Assessment of the Anti-Biofilm Potency of *Psidium guajava* on Two Strains of *Pseudomonas fluorescens*

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Abstract

Psidium guajava, more commonly known as Guava is an evergreen plant of the family *Myrtaceae*. It is a traditional plant used to treat various gastrointestinal infections such as typhoid fever, and also known to inhibit the growth of certain bacteria including *Staphylococcus aureus*. *Pseudomonas fluorescens* is an organism capable of surviving in heavy metal contaminated environment. One common type of heavy metal contaminant is Chromium(Cr), known to cause serious ecological and health problems. The most toxic and soluble form of chromium is Cr (VI) also called hexavalent chromium, while Cr (III) is less toxic. *Pseudomonas fluorescens* is a common bacterial strain known to not only survive in chromium contaminated soil, but is also capable of converting toxic chromium (VI) to chromium (III) due to its ability to form biofilm. The objective of this research is to investigate the effectiveness of *Psidium guajava* in reducing the formation of biofilm by *Pseudomonas fluorescens,* thereby enhancing its movement in porous media, such as soil. This pilot research project was conducted in collaboration with Savannah River National Laboratory, and investigated the remediation of chromium (VI). Static biofilm assays were conducted using methanolic and chloroform extracts of *Psidium guajava* . Results indicated that the extracts of *Psidium guajava* did reduce biofilm formation in *Pseudomonas fluorescens, pf-01* strain. However, the guava extract did not have an inhibitory effect on biofilm formation in the *pf-5* strain.

Introduction

Pollution can be defined as the introduction of contaminants into the environment. One prevalent act of pollution is the presence of heavy metals in soils and water sources. A major type of heavy metal is Chromium(Cr). It is mostly released into the environment from chrome leather tanning processes. The most toxic and soluble form is hexavalent chromium, Cr (VI), while Cr (III) is naturally occurring and less toxic (Chen, 2010). Chromium (III) is an essential dietary nutrient found in humans. Hexavalent chromium, however is carcinogenic, and can cause gastrointestinal diseases in humans, and developmental and reproductive defects in animals and plants. Atmospheric release of chromium has been linked to lung cancer, and further studies are also investigating the relationship between Cr(VI) contaminated water and increased carcinogenic risks in humans (Davidson et. al., 2010).

Pseudomonas fluorescens is a common bacterial strain known to be able to survive in chromium contaminated soil, and convert the toxic chromium (VI) to chromium (III) due to its ability to form biofilm (DeLeo, 1993). Gomashe et.al. (2014) reported that extracts from various part of *Psidium guajava* significantly inhibited biofilm formation in *Streptococcus mutans,* an oral plaque forming bacteria. Bioremediation refers to the use of microorganisms, plants and their by–products to break down pollutants or hazardous compounds in the environment (Wenzel, 2009).

It is therefore imperative that we find ways to convert Cr (VI) to Cr (III) in the environment. Conventional methods of removing heavy metals and other pollutants in the environment include, excavation, solvent extraction, reburial etc. (Salem, 2011). However, these methods are not only expensive, they do not guarantee permanent removal of the toxic materials in the environment. A lot of emphasis has been placed on discovering alternatives to these conventional methods of remediating heavy metal contamination. The use of living organisms for bioremediation of pollutants have become effective alternatives to the conventional methods, because it is economical, sustainable, and eco-friendly.

This project therefore investigated the effectiveness of *Psidium guajava* in decreasing the formation of biofilm by *Pseudomonas fluorescens* thereby enhancing its mobility in porous media , such as soil, and consequently improve its efficiency in converting the toxic chromium (VI) to chromium (III).

Materials and Methods

Preparation of tissue and extraction of phytochemicals;

Psidium guajava leaves were obtained from Nigeria in West Africa. The leaves were sterilized in 10% Clorox solution, rinsed in distilled water, and then air dried. Four grams of the dried ground leaf sample was transferred to centrifuge tubes containing 20 ml of 99% methanol or Chloroform respectively. The tubes were then allowed to shake over night at room temperature in an open shaker. Each sample was then centrifuged at 4000 rpm for 10 minutes. The supernatant of each sample was removed and transferred to micro- centrifuge tubes and allowed to dry in the centrivap overnight. The samples were reconstituted to 50mg/ml for use in static biofilm assay.

Bacterial culture;

Individual bacterial strains was streaked from the freezer stock onto Luria Bertani (LB) plates and grown in incubator (Precision PS scientific SN 22-AD-2) at 25^{0}C for 48 hours until isolated colonies appeared. A single colony of *P. fluorescens* was inoculated into 3 mL of LB broth in a 15 ml conical tube and then placed in an open-air shaker to grow overnight at 25^{0}C.

Preparation of extract for biofilm assay;

For individual strains and extract, experiment was performed in triplicate. A labeled polyvinyl chloride (PVC) 96 well microtiter plate was used. A single row was used for a specific bacterial strain and extract which included: 10 dilutions, a biofilm control (BC, broth with bacteria-no extract) and a sterility control (SC, broth only). Fifty µl of LB broth was added to first 11 wells in a row while 100 µl of LB broth was added to well 12. The appropriate amount of LB broth was added to a single tube of dried extract for re-suspension to make an equivalent concentration of 100 mg/L.

Static Biofilm assay:

Each bacterial strains were diluted 1:100 LB Broth. Fifty µl was transferred from well 1 to 11, well 12 served as the positive control. The plates were incubated at 25° C for 48 hours. The planktonic bacteria was removed from the plates and washed thoroughly with water. Each well was stained with 125 µl of 0.1% crystal violet solution and incubated at room temperature for 10 minutes. 200 µl of 95% ethanol was added to each well and allowed to solubilize for 15min. 125 µl of the crystal violet/ethanol solution from each well was pipetted to a flat- bottom 96 well plate for optical density reading. The optical density (OD) of each of these 125 µl samples was measured at a wavelength of 562 nm using a micro-plate reader. Inhibition mediated reduction of biofilm was then calculated by the following formula.

$$\% \text{ of inhibition} = \frac{[\text{Optical density in control (OD) - optical density (OD) in treatment}]}{\text{Optical density (OD) in control}} \times 100$$

Results

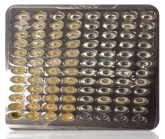

Fig 1:PVC plate with *psidium guajava* extract against *pf-01* and *pf-5*

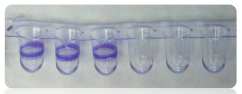

Fig 2 :Biofilm formed at air-liquid interface after addition of 0.1% Crystal violet

Inhibition of biofilm formation in *pf-01* strain by chloroform extract of *Psidium guajava*

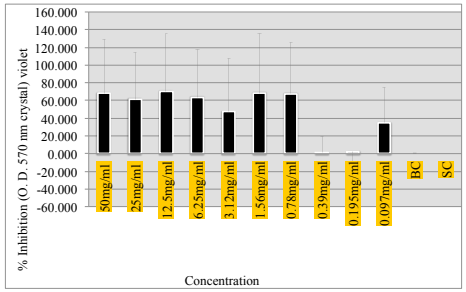

Fig 3: Biofilm assay showing % inhibition of chloroform extract of *Psidium guajava*

Inhibition of biofilm formation in *pf-01* strain by methanoic extract of *Psidium guajava*

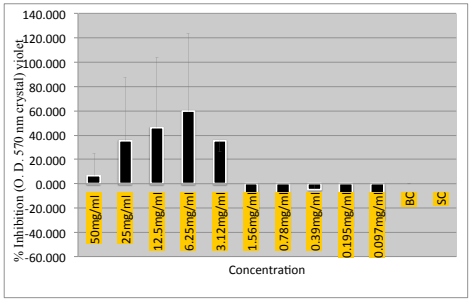

Fig 4: Biofilm assay showing % inhibition of methanolic extract of *Psidium guajava*

Conclusion

- Both the chloroform and methanolic extract of *Psidium guajava* impeded biofilm production in *pf-01* strain. However, the chloroform extract exhibited higher levels of inhibition than the methanolic extract.
- The methanolic and chloroform extracts of *Psidium guajava* did not have an inhibitory effect on biofilm formation in the *pf-5* strain.
- This suggest that for bioremediating purposes, *pf-1* would move more freely in porous media (due to reduction in biofilm formation) when exposed to methanolic or chloroform extracts of *Psidium guajava*, thereby enhancing its bioremediating capacity.

Analysis of inhibition of chloroform extract of *Psidium guajava* against biofilm formation in pf-5

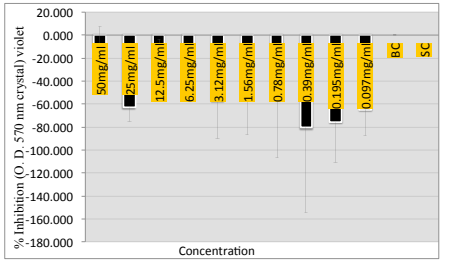

Fig 5: Biofilm assay showing % inhibition of chloroform extract of *Psidium guajava*

Analysis of inhibition of methanolic extract of *Psidium guajava* against biofilm formation in *pf-5*

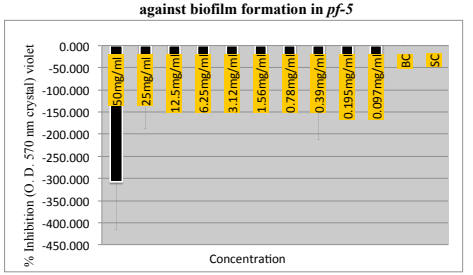

Fig 6: Biofilm assay showing % inhibition of methanolic extract of *Psidium guajava*

Future Plan

- Use of guava for phytoremediation
- Testing other extracts for possible inhibitory potentials, and also combining them with *Psidium guajava* to evaluate their synergistic effects in inhibiting biofilm formation.
- Increase the concentration of the extract for testing % inhibition in pf-5.
- Use of Nuclear Magnetic Resonance (NMR) to identify the phytochemicals present in the extracts used..
- The identified phytochemical will then be tested individually to ascertain their anti-biofilm potentials..

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