International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Quorum Quenching Activity of Pigments Produced by Actinomycetes

A. Parimala Gnana Soundari[1], C. Nagarajan[2], V. Manon Mani[3], V. Brindha Priyadarisini[4]

Clinical Biotechnology Lab, Department of Microbial Biotechnology, Bharathiar University, Coimbatore-641046, Tamil Nadu, India

Abstract: ***Quorum sensing is a cell to cell communication system prevailed by the microorganisms for its survival. Different quorum sensing molecules like AHL, Oligopeptides and Autoinducers are produced for this communication. Communication between cells leads to the formation of biofilm. These biofilms are first step for the virulence of pathogen and also imparts resistance to the pathogen. Hence this study was designed to identify compounds from Actinomycetes that can disrupt the biofilm formation by the organism through quorum quenching activity. Among the eight pigmented actinomycetes used C5-5Y showed higher activity against the test pathogens (Staphylococcus aureus, Streptococcus mutants and Salmonella sp.). The yellow pigment produced by the strain was extracted from biomass with acetone and was partially purified through TLC; This partially purified yellow pigment was found to show inhibition of Gram positive bacteria Staph. aureus and Strep. Mutants by quenching the quorum sensing molecules. This result indicates the potency of the pigment in controlling biofilm formation by the Gram positive pathogens.***

Keywords: Quorum sensing molecules, Quorum quenching, Biofilm, Actinomycetes, Pigments.

1. Introduction

In the early 1970s it was discovered that bacteria possess the ability to communicate with one another which allow colonies of bacteria to regulate collective behaviour. In effect, it allows a colony to function as a single organism. This process of cell to cell communication is called quorum sensing (Nealson et al., 1979). Movement, growth rates, biofilm formation and the production of protective chemicals are some such examples (Reisner et al., 2003). These chemical signals are important for the establishment of infection and can serve as a switch to a pathogenic state (Zhu et al., 2003).

There are three types of quorum sensing system in bacteria such as AHL (AI-1), Oligopeptide and universal Autoinducer (AI-2). A large number of gram-negative quorum-sensing systems studied so far utilize N-acyl homoserine lactones as signal molecules (Smith et al., 2003). The gram positive organism uses LuxI/LuxR signalling molecules (Horinouchi et al., 1994). Inactivation of quorum sensing system is generally known as quorum quenching. The advantage of quorum quenching is to inhibit the virulence of pathogen. Many of the compounds from plants and microbes have been reported to possess quorum quenching properties (Teplitski et al., 2011). Actinomycetes are aerobic, free living filamentous bacteria which are reported to be the predominant producers of industrially important secondary metabolites and the metabolites were found to exhibit good quorum quenching properties (Wang et al., 2009). The main objective of this study is to prove the quorum quenching property of the pigment produced by actinomycetes isolates.

2. Materials and Methods

2.1 Strains used in this Study

Eight pigment producing Actinomycetes strains available in our laboratory culture collection (CBT, BU) were taken to study the quorum quenching property of the isolates and pigment. To achieve the goal, based on the literature five common pathogen showing quorum sensing property was selected for this study.

2.2 Screening for Antagonistic Activity

The pigment producing strains were screened initially for antagonistic activity against the selected test pathogens by cross streaking method (Rahman et al., 2011). Actinomycetes isolates were streaked across diameter on starch casein nitrate agar plates. After incubation at 37˚C for 7 days, overnight cultures of the test pathogens having 0.1 O.D at 600nm were streaked perpendicular to the central strip of actinomycetes culture and incubated at 37˚C for 24 hrs. Zone of inhibition was measured. The antagonistic activity was further confirmed with well diffusion assays against the pathogens.

2.3 Production and Extraction of the Pigments

The strain C5-5Y was inoculated in starch casein nitrate broth and kept in shaker for 7 days at 180 rpm in room temperature. Seven day old culture was taken and biomass was harvested by centrifugation at 5000 rpm for 10 minutes. The pigments from the biomass and culture filtrate were extracted using 9 solvents (Methanol, Petroleum ether, Acetone, Ethyl acetate, Chloroform, Propanol, Ethanol, Isoamyl alcohol and Diethyl ether) to identify the efficient solvent for extraction. The solvents were added in the ratio of 40%v/v to the culture filtrate and 30%w/v to the biomass and incubated in water bath at 60˚C for 20mins to enhance the extraction.

2.4 Antimicrobial Activity of Pigment Extracts

The pathogens *Staphylococcus aureus, Streptococcus mutants,* and *Salmonella* were grown and maintained in nutrient broth. Overnight cultures having 0.1O.D at 600nm which is equivalent to a cell population of about 10^6 cells/mL (Mc Farland standard) was spread plated on to the Muller Hinton agar plates. Twenty milliliters of pigment extracts from the culture filtrate and the biomass extracted using

different efficient solvents were poured in the wells on the spreaded Muller Hinton agar plates along with the respective solvents as positive controls. The plates were incubated at 37˚C for 24 hrs and antimicrobial activity was detected by the presence of a clear zone. Zone of inhibition was recorded by measuring the diameter of the inhibition zone.

2.5 Anti-quorum sensing activity of pigment extract from culture filtrate and biomass

2.5.1 For Gram-positive bacteria

2.5.1.1 Inhibition of protease activity

Protease activity was detected using skim milk agar plates. *Staph. aureus* and *Strep. mutants* were inoculated in tryptic soy broth (TSB) containing chloroform and acetone extracts and incubated at 37˚C at 180 rpm in an orbital shaker. Cultures were centrifuged at 13,000 rpm for 15 min and culture filtrate was collected and stored at -20˚C until further use. Small (3mm diameter) wells were cut on the skim milk agar plate and were filled with 20µl of suspensions of staphylococcal and streptococcal culture filtrates. Digestion of the substrate was indicated by the formation of clear area surrounding the well. The zone of clearance was measured after an overnight incubation at 37˚C.

2.5.1.2 Inhibition of lipase activity

Inhibition of lipolytic activity was done by plate assay. Tributyrin agar medium was prepared and sterilized by autoclaving for 20min at 121˚C. Pathogen cultures were grown on the medium containing 20µl of chloroform pigment extract, acetone pigment extract while cells grown in a media without extracts served as control. Culture filtrate of the treated and untreated pathogens was added to appropriate wells cut onto the tributyrin agar plates. The plates were incubated at 37˚C for 48hrs and evaluated for the appearance of zone of clearance. The diameter of zone of clearance was measured in cm.

2.5.2 Gram-negative bacteria

2.5.2.1 Carbapenem activity

Carbapenem production was detected on agar plates using the *E.coli* strain. 1ml of test strain *Salmonella spp* was grown in the medium containing pigment extract. After incubation, cells were pelleted by centrifugation at 3000 rpm for 5mins. Aliquot (20µl) of culture was dispensed to wells cut onto agar plate seeded with a lawn of *E.coli* culture and the plates were incubated overnight at 30˚C. Zone of inhibition indicates the anti-quorum sensing activity of the pigment.

2.6 Partial purification of the crude extract by TLC

The crude extract was prepared by grinding the biomass using acetone which was concentrated and spotted in TLC plates. The compounds were separated using ethyl acetate: petroleum ether (4:1) as solvent system. The spots were scraped out of the TLC plates and dissolved in acetone, concentrated, checked for its antimicrobial activity.

2.7 Minimal inhibitory concentration

The minimum inhibitory concentration of the partially purified compound required against *Staph. aureus* and *Strep. mutants* was determined using the agar disc diffusion method. Dilutions of the purified compound were done to obtain 5µl-25µl/mL concentration. Different dilutions of purified compound were added to the disc and placed on the agar plates seeded with the pathogens. The least concentration of the purified compound that had inhibitory effect was taken as the minimum inhibitory concentration (MIC) of the compound.

2.8 Anti-quorum sensing activity of partially purified compound

The antiquorum sensing activity was detected through protease activity, lipase activity, cell surface hydrophobicity and biofilm formation. Lipase and protease activity were tested similarly as mentioned earlier except for the concentration of partially purified compound (10µl/mL).

2.8.1 Cell surface hydrophobicity

Effect of the partially purified compound on cell surface hydrophobicity of *Staph. aureus* and *Strep. mutants* were measured by bacterial adhesion to hydrocarbon (Zhang et al., 2007). Bacterial cells grown in TSB broth were adjusted to an O.D of 1.0 at 600nm and were used for inoculation. The broth was inoculated with bacterial cultures and incubated at 37˚C for 24hrs. Bacterial cultures grown in the broth without compound served as negative control and with acetone served as positive control. Grown cultures were centrifuged at 5000rpm for 15min. 1 mL of hexadecane was added to 4mL of cell suspension in a test tube and was vortexed for 1 min. The mixture was allowed to settle for 30min for the formation of layers and the OD of aqueous phase at 600nm was measured. The hydrophobicity index (HI) of microbial cells was calculated by the formula described by Serebryakova *et al.*,(2002). The results were expressed as the proportion of the cells which were excluded from the aqueous phase, determined by the following equation, which refers the percentage of cells that migrates towards the organic phase.

$$Cell\,surface\,hydrophobicity = \frac{OD\ Initial - OD\ Final}{OD\ Initial} \times 100$$

2.8.2 Biofilm formation

2.8.2.1 Microscopic Method

Biofilm formation was the first step in pathogenic infection. The ability of the organism to form a biofilm can be detected following the method of Lembke *et al*., (2006). Biofilm (*Staph. aureus, Strep. mutants*) were allowed to grow on the medium with glass pieces in glass test tubes supplement with the 10µl/ml of compound. Medium with acetone served as positive control. Cultures were incubated at 37˚C for 24h. After incubation, the glass beads were washed four times with 0.85% normal saline solution (NSS) and glass beads were stained with 0.1% crystal violet solution for 10 min. stained glass beads were observed at a magnification of 40X for the formation of biofilm using a phase contrast microscope (Kozo optics).

2.8.2.2 Quantification method

The effect of the partially purified compound on biofilm formation of *Staph. aureus* and *Strep. mutants* were investigated by adding the acetone extract into glass test tubes containing TSB supplemented with 0.25% glucose. Subsequently, the tubes were inoculated with the bacterial

suspension and incubated at 37˚C for 24h. The bacterial growth was quantified in a spectrophotometer at 415nm. The tubes containing the media and the acetone served as control.

2.8.2.3 Adherent assay: tube method

Glycocalyx production was determined. *Staph.aureus* and *Strep.mutants* were inoculated into 5ml of TSB glass tubes in triplicate. Saccharide free basal medium (TSB without glucose) that lacks the substrate for polysaccharide was used as a control. Culture was inoculated at 37˚C for 20-24h and the contents were aspirated. One tube was examined unstained and others stained with crystal violet tryphan blue. Slime positivity was judged by the presence of visible unstained or stained film lining the wall of the tube. Formation of a ring at the liquid air interface was not considered as positive test.

2.9 Effect of partially purified compound against bacterial growth curve

The effect of partially purified compound on cell proliferation of *Staph.aureus* and *Strep. mutants* was determined. An overnight culture of *Staph.aureus* and *Strep.mutants* were diluted to obtain 0.1O.D at 600nm. Anti-biofilm activity and the effect of growth were monitored at 4hrs intervals until a final time point of 24hrs.

3. Results and Discussion

3.1 Pathogens used in this study

Five quorum sensing pathogens considered in this study were procured from PSG Hospital, Coimbatore among which two were gram positive (*Staph. aureus, Strep. mutans*) and three were gram negative (*E.coli, Salmonella, Pseudomonas*). These pathogens were tested for their virulence against eight pigment producing Actinomycetes lab culture collection (K2-2B, PA-2B, K1-1Y, K2-1L, C5-5Y, V1-1R, V1-4G and K4-8P) isolated from Industrial areas.

3.2 Screening for Antagonistic activity

The pathogens were tested for their virulence against eight pigment producing Actinomycetes cultures (K2-2B, PA-2B, K1-1Y, K2-1L, C5-5Y, V1-1R, V1-4G and K4-8P). Among which two strains (K2-2B and K2-1L) showed activity against two pathogens, V1-1R showed activity against one pathogen and C5-5Y showed activity against three pathogens. Hence C5-5Y was employed for further studies (Table 1). The results were confirmed further with well diffusion assay method. This inhibition of the pathogen may be due to the production of secondary metabolite by the Actinomycetes strain. Such antagonistic metabolites are reported to be produced by the microorganism against *Staphylococcus aureus* by Jonson *et al.,* (1968); Oskay (2009).

Table 1: Screening of antagonistic activity of pigmented Actinomycetes strains

Pathogens	*K2-2 Brown*	*PA2 Brown*	*K1-1 Yellow*	*K2-1 Red*	*C5-C5 Yellow*	*V1-1 Red*	*V1-4 Green*	*K4-8 Pink*
E.*coli*	-	-	-	-	-	-	-	-
Pseudomonas sp	-	-	-	-	-	-	-	-
S.*aureus*	0.8cm	-	-	1.2cm	2.8cm	-	-	-
Streptococcus mutants	-	-	-	-	2.9cm	-	-	-
Salmonella	1.3cm	-	-	1.7cm	2.8cm	-	-	-

3.3 Production and Extraction of the pigments

The C5-5Y strain was inoculated in starch casein nitrate broth. The pigments from the biomass and culture filtrate were extracted using 9 different solvents (Methanol, Petroleum ether, Acetone, Ethyl acetate, Chloroform, Propanol, Ethanol, Isoamyl alcohol and Diethyl ether). The Pigments from culture filtrate was found to get extracted with Chloroform, Ethyl acetate, Isoamyl alcohol while from biomass with solvents Acetone, Ethanol and Propanol.

3.4 Antimicrobial activity of extracted pigments

The Pigment extracts from both Culture filtrate (Table 2) and biomass (Table 3) was checked for its antimicrobial activity against Staph. aureus, Strep. mutans and Salmonella spp. various solvent extracts from the microorganism showed an antimicrobial activity. Solvents extract with ethyl acetate (Ilic et al., 2005) and chloroform (Thangadurai et al., 2004) when employed to showed to extract the antimicrobial principle from Actinomycetes culture when checked with pathogens.

Among the three extracts from culture filtrate, the chloroform extract showed efficient antimicrobial activity (Table 2) against all the pathogens and no zone of inhibition was observed with control whereas solvents Ethyl acetate and Isoamyl alcohol showed minimal inhibition activity against pathogens, so the chloroform extract was taken for further studies.

Of the intracellular pigment extract, the Acetone extract showed efficient antimicrobial activity against all the pathogens with minimum zone of inhibition when used as a positive control. Methanol and Propanol showed inhibition against pathogens when used as positive control, so Acetone extract was taken for further studies.

Table 2: Antimicrobial activity of pigment extracts from culture filtrate of C5-5Y

pathogens	*Extract*	*Zone of inhibition*	*Positive control*	*Zone of inhibition*
S.*aureus*	Chloroform extract	2.5cm	Chloroform	-
	Ethylacetate extract	2.7cm	Ethyl	1.2cm
	Isoamyl alcohol	2.8cm	Isoamyl	2.8cm
S.*mutants*	Chloroform extract	2.4cm	Chloroform	-
	Ethylacetate extract	2.5cm	Ethyl	1.7cm
	Isoamyl alcohol	2.8cm	Isoamyl	2.8cm
salmonella	Chloroform extract	2.1cm	Chloroform	1.0cm
	Ethylacetate extract	2.3cm	Ethyl	-
	Isoamyl alcohol	2.8cm	Isoamyl	1.6cm

Table 3: Antimicrobial activity of pigments extracted from Biomass

Pathogens	*Extract*	*Zone of inhibition*	*Control*	*Zone of inhibition*
Staphylococcus aureus	Acetone	2.3cm	Acetone	0.5cm
	Methanol	2.3cm	Methanol	0.7cm
	Propanol	2.2cm	Propanol	0.8cm
Streptococcus mutants	Acetone	2.2cm	Acetone	0.3cm
	Methanol	2.4cm	Methanol	0.6cm
	Propanol	2.4cm	Propanol	0.9cm
Salmonella	Acetone	1.9cm	Acetone	0.6cm
	Methanol	2.2cm	Methanol	0.6cm
	Propanol	2.0cm	Propanol	0.9cm

3.5 Anti-quorum sensing activity of extracellular and intracellular pigment extracts

3.5.1 Gram positive bacteria

3.5.1.1 Inhibition of protease

Proteolytic activity of *Staph. aureus* and *Strep. mutants* were observed by the formation of zone of clearance around the colonies. Clearance zone of 2.7cm and 2.9cm were observed with *Staphylococcus aureus* and *Streptococcus mutants* respectively. However the inhibition of proteolytic activity was confirmed by the absence of zone of clearance by the chloroform extract treated *Staph. aureus* and *Strep. mutants* culture filtrates. The peptides seem to play important role in quorum sensing hence contributing microbicidal activity. The protease produced by the bacterial pathogens inactivates these peptides representing bacterial defence systems (Projan *et al.,* 1997).

3.5.2 Inhibition of Lipase

The lipase is an important lipolytic enzyme of *Staph. aureus* that contributes significantly to the pathogenesis. Lipolytic activity of culture filtrates of *Staph. aureus* and *Strep. mutants* showed a zone of clearance of 2.2cm and 2.5cm around the colonies respectively. However the inhibition of lipolytic activity was observed by the reduction in the diameter of zone of clearance with extract treated *Staph. aureus* and *Strep. mutants* added wells.

3.2 Gram-negative bacteria

3.2.1 Carbapenem activity

The plate showed the absence of zone of clearance by the culture filtrate of treated Salmonella which indicated no production of carbapenem. Hence the pigment extract does not have any quenching activity against Salmonella quorum sensing molecule. The inhibition by the crude pigment might me by other means.

3.7 Partial purification of the crude pigment by TLC

The crude extract of the biomass using acetone, was concentrated and spotted in TLC plates. The compounds were separated using ethyl acetate: petroleum ether (4:1) as mobile phase. Two yellow spots were obtained at the Rf values 0.8 and 1.16.

3.8 Antimicrobial activity of partially purified compound

The antimicrobial activity was checked for these two spots and the spot having the Rf value 0.8 showed activity. This band was scraped out of the TLC plates and dissolved in acetone, concentrated and was stored taken for further use. *Strep. mutant* was found to be highly susceptible to the compound with the zone of inhibition of 3.1cms (Table 4). *Staphylococcus aureus* and *Salmonella spp* showed zone of 2.8cm in diameter was observed against the compound.

Table 4: Antimicrobial activity of Partially purified compound

Organism	*Compound (C5-5Y)*	*Control (Acetone)*
Salmonella	2.8cm	-
S.*aureus*	2.8cm	-
S.*mutants*	3.1cm	-

3.9 Minimal inhibitory concentration

The minimum inhibitory concentrations of the partially purified compound against *Staph. aureus,* and *Strep. mutants* was determined using the agar disc diffusion method. The concentration of the partially purified compound used was 5µL-25µL/mL. The least concentration of the partially purified compound that had inhibitory effect was 5µL/mL with a zone of clearance of 1.5cm. Since the positive control used had some inhibitory zone of around 0.7cm against pathogen cultures, we have taken 10µL/mL as minimum inhibitory concentration (Table 5).

Table 5: Minimum inhibitory concentration (MIC) of partial purified compound (C5-5Y) against pathogens

S.No	*Extract concentration*	*Staphylococcus aureus*	*Streptococcus mutants*
1	5µl	1.5cm	1.5cm
2	10 µl	1.9cm	1.9cm
3	20 µl	2.1cm	2.0cm
4	25 µl	2.2cm	2.2cm
5	Control	0.7cm	-

3.10 Anti-quorum sensing activity of partially purified compound

The anti-quorum sensing activity was detected through protease and lipase activity, cell surface hydrophobicity and bioflim formation. The inhibition of the proteolytic activity was confirmed by absence of zone of clearance and of lipolytic activity by the reduction in the zone of clearance of about 0.5cm for *Staph. aureus* and 0.4cm for *Strep. mutants* when compared to the controls.

3.11.1 Cell surface hydrophobicity

The adherence capacity of pathogens to host cells depends on the bacterial surface properties like hydrophobicity (Magnusson, 1989). It is the major determinant of biofilm formation in *Staph.aureus* and *Strep.mutants*. The partially purified compound was found to significantly reduces the cell surface hydrophobicity of *Staph.aureus* and *Strep.mutants*. The control showed high hydrophobicity of 89% and 91% to *Staph.aureus* and *Strep.mutants* respectively. The hydrophobicity percentage of the solvents were recorded as 76 and 84 whereas the treated cells showed minimum hydrophobicity levels of 24% and 23% respectively indicating the efficacy of partially purified compound in inhibiting the quorum sensing activity of the pathogens.

3.11.2 Biofilm formation

3.11.2.1 Microscopic method

The anti-bioflim activity of partially purified compound against *Staph. aureus* and *Strep. mutants* were observed through light microscopy and it was found to be significant. It was found that the partially purified compound has the ability to disturb the biofilm architectures when compared with control hence an inhibition in their growth on the glass beads were observed.

3.11.2.2 Adherent assay: Quantification and Tube method

The level of *Staph. aureus* and *Strep. mutants* biofilm formation treated with partially purified compound at 10µL/mL concentrations was quantified. The level of their biofilm markedly decreased to O.D 0.068 and O.D 0.087 from O.D 0.879 and O.D 0.708, when treated with partially purified compound from their respective controls. The adherent mode of bacteria on the surfaces of biomaterials has been well documented as one of the important causes of prosthetic device related infection that are refractory to antimicrobial therapy (Nickel *et al.,* 1994). The slow growth of biofilm bacteria and EPS or glycocalyx acting as barrier to the penetration of antibacterial agents (Farber et al., 1998) are considered to be responsible for failure of antimicrobial therapy for these infections. Three possible mechanisms of treatments may be effective on biofilm formations i). Electrostatic interference with the adhesion of bacteria and/or glycocalyx to the substratum, ii). Activaion or release of enzymes to disturb the EPS in the biofilm, iii). Inhibition of the formation of new glycocalyx.

This study reveals the effectiveness at a lower concentration (10µL /mL) of partially purified compound on the biofilm formation. It has been suggest that the effect will be improved by increasing the concentrations; further purification will be effective on inhibition of pathogenesis caused by larger inoculums of pathogens. The success of natural compounds in inhibiting cell attachment is a promising tool for reducing microbial colonization on various surfaces (Bavington *et al.,* 2005).

3.12 Effect of partially purified compound against bacterial growth curve

When the partially purified compound was added at the beginning of the cell cycle (0hrs) no change was observed in the growth of *Staph. aureus* and *Strep. mutants* for few hours. But after 8hrs there was a significant control in the growth of *Staph. aureus* and *Strep. mutants* respectively in the presence of the compound when compared to their positive control (acetone) and negative control (Fig. 1,2.). The inhibiton of growth started from 12hrs for *Staph. aureus* and *Strep. mutants* after incubation. These results confirm that the partially purified compound controls the growth of *Staphylococcus aureus* and *Streptococcus mutants* by quorum quenching activity which is essential for their multiplication and pathogenesis

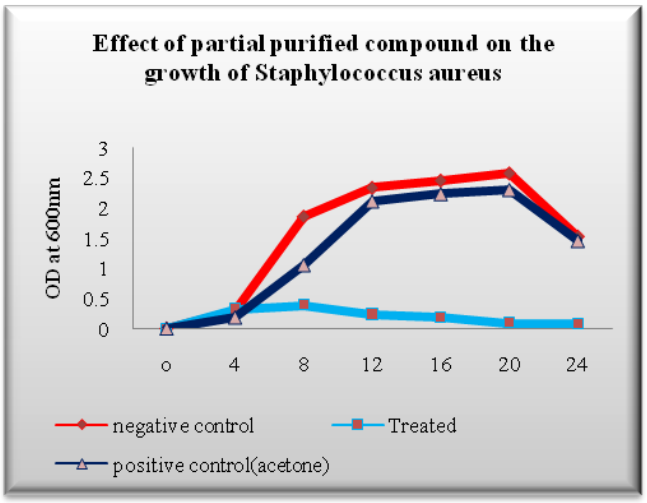

Figure 1: Effect of partial purified compound on the growth of Staphylococcus aureus

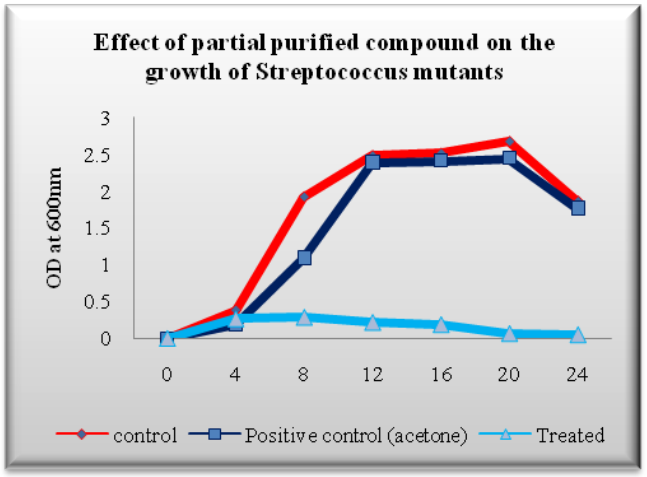

Figure 2: Effect of partial purified compound on the growth of Streptococcus mutants

4. Conclusion

From the results, it is evident that the yellow pigment produced by the strain C5-5Y exhibited efficient quorum quenching activity against two important clinical pathogens such as Staph. aureus and Strep. mutants which are main pathogens causing nosocomial infections. Hence, it is suggested that this compound can be used as a sterilizing agent for surgical instruments for preventing nosocomial infection. Further, the mechanism of inhibition of quorum sensing molecules by the compound has to be explored to use this pigment as a therapeutic agent.

References

[1] Bavington C and Page C. Stopping bacterial adhesion: a novel approach to treating infections. *Respiration* 72.4 (2005): 335-344.
[2] Farber, Bruce. Method of reducing medical device related infections. U.S. Patent No. 5,716,406. 10 Feb. (1998).
[3] Horinouchi, S and Beppu T. A-factor as a microbial hormone that controls cellular differentiation and

secondary metabolism in Streptomyces griseus. Mol. Microbiol. 12(1994): 859-864.
[4] Ilic, Vesna. The influence of silver content on antimicrobial activity and color of cotton fabrics functionalized with Ag nanoparticles. *Carbohydrate Polymers* 78.3(2009): 564-569.
[5] Johnson, Leroy. E and Alma Dietz. Kalafungin. a new antibiotic produced by Streptomyces tanashiensis strain Kala. *Applied microbiology* 16.12(1968): 1815-1821.
[6] Lembke, Cordula, "Characterization of biofilm formation by clinically relevant serotypes of group A streptococci." *Applied and environmental microbiology* 72.4(2006): 2864-2875.
[7] Nealson, K.H and Hastings. Bacterial bioluminescence: its control and ecological significance. *Microbiol. Rev.* 43(1979):496–518
[8] Nickel, J. Curtis, et al. Bacterial biofilms: influence on the pathogenesis, diagnosis and treatment of urinary tract infections *Journal of Antimicrobial Chemotherapy* 33.suppl A (1994): 31-41.
[9] Oskay, Mustafa, Antifungal and antibacterial compounds from Streptomyces strains, *African Journal of Biotechnology* (2009) 8.13.
[10] Projan, Steven. J and Richard. P. Novick. The molecular basis of pathogenicity. *The staphylococci in human disease. Churchill Livingstone, New York, NY* (1997): 55-81.
[11] Rahman, M.A., M.Z. Islam and M.A. Ul-Islam., 2011. Antibacterial activities of actinomycete isolates collected from soils of Rajshahi, bangladesh. Biotechnol. Res.Int., 1-6.
[12] Reisner, A., Haagensen .J.A, Schembri M.A, Zechner.E.L and Molin.S. Development and maturation of Escherichia coli K-12 biofilms. Mol. Microbiol. 48(2003): 933-946.
[13] Serebryakova, E. V., et al. Evaluation of the hydrophobicity of bacterial cells by measuring their adherence to chloroform drops. *Microbiology* 71.2 (2002): 202-204.
[14] Smith, K.M., Bu .Y and Suga.H. Library screening for synthetic agonists and antagonists of a Pseudomonas aeruginosa autinducer. Chem. Biol. 10 (2003):563-571.
[15] Teplitski, M., Robinson and Bauer.W.D. Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviours in associated bacteria. Mol.Plant Microbe. Interact. 13 (2000):637-648.
[16] Thangadurai, D. Antimicrobial screening of *Decalepis hamiltonii wight* and arn.(Asclepiadaceae) root extracts against food-related microorganisms. *Journal of food safety* 24.4 (2004): 239-245
[17] Wang, Y. Tang.S.K, Lou.K, Mao.P.H, Jin.C.L.X Jiang, Xu. L.H and Li W.J. Paracoccus saliphilus sp. nov., a novel halohilic bacterium isolated from a salt lake. Int. J. Syst. Evol. Microbiol. 59 (2009):1924-1928.
[18] Zhang, Xue-Song, Rodolfo García-Contreras, and Thomas K. Wood. YcfR (BhsA) influences Escherichia coli biofilm formation through stress response and surface hydrophobicity. *Journal of bacteriology* 189.8 (2007): 3051-3062.
[19] Zhu, J and Mekalabos J.J. Quorum sensing-deptendednt biofilms enhance colonization in Vibrio chloerae. Dev.Cell. 5 (2003):647-656.