogenes* strain NBRC 3113 and *Streptomyces levis* strain NRRL B-16370 showed antibiofilm activity against various biofilm forming organisms. The extracts are non-proteinaceous in nature. *Streptomyces viridochromogenes* strain NBRC 3113 extract is heat resistant and *Streptomyces levis* strain NRRL B-16370 extract is heat labile. In the presence of extract, the bacteria showed decrease in their adherence property to hydrocarbons. The extracts are dose dependent and non-toxic. Further purification and characterization of the active component in the extracts would reveal the potential role in inhibition of biofilm and the component can be used to develop an active antibiofilm agent.

FUTURE PROSPECTS

Purification of the active component from the crude extracts and their structural identification.

Identifying the mechanism by which inhibition is occurring.

Study of gene expression, involved in biofilm formation, in presence of crude extract.

Testing the same extracts against other biofilm forming organisms to prove their broad spectrum antibiofilm capacity.

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