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To study the effect of guava leaf extract on biofilm formation in *Pseudomonas Aeruginosa*

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I. Introduction:

Pseudomonas Aeruginosa:
Pseudomonas aeruginosa is a gram negative bacteria with unipolar motility which causes various diseases in animals including humans. It is an aerobic bacteria and an opportunistic one for both plant and animals. It is present in water bodies, soil environment and manmade environment too. It gives positive result with citrate, oxidase and catalase test. It can prevail not only in natural environment but also in HYPOXIC atmosphere. It lives on a variety of organic matter obtained from animals and mainly infects areas with damaged tissues or with less immunity. The most basic symptoms include inflammation and sepsis. The infection in organs like lungs, kidney, urinary tract can prove to be fatal.It is also responsible for causing gingivitis in humans which results in bleeding gums and loosening of teeth.
It forms biofilms which are responsible for providing antibiotic resistance to the bacteria.

Biofilms:

A biofilm is composed of living, reproducing microorganisms, such as bacteria, that exist as a colony, or a community. In other words, biofilms are alive and have a complex social structure that both protects them and allows them to grow.

A biofilm forms when certain microorganisms adhere to the surface of some object in a moist environment and begin to reproduce. The microorganisms form an attachment to the surface of the object by secreting a slimy, glue-like substance. Biofilms can form on just about any imaginable surface: metals, plastics, natural materials (such as rocks), medical implants, kitchen counters, contact lenses, the walls of a hot tub or swimming pool, human and animal tissue etc .Indeed, wherever the combination of moisture, nutrients, and a surface exists, biofilms will likely be found as well.

A biofilm community can be formed by a single kind of microorganism, but in nature biofilms almost always consist of mixtures of many species of bacteria, as well as fungi, algae, yeasts, protozoa, and other microorganisms, along with non-living debris and corrosion products. For example, over 500 bacterial species have been identified in typical dental plaque biofilms.

GUAVA:

The important constituents of guava are vitamins, tannins, phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols and triterpenoid acids. Leaves contain phenolic compounds, isoflavonoids, gallic acid, catechin, epicatechin, rutin, naringenin, kaempferol having hepatoprotective, antioxidant, anti-inflammatory, antispasmodic, anticancer, antimicrobial, anti-hyperglycemic, analgesic actions.The leaf contain two important flavonoids **quercetin** known for its spasmolytic, antioxidant, antimicrobial, anti-inflammatory actionsand **guaijaverin** known for its antibacterial action.Pulp contains ascorbic acid, carotenoids (lycopenes, β-carotene) possessing antioxidant, anti-hyperglycemic, antineoplastic. The seed contains glycosides, carotenoids, phenolic compounds having antimicrobial actions.

Guava is proven for its antidiarrheal, antimicrobial, antiparasitic, antitussive, hepatoprotective, antioxidant, antigenotoxic, antimutagenic, antiallergic, anticancer and anti-hyperglycemic effects.Acclaimed as the “poor man's apple of the tropic “guava has been used for various purposes in different regions of the world. It has been used in the treatment of diarrhea, dysentery, menstrual disorders, vertigo, anorexia, digestive problems, gastric insufficiency, inflamed mucous membrane, laryngitis, skin problems, ulcers, vaginal discharge, cold, cough, cerebral ailments, nephritis, jaundice, diabetes, malaria and rheumatism to mention a few.
Guava leaf extract can be used in order to decrease the amount of bioflim formation and hence it can be incorporated in formulations for the treatment of gingivitis and other diseases.

ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF *PSEUDOMONAS AERUGINOSA*
PROTOCOL USED:

Pseudomonas Isolation Agar is used for the isolation of *Pseudomonas aeruginosa* and other *Pseudomonas* spp.

Principles of the Procedure :

Enzymatic Digest of Gelatin provides nitrogen, vitamins, and carbon in Pseudomonas Isolation Agar. Magnesium Chloride and Potassium Sulfate promote production of pyocyanin. Irgasan, an antimicrobial agent, selectively inhibits Gram-positive and Gram-negative bacteria other than *Pseudomonas* spp. Glycerol serves as an energy source. Agar is the solidifying agent.

Formula / Liter Supplement /Liter

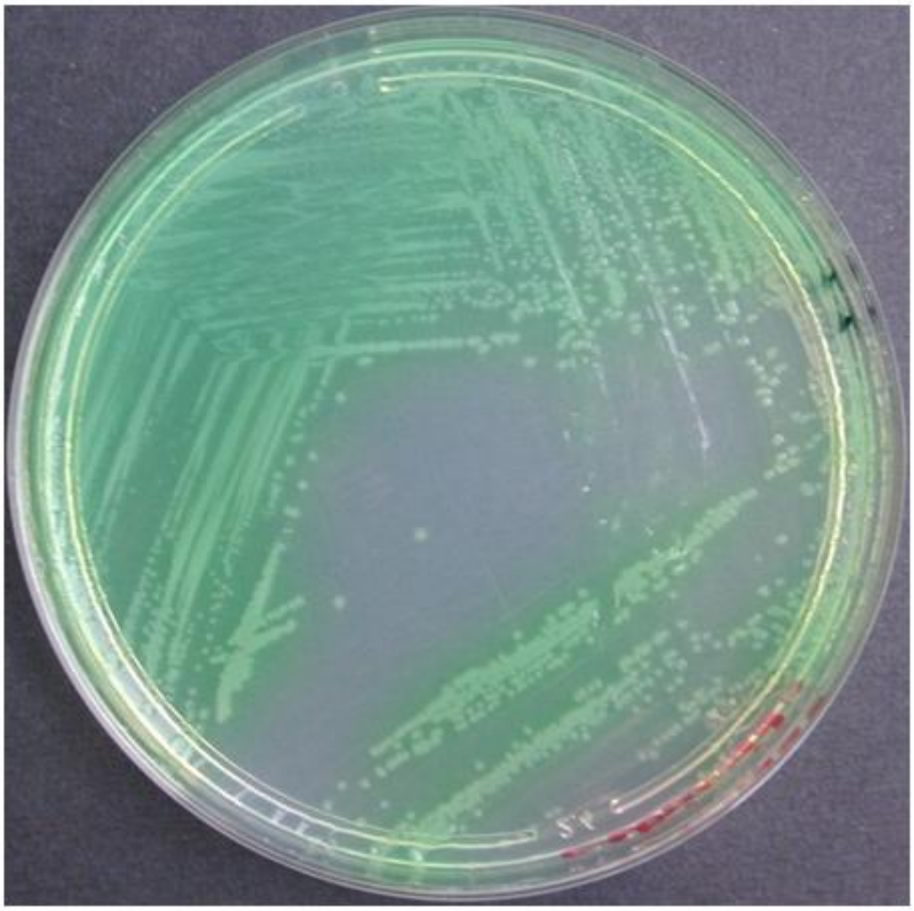
Enzymatic Digest of Gelatin ..... 20 g
Glycerol 20 mL
Magnesium Chloride ..... 1.4 g
Potassium Sulfate ..... 10 g
Irgasan..... 0.025 g
Agar ..... 13.6 g
Final pH: 7.0 ± 0.2 at 25 degree Celsius.

Directions :

1. Suspend 45 g of the medium in one liter of purified water containing 20 mL of glycerol.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121 degree Celsius for 15 minutes.

II. Result:

Presence of good growth was observed .*Pseudomonas aeruginosa* colonies are found to be green to blue-green with pigment that diffuses into the medium.



ISOLATION OF THE LEAF EXTRACT:

PROTOCOL USED:

Preparation of water extracts

Sample of 100 g guava leaves in 1.5 L distilled water was boiled for 4 h. The sample was then filtered using Whatman filter paper No. 4. The filtrate was concentrated in an evaporator at 60°C . The resulting extracts were stored at 4°C until the analysis.

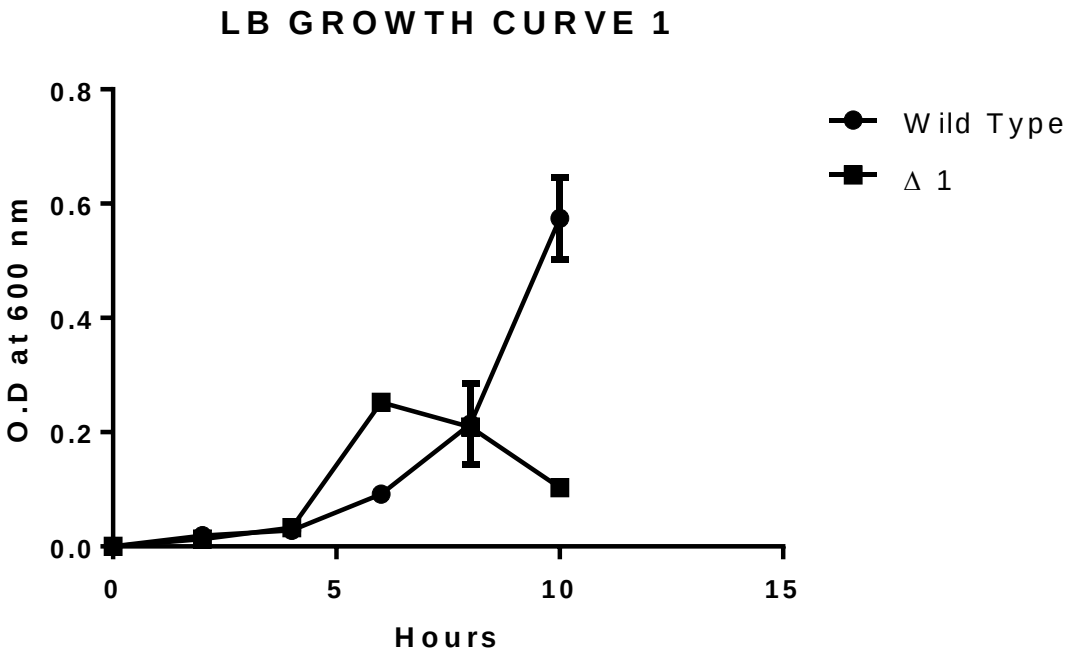
GROWTH CURVE (IN LB MEDIUM)

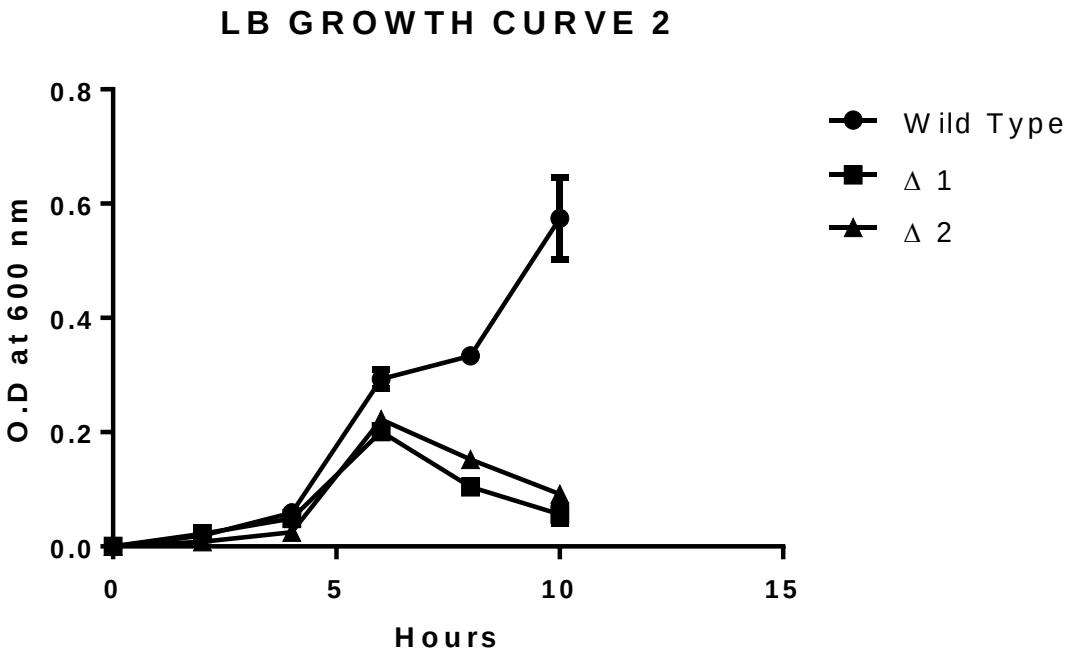
PROTOCOL USED:

1. Wild type is inoculated and kept for growing overnight.
2. 1 LB tube was inoculated with wild type which was further duplicated into WT1 and WT2. 1LB tube was inoculated with the wild type + Leaf extract (200 ul) which is further duplicated giving 2 more samples.
3. Dilution factor is 1:100.
4. 0 hrs OD values are taken for all the samples.
5. Various time points and dilution factors are given below in tabulated form.

TIME POINTS	DILUTION FACTOR
0 hrs	10^{0} and 10^{-1}
2 hrs	10^{0} and 10^{-1}
4 hrs	10^{-1} and 10^{-2}
6 hrs	10^{-2} and 10^{-3}
8 hrs	10^{-3} and 10^{-4}
10 hrs	10^{-4} and 10^{-5}

OD is taken at each time point and graph is plotted using graph prism software.


LB GROWTH CURVE 1
O.D at 600 nm
0.8
0.6
0.4
0.2
0.0
0
5
10
15
Hours
Wild Type
Δ 1



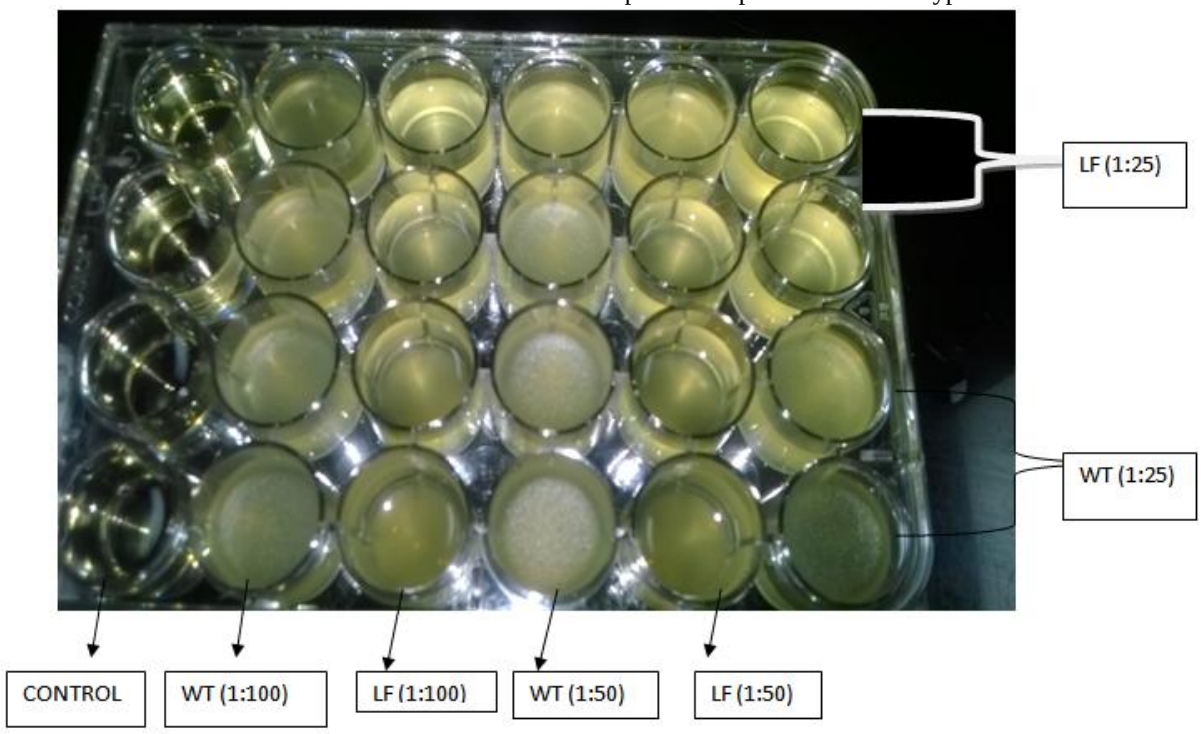
Biofilm Assay

PROTOCOL USED:

1. 24 well plates is taken and in each well 2ml of bioflim medium is added.
2. First column is inoculated with overnight wild type culture with a dilution factor of 1:100. 2nd column is inoculated with wild type + leaf extract culture with a dilution factor of 1:100.
3. 3rd column inoculated with WT (1:50), 4th column with **WT+leafextract**(1:50).
4. 2 wells of 5th column inoculated with WT (1:25) and other two wells with WT+ leaf extract (1:25).
5. The 24 well plates are kept undisturbed for 3 days to visualize the biofilm.

RESULT:

Pellicle formation was absent in the WT + leaf extract sample as compared to the wild type.



SWIM AND SWARM ASSAY

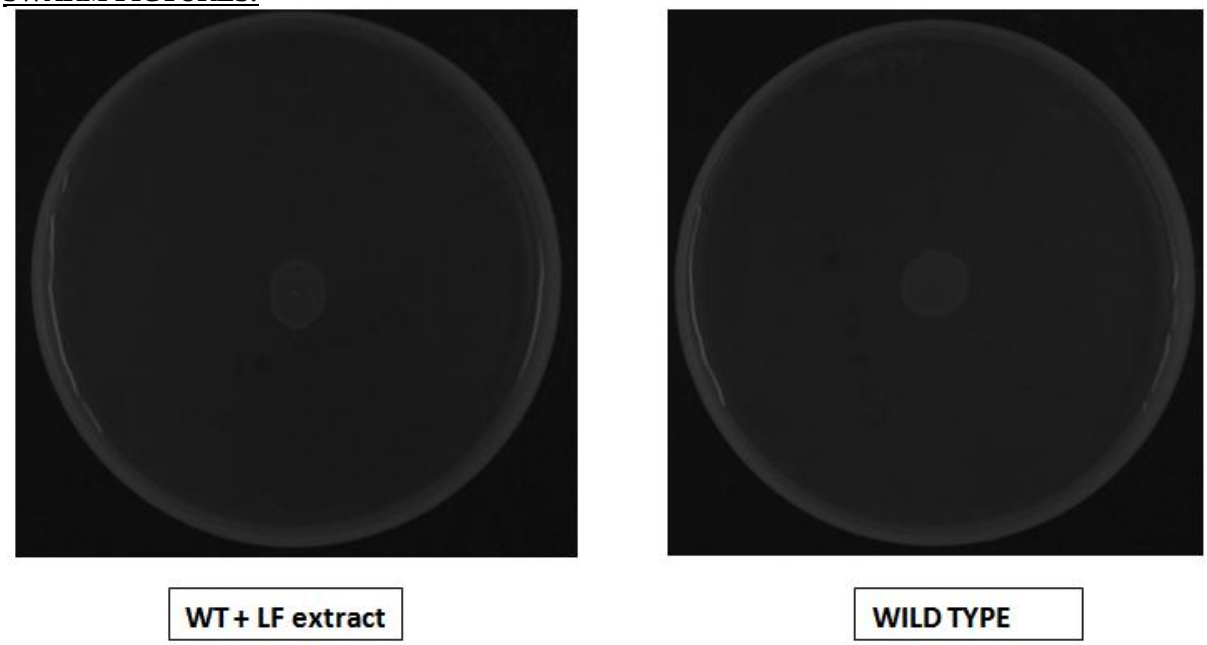
SWIM MOVEMENT: Flagella regulated movement of Pseudomonas inside a liquid or semi solid medium is called swimming. It is planktonic in nature. The bacteria are present in the dispersed form.
SWARM MOVEMENT: Movement of bacteria over the top of the surface of a solid or semi solid medium using flagella in high densities as aggregates in known as swarm movement.
ADVANTAGE: Provides resistance against antibiotics (Clusters or high density population is more favorable to cope with high antibiotic stress).

PROTOCOL USED:
1. LB agar with 0.5% Agar concentration is used to form Swarm plates.
2. LB agar with 0.3% Agar concentration is used to form swim plates.
3. 2 Swim and 2 swarm plates are formed.
4. At the centre of each plate 20µl of the sample is poured.
5. 2 plates with WT and 2 plates with WT + leaf extract.
6. Kept for 5 hrs incubation without disturbing.
7. Images are obtained in the Gel-Doc with filter 1 and transilluminator white light after 5 hrs.

RESULT:
WT+ leaf extract sample have less diameter of the swim and swarm dot as compared to the wild type.

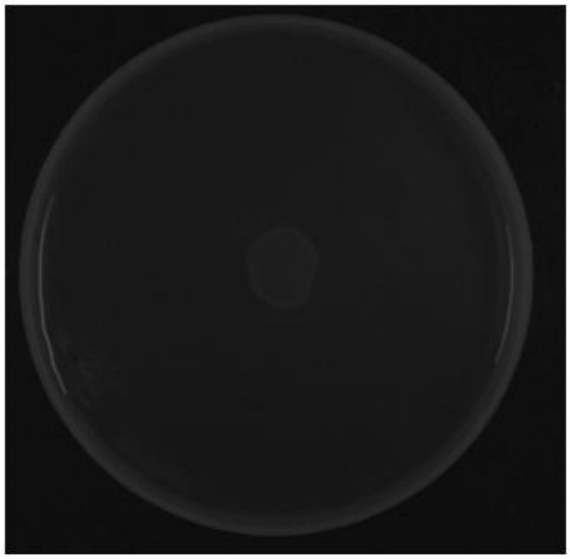
SWIM AND SWARM PICTURES

SWARM PICTURES:



SWIM ASSAY PICTURES:

WT + LF extract



WILD TYPE

III. Conclusion:

It is found out that the biofilm get inhibited in the presence of guava leaf extract and the motility of the bacteria also reduces significantly. Therefore the use of guava leaf extract in toothpastes is favourable to treat gingivitis.