International Journal of Current Research in Medical Sciences

ISSN: 2454-5716

www.ijcrims.com

Volume: 1- Issue: 1 July 2015

Research Article

# Biofilm resistance to antimicrobial agents by *Streptomyces* sp.

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## Abstract

The study focused about mainly the isolation of *Streptomyces* species, a group of Actinomycetes from soil samples collected from various places of both Tamil nadu and Kerala. Soil samples were initially serially diluted and plated on Actinomycetes isolation agar and incubated at $28^0$c for 5-7 days and then in ISP 5 medium incubated at $28^O$c- for 7-14days. About 30 *Actinomycetes* isolates were initially isolated and among them only five actinomycetes were screened by cross streak method against specific stranded strains. They are characterized and grouped to the genus of *Streptomyces* by their colony morphology, spore morphology, growth on selective media and the biochemical characteristics. The presence of polyunsaturated fatty acids were examined in all potential strains by PUFA analysis. The potential strains were mass cultivated in starch casein broth at $24^o$C for 4- 7 days. S7 strain produced orange colored diffusible pigment in the starch casein broth The biofilm study was conducted by crystal violet assay by using stranded stains. The microtiter plate method was done in 12- well polysterene plate and the crystal violet assay was subjected to determine the density of cells by reading at UV spectrophotometer at 490nm. Dense biofilm was observed in *Staphylococcus* sp. and *pseudomonas* sp. And medium in other bacterial strains . Minimum inhibitory concentration testing (MIC 50) of five different actinomycetes supernatant were determined. MIC50 of S7 was found to be as 150 µl for *Staphylococcus* sp., 200 µl for *Pseudomonas* sp., for *E.coli* and *Bacillus* it was observed as 100 and 75 µg/ml concentrations. Biofilm inhibitory concentration testing: at 300 µl concentration inhibition was observed as which means that the S7 concentration for biofilm was greater than sessile or planktonic cells. Silver nanoparticles was produced from the highly potential strain S7 and its antibiogram was noted .

**Keywords:** *Streptomyces* species, Dense biofilm, microtiter plate method **.**

## Introduction

Biofilm on indwelling medical devices may be composed of Gram-positive or Gram-negative microorganisms. These organisms may originate from the skin of patient, or health-care workers, tap water to which entry ports are exposed or other sources in the environment. Biofilms may be composed of single species or multiple species, depending on the device and its duration of action. Catheters may be inserted for administration of fluid, blood products, medications, nutritional solution, and hemodynamic monitoring (Rodney Lacret *et al*., 2015).

Bacterial cells undergo a number of physiological and phenotypic changes following attachment to a solid surface. These lead to higher cell resistance to antimicrobial agents. Bacteria living in biofilms can be up to 1000 times more resistant to antibacterial compounds (such as disinfectants, antibiotics, surfactants) than planktonic cells (Czaczyk *et al*., 2007). Biofilm formation occurs as a consequence of the device being colonized by the microorganisms. Biofilms are defined as communities of microorganisms that grow on an abiotic or biotic surface and are embedded in a matrix consisting of an extracellular polymeric substance. The increased resistance to antimicrobial therapy is associated with the biofilm mode of growth and explains why biofilm-associated infections often results in treatment failure (Punithavathy *et al*., 2012).

Biofilms are defined as structured microbial communities that are attached to a surface and encased in a matrix of exopolymeric material. This is of particular significance since it is now estimated that a significant proportion of all human microbial infections involve biofilm formation (Gordon Ramage *et al*., 2005).

Conventionally, antibiotics used to treat these biofilm-forming pathogens are not targeted against the recalcitrant biofilms; rather they target their planktonic counterparts, which create selective pressure on bacteria and gets resistance against the particular drug (Shanmugaraj Gowrishankar*et al*., 2012)

The biofilm communities of *S. pyogenes* communities tend to exhibit significant tolerance to antimicrobial challenge during infections. The strains use the biofilm to evade the antibiotic effect, thereby becoming resistant to the treatment. Penicillin remains the antibiotic of choice for *S. pyogenes* infections based of its narrow spectrum of effect, efficacy, good safety profile and low cost as there is an increase in the resistance mechanisms of *S. pyogenes* to antibiotics, much effort is being exerted to identify novel compounds with effective antibacterial properties. Lipids act an alternative source of biofilm inhibiting compound; using soil isolates of actinomycetes (Manickam Rajalakshmi *et al*., 2014).

## Materials and Methods

### Collection of soil samples

The soil samples were collected in sterile plastic bags from various places of Tamil nadu and Kerala. The sediment samples were serially diluted and plated on actinomycete isolation agar and incubated at 28°C for 4-5 days (Amit Pandey *et al*., 2011).

### Characterization of the isolates

Based on the morphological differences they are streaked on ISP 5 media and the isolates are subjected to biochemical tests for further analysis (Ajijur Rahman., *et al*., 2011)

### Primary screening for antimicrobial activity of isolated actinomycetes

The isolated *Streptomyces sp.* was screened for their antagonistic activity against pathogenic microorganisms by using primary screening. Primary screening was done by cross streak method and the lead isolates were selected and studied further. *Streptomyces sp.* isolates obtained were grown by streaking horizontally on the muller hinton agar medium plate and incubated for 7 days at room temperature. After incubation, test organisms were streaked perpendicular to that growth and then incubated at $37^{o}$ C for 24 hours. The inhibition of growth of the test organisms were observed after incubation period. Those *Streptomyces sp.,* isolates showed broad spectrum antibacterial activities were selected for secondary screening (Raghava Rao *et al*., 2013).

### Antibiofilmic activity of isolates

### Biofilm formation by microtiter plate method

The static biofilm model was followed with some modifications. Briefly, the 24-well microtiter plate was taken, to this 3ml of nutrient broth was added and 1 ml of each stranded strains were

added along with coverslips and kept incubation at room temperature for 72 h for the formation of well formed biofilm, wells containing broth alone were used as negative controls. The crystal violet assay was conducted as previously described with some modifications. The coverslips were carefully removed from each well of the 24-well plates (static biofilm formation), rinsed gently with double distilled water, and placed into the corresponding wells of a new 24-well plate containing 1 ml of 4.0% crystal violet solution (wt/vol). After incubation at room temperature for 30 min, the crystal violet solution was discarded and it was rinsed double distilled water, and then placed in the corresponding wells containing 1 ml of 95% ethanol. The plates were incubated at room temperature for 1 h and the extracted crystal violet, which indicates the strength of the biofilm, was measured at an absorbance of 595 nm. The coverslips were then documented in a light optical microscope (Manickam Rajalakshmi *et al*., 2014).

### Biofilm Inhibition Assay

Overnight cultures of bacterial strains were incubated on 24 well microtitre plate containing nutrient broth with and without *Streptomyces sp*. extracts. Plate was incubated without agitation at 37 °C for 18 h. After incubation, planktonic cells were discarded and the adherent cells on the slide were gently rinsed twice with deionized water and air dried. The biofilm was stained with 0.1% crystal violet solution for 5 min and then rinsed twice with deionized water. Finally it was resuspended in 1 ml absolute ethanol and the absorbance was observed at 570 nm (Manickam Rajalakshmi *et al*., 2014).

## Results and Discussion

### Antibiofilmic activity of isolates

### Biofilm formation by microtiter plate method

The microtiter plate method was done for crystal violet assay- biofilm formation assay. Under the condition of the study, *Staphylococcus sp.* and *Pseudomonas sp.* showed different levels of biofilm forming ability (Photo 8& 9). Of these high values were reported in *Staphlococcus* and *Pseudomonas* species were 0.45-0.36. All the other bacterial strains showed moderate to low biofilm activity.

### Biofilm Inhibition Assay

The biofilm formed were disturbed by the strain S7 to a maximum inhibition. Other strains showed minimum inhibition against the well formed biofilms. The bioactive capabilities of CAA have not yet been explored and the present study shows that CAA may be promising candidates with potential antibiofilm activity against *S. pyogenes*. The metabolites of CAA extracts inhibited biofilm pyogenes. The bioactive compounds present in the extracts, like berberine sulfate might have interfered with the adherence of *S. pyogenes* by releasing the adhesin, lipoteichoic acid (LTA) from the streptococcal cell surface. An important step in biofilm development is the formation of the characteristic biofilm architecture (Paramasivam Nithyanand *et al*., 2010). The present study also focused on the antibiofilmic activity of the isolated potential strains.

Figure 1. biofilm forming ability of the bacterial strains

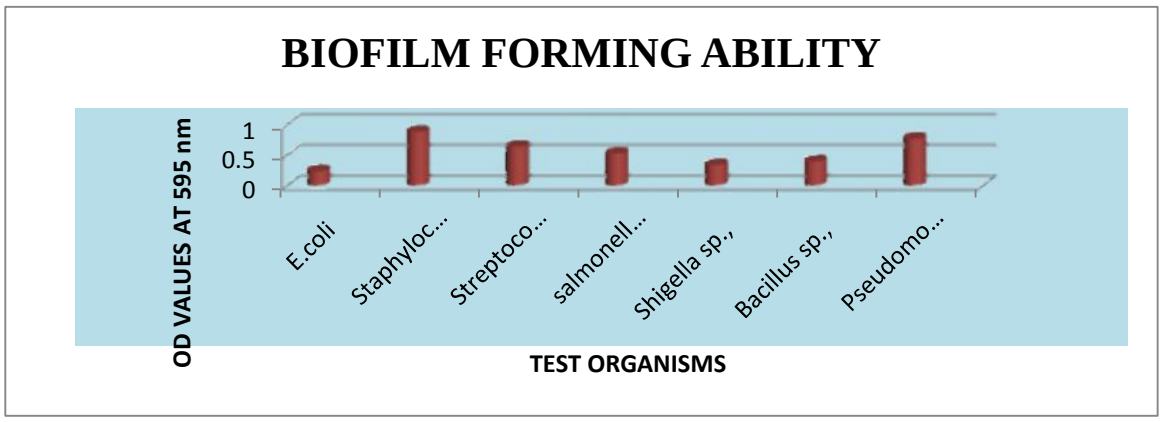

Table. 1 Biofilm forming ability of the bacterial strains

| Bacteria Employed | OD VALUES at 595 nm |
|---|---|
| ***E.coli*** | 0.25 |
| ***Staphylococcus aureus*** | 0.89 |
| ***Streptococcus sp.,*** | 0.65 |
| ***Salmonellas sp.,*** | 0.54 |
| ***Shigella sp.,*** | 0.35 |
| ***Bacillus sp.,*** | 0.41 |
| ***Pseudomonas sp.,*** | 0.78 |

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