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Evaluation of Chemotaxis using Soft Agar Plate Method

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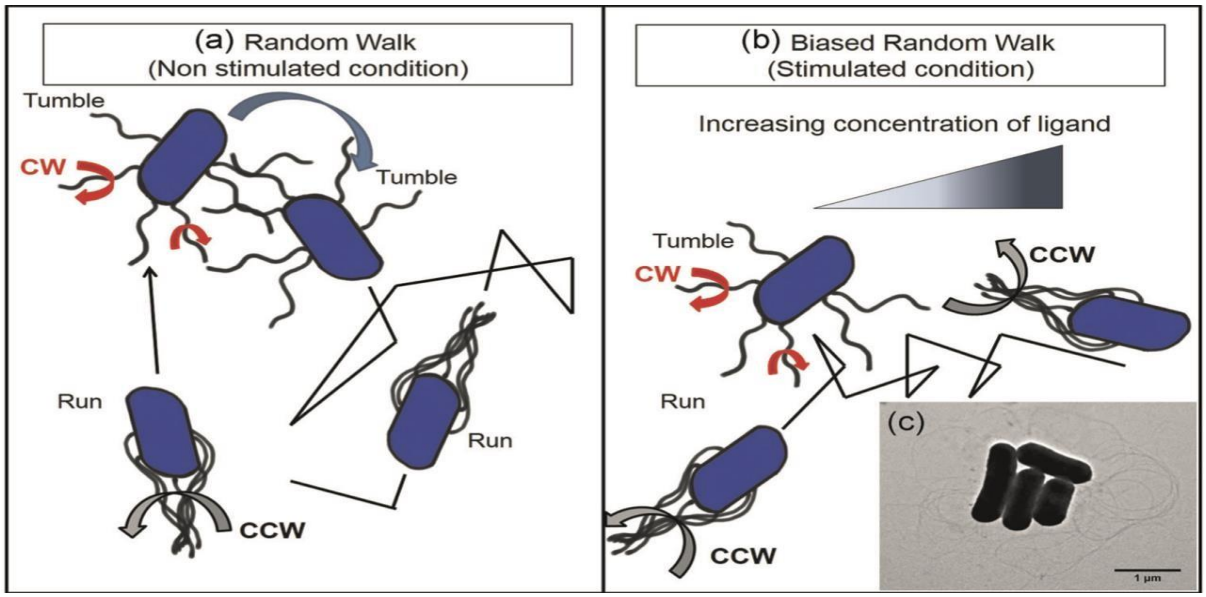
ABSTRACT:

The movement of cells in response to a chemical gradient is known as chemotaxis. An assay that is frequently used to analyze chemotaxis is the soft agar plate method. In this study, we used the soft agar plate method to examine the chemotactic migration of bacteria. On soft agar plates with a chemical attractant, bacteria were injected, and over time, their migration toward the attractant was seen. We discovered that the bacteria clearly responded to the attractant by migrating in that direction and producing discernible colonies. Our findings show how the soft agar plate approach may be used to examine the chemotactic behavior of bacteria, and they also imply that it can be used to study the chemotactic behavior of other cell types. Chemotaxis is a biological mechanism in which specialized sensory receptors on the surface of bacteria detect chemical gradients. These receptors set off a series of events that alter the bacterial flagella motor and cause the bacterium to migrate in the direction of the chemical gradient. Several crucial bacterial processes, such as nutrition intake, host colonization, and biofilm formation, depend on chemotaxis.

INTRODUCTION:

An essential laboratory method for assessing the efficacy of medications against bacterial infections is antimicrobial susceptibility testing. In this technique, bacterial colonies are grown in a soft agar medium containing a certain number of antibiotics. It is possible to determine the bacterium's susceptibility to various antibiotic concentrations by measuring the gradient of decreasing antibiotic concentration created by the antibiotics diffusing through the agar. This test can assist medical professionals in selecting the most efficient antibiotic therapy for a specific infection, lowering the risk of the emergence of antibiotic resistance and improving patient outcomes. The soft agar plate method is widely used because it is an easy, affordable, and reliable way to determine how susceptible bacteria are to antibiotics.

The movement of bacterial colonies in response to chemical gradients is known as chemotaxis. Bacteria can use this fundamental mechanism to move toward or away from particular chemical stimuli in their surroundings. Bacteria need to be able to detect and respond to chemical cues in order to survive in complex settings.



State of the art of bacterial chemotaxis. Journal of Basic Microbiology, 61(5), pp.366-379

AIM and OBJECTIVES:

The aim and objectives of carrying out the soft agar plate method experiment ofPseudomonas, Bacillus, and E. coli strains with the presence of antibiotics is to:

- To determine the antimicrobial susceptibility of pseudomonas aeruginosa, bacillus subtilis, and Escherichia coli to different classes of antibiotics.

- Chemotactic pattern of bacteria under antibiotic stress and different agarconcentrations.

- To study chemo repellence property of antibiotics.

- To determine the zone of inhibition of each antibiotic, which is the area surrounding the antibiotic disk on the agar plate where bacterial growth isinhibited.

In general, the goals of conducting an experiment using the soft agar plate method with these bacterial strains and antibiotics are to provide critical datafor deciding which antibiotic treatments are most successful, keeping track of the emergence of antibiotic resistance, and ensuring the safety and efficacy of antibiotics used in clinical settings.

MATERIALS and METHODS:

Antimicrobial susceptibility tests require a few materials to be conducted accurately. The specific materials required may vary based on the type of testbeing conducted, but some common materials include:

- Microorganisms: The microorganisms that need to be tested for susceptibility to antibiotics such as E.Coli, Pseudomonas, Bacillus etc.

- Antibiotics: A range of antibiotics that need to be tested against the microorganisms such as Tetracycline, Erythromycin, NFX, LVX, OFX etc.

- Mueller Hinton agar: This is a type of nutrient agar commonly used in Antimicrobial Susceptibility testing.

- Microorganisms are transferred onto the agar plate using an inoculating loopor swab.

- Tryptone: used to make bacterial suspensions for testing,.

- Incubator: Used to provide optimal temperature and conditions for thegrowth of microorganisms.

- Measuring cylinders or pipettes: Used to measure and dispense the required volumes of antibiotic solutions, broth,

- Petridishes: 6mm and 9 mm Petridishes are used to observe the cell growthof microorganisms in agar medium.

- Centrifugation machine with rpm up to 4000 rpm is required to form pellet of the inoculated overnight culture.

- Shaker is required to keep the inoculated culture overnight and helps in theformation of cell growth.

- Autoclave machine is required to autoclave the Petridishes, tryptone brothand the agar media before proceeding with the experiment for sterilizationpurpose.
- Weighing scale: To measure the amount of tryptone and agar taken to beused for the experiment.

- Dryer: Once the experiment is been completed the flasks, Petridishes, measuring cylinders are washed and kept in the dryer to be used for futureexperiments.

METHODS:

A detailed procedure for using the **soft agar plate method** to test antibioticresistance for different bacterial strains:

Required materials are:

Mueller-Hinton agar platesAntibiotic discs
Test organismSterile swabs
Soft agar (0.7-0.75%)
Incubator set to appropriate temperature for test organism

- Choose a strain of bacteria that you want to examine for antibioticresistance.

- Using a sterile swab, inoculate a Mueller-Hinton agar plate with a lawn culture of the chosen bacterial strain. To accomplish this, wet the swab withsterile saline before sweeping it across the agar plate's whole surface.

- Using sterile forceps, press antibiotic discs onto the agar plate's surface. Based on the particular resistance profile of the bacterial strain you are testing, select a set of medications. To choose which antibiotics to use and atwhat concentration, consult a chart or other instructions.

- Allow the antibiotic to permeate into the agar for 30 minutes while incubating the agar plate at the right temperature for the particular bacterial strain. Depending on the bacterial strain being tested, the incubation temperature and duration may change.
- Prepare a soft agar solution by melting agar in a water bath or microwaveand chilling it to 45–50°C while the plate is incubation.

- The plate should then be gently covered with a layer of soft agar (0.7-0.75%)by pouring the agar on top of the plate after the antibiotic discs have been incubated for 30 minutes. Make sure the soft agar solution is distributed uniformly across the plate's surface.

- Incubate the plate for an additional 24-48 hours at the proper temperature forthe bacterial strain being tested after allowing the soft agar to solidify.

- Watch the zones of inhibition that surround the antibiotic discs after incubation. A distinct zone of inhibition will be visible all the way around the disc if the bacterial strain is susceptible to the antibiotic. There won't be any discernible zone of inhibition surrounding the disc if the bacterial strain is resistant to the antibiotic.

- Measure each zone of inhibition's diameter and compare it to accepted limits for susceptibility or resistance. To interpret the findings, use a chart or somerules.

- Use the right temperature and incubation period for each bacterial strainwhen repeating the process with additional strains.

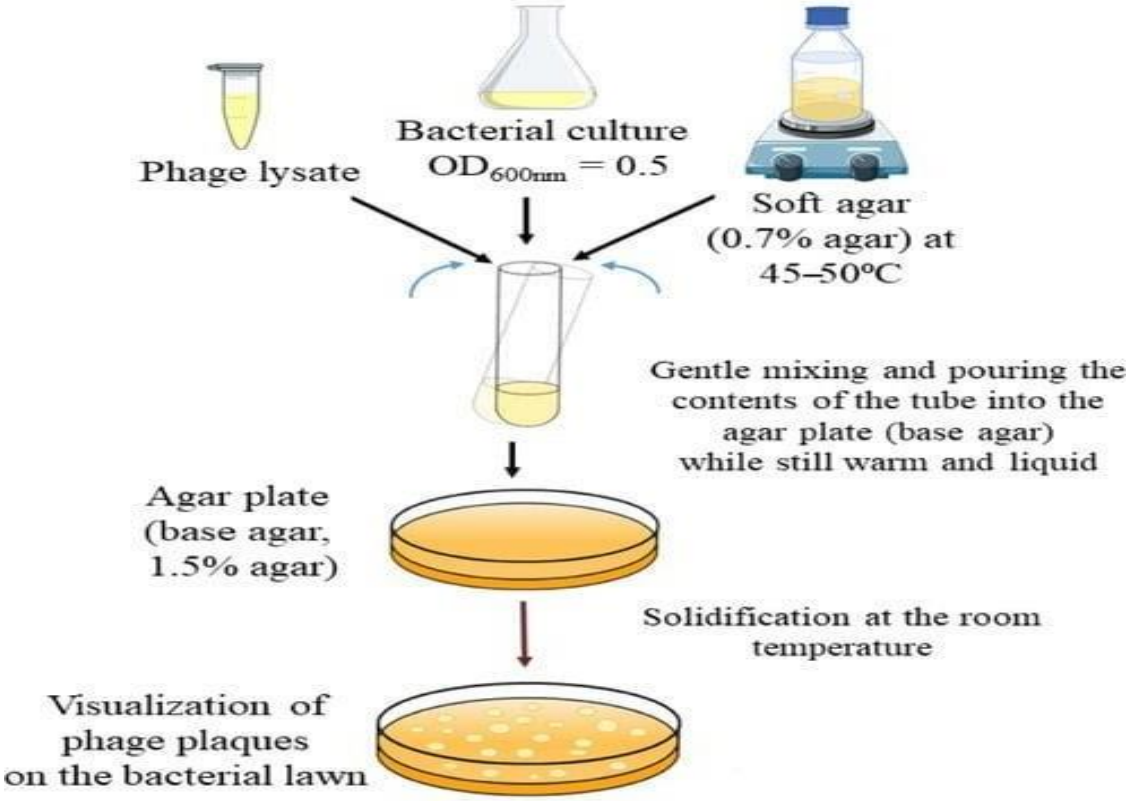


Fig 1- Standard double-layer agar (DLA) method visualization (prepared based on the methodology described by Kropinski et al. [24]).

PREPARATION PROCESS OF 0.2% TRYPTONE AGAR MEDIA :

Materials:

Agar powder
Nutrient broth or other appropriate liquid mediaDistilled water
Autoclave or pressure cooker Sterilized culture tubes or plates

PROCEDURE:

- Measure out the correct quantity of agar powder. For a 0.2% agar media, 100 milliliters of liquid medium will require 0.2 grams of agarpowder.
- In a flask or beaker, mix the liquid media with the agar powder.
- Considering the amount of the liquid media as well as the volume of the agar powder, add distilled water to the combination until the appropriate volume is reached. In order to create 500 milliliters of 0.2% agar media, for instance, you must first combine 0.1 grams of agar powder with 500 milliliters of liquid media. Next, you must addenough distilled water to make up the remaining 500 milliliters.
- Until the agar powder is completely dissolved, thoroughly combinethe liquid media and agar powder.
- Fill sterile culture tubes or plates with the agar media, being careful not to introduce any impurities.
- After sterilizing the media in an autoclave or pressure cooker, place sterilized caps or lids on the culture tubes or plates. The volume of themedia and the type of container being used should be taken into consideration while applying the manufacturer's recommended sterilizing procedures.
- Before using, let the media cool and harden.
- The agar media should be kept in a refrigerator or other environmentthat is suitable for the type of microorganism being cultivated.

PREPARATION PROCESS OF 2% TRYPTONE AGAR MEDIA:

Materials:

Agar powder
Nutrient broth or other appropriate liquid mediaDistilled water
Autoclave or pressure cooker Sterilized culture tubes or plates

PROCEDURE:

- Measure out the correct quantity of agar powder. 100 milliliters of liquidmedia will require 2 grams of agar powder for a 2% agar media.

- In a flask or beaker, mix the liquid media with the agar powder.

- Considering the amount of the liquid media as well as the volume of the agarpowder, add distilled water to the combination until the appropriate volume is reached. In order to create 500 milliliters of 2% agar media, for instance, you must first combine 10 grams of agar powder with 500 milliliters of liquid media. Next, you must add sufficient distilled water to make up the remaining 500 milliliters.

- Until the agar powder is completely dissolved, thoroughly combine the liquid media and agar powder.

- Fill sterile culture tubes or plates with the agar media, being careful not tointroduce any impurities.

- After sterilizing the media in an autoclave or pressure cooker, place sterilized caps or lids on the culture tubes or plates. The volume of the media and the type of container being used should be taken into consideration while applying the manufacturer's recommended sterilizing procedures.

- Before using, let the media cool and harden.

- The agar media should be kept in a refrigerator or other environment that issuitable for the type of microorganism being cultivated.

PREPARATION PROCESS OF STOCK SOLUTION OF OFLOXACINANTIBIOTIC:

- Be sure to figure out how much ofloxacin is needed to make the stock solution. Should weigh out 10 mg of ofloxacin (1000 g/mL x 10 mL / 0.99)if wanted to construct a 1000 g/mL stock solution and ofloxacin was available in powder form with a 99% purity.
- Add the calculated quantity of sterile, dry, and clean ofloxacin to thecontainer.

- To make up the volume of the stock solution, add a sufficient amount of solvent to the container. For instance, you would add 10 mL of solvent tocreate a 10 mL stock solution.

- Until the ofloxacin powder is fully dissolved in the solvent, thoroughlycombine the contents of the container.

- Include the relevant details on the label for the container, such as the compound's name, concentration, and preparation date.

- Depending on how soluble Ofloxacin is, a certain solvent will be chosen tomake the stock solution. For stock solutions, water, methanol, and ethanol are often used solvents.

PREPARATION PROCESS OF STOCK SOLUTION OF NORFLOXACINANTIBIOTIC:

- Establish the quantity of Norfloxacin needed to create the stock solution. For instance, you would need to weigh out 10 mg of Norfloxacin (1000 g/mL x 10 mL / 0.99) if wanted to manufacture a 1000 g/mL stock solution and the Norfloxacin was available in powder form with a purity of 99%.

- Norfloxacin should be added in the calculated quantity to a sterile, clean,and dry container.

- To make up the volume of the stock solution, add a sufficient amount of solvent to the container. For instance, you would add 10 mL of solvent tocreate a 10 mL stock solution.

- Thoroughly combine the contents of the container until the Norfloxacinpowder is fully dissolved in the solvent.

- Include the relevant details on the label for the container, such as the compound's name, concentration, and preparation date.

- Norfloxacin's solubility will determine the solvent to be used to make the stock solution. Typically, water, methanol, and ethanol are utilized as stocksolutions' solvents.

PREPARATION PROCESS OF STOCK SOLUTION OF OFLOXACINANTIBIOTIC:

- Determine how much ofloxacin is needed to create the stock solution. Forinstance, if the ofloxacin is 99% pure and is accessible as a powder, you would need to weigh out 10 mg of ofloxacin (1000 g/mL x 10 mL / 0.99).

- To a clean, dry, and sterile container, add the appropriate amount ofofloxacin.

- To make the container's volume equal to the volume of the stock solution, add a suitable amount of solvent. You would add 10 mL of solvent, for instance, to create a 10 mL stock solution.

- As soon as the Ofloxacin powder is entirely dissolved in the solvent, thoroughly combine the container's contents.

- Include the compound's name, its concentration, and the date it was prepared on the label of the container.

- The solubility of ofloxacin will determine the solvent to be used to make thestock solution. Water, methanol, and ethanol are typical solvents used for stock solutions.

PREPARATION PROCESS OF MAKING AN ANTIBIOTIC DISC:

- There are two types of paper available: Whatmann paper and Glass MicroFiber. Antibiotic discs can be made with both the types of paper available.

- Cut out the papers in the form of round discs with a punching machine fromthe sheets of Whatmann

and Glass Micro Fiber.

- Collect all the round discs of paper cut out and segregate the Whatmann andGlass Micro Fiber pieces of paper seperately in various containers sealed tightly.

- Use clean and sterile petridishes, wipe the lid and the bottom of petridishthoroughly with ethanol.

- Transfer the cut out paper discs of whatmann and GMF in separate to the lidof the petridish carefully.

- Once the paper bits are kept in the lid close the petridish with the bottomkept over the lid.

- Keep all the petridishes for autoclave with a beaker and petridishes to bekept on top of the beaker for autoclave.

- Once the autoclave is over, wait for the temperature to cool down.

- Remove the lid and separate the paper discs seperately in each petrdish and keep it in Furnace, Infrared or Dryer so that the excess water gets dried before the stock solution is been transferred.

- Once the paper discs are dried, prepare the stock solution required forpreparing various antibiotics.

- The paper discs are to be arrranged in rows and columns kept over aspreaded parafilm for the stock solution to be transferred.

- Using a 10 microlitre pippete, transfer 6 microlitre of stock solution to eachpaper bit seperately for NFX, LVX and OFX.

- Once the stock solution is been transferred to the paper disc, carefully markthe petridishes with the antibiotic name and date of preparation.

- Transfer the petridishes to the incubator maintained around 37 degree celsiusand let it be kept inside the incubator for around 2 hours of duration.

- Once kept inside the incubator for 2 hours, transfer the Antibiotic discs to a container marked carefully with name of antibiotic, date of preparation and keep the antibitoics stored in a 4 degree celsius fridge.

RESULTS:

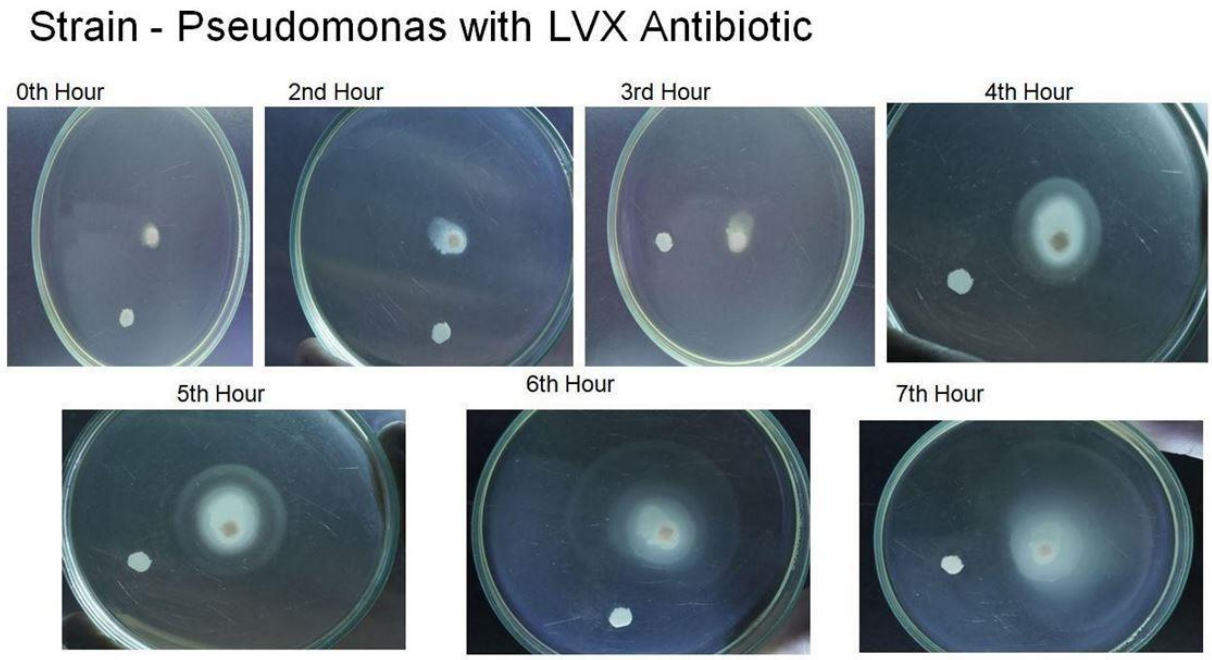


Fig 4- The result of Pseudomonas cell growth on 0.2% agar media with LVX antibiotic.

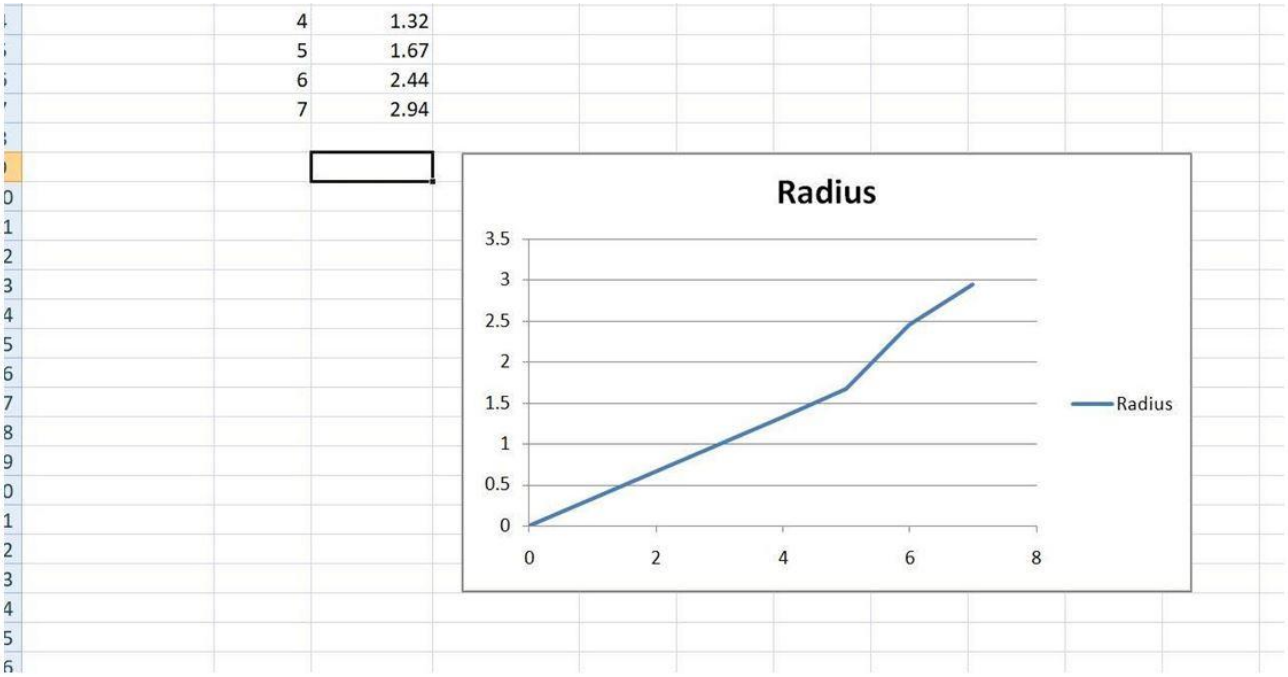


Fig 4.1-From the experimental results, using ImageJ, the radius of outer circle formed of Pseudomonas strain away from LVX antibiotic is calculated and the graph is plotted using radius value. X axis measures Radius and Y axis measures time.

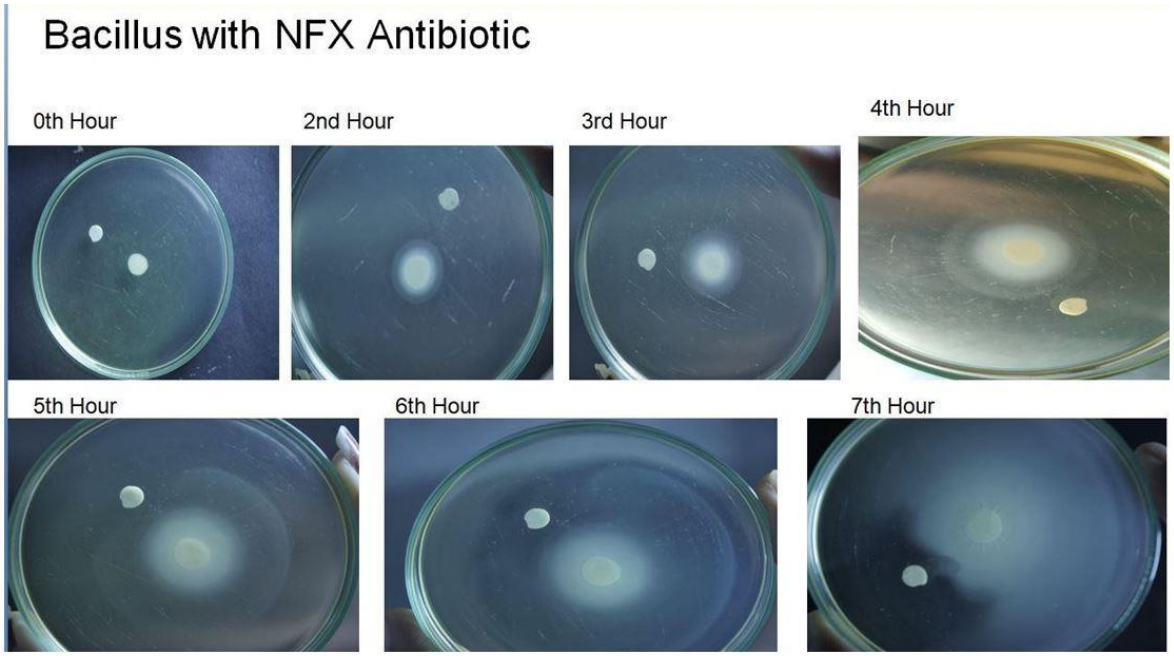


Fig 9- **The result of Pseudomonas cell growth performed on 0.2% agar media in the presence of OFX antibiotic is observed starting from 0th hour till 7th Hour. As cells moves towards the antibiotic, "Zone of Inhibition " can be observed.**

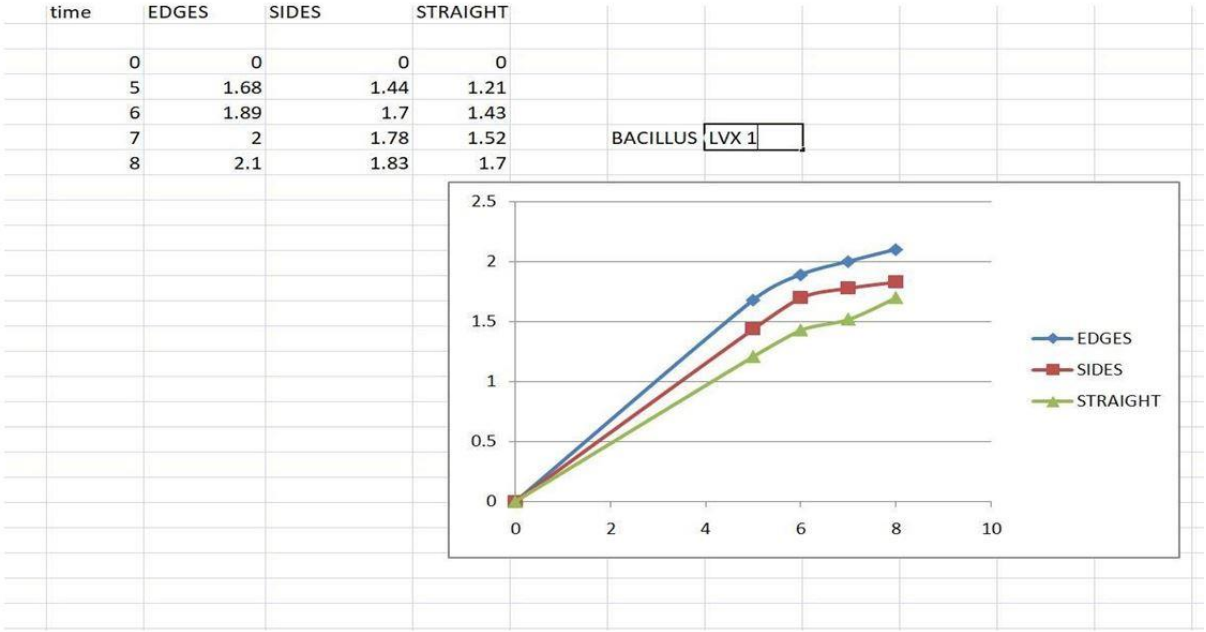


Fig 17.2- From the experimental results, using ImageJ, the radius of circle formed Bacillus towards the LVX antibiotic is calculated and the graph is plotted using edges, sides and straight values.

DISCUSSION:

Cells can chemotactically migrate in either direction along a chemical gradient. Chemotaxis enables microorganisms to migrate in either a nutrient- or toxin- removal direction. Bacteria have unique receptors on the surface of their cells thatcan track changes in the chemical composition of their surroundings. The flagella of the cell get this information from these receptors, and they can subsequently alter their rotation to move the cell in the desired direction.
To assess a bacterial strain's resistance to antibiotics, researchers frequently utilizethe soft agar plate method. A soft agar medium is used to suspend the bacterial cells, and they are then placed on a solid agar plate that has been pre-treated with the desired antibiotic. On a solid agar plate, if the bacterial strain is susceptible to the antibiotic, it will not grow there. On the other hand, if the bacterial strain is resistant to the antibiotic, it will be able to thrive while being present on the solid agar plate. The minimum inhibitory concentration (MIC) of an antibiotic for a specific bacterial strain can be found using the soft agar plate method. To do this, aseries of soft agar plates with progressively higher antibiotic doses are prepared, and the bacterial growth on each plate is then observed. The experiment is done in 0.2% agar media, as the cells find it easy for chemotaxis movement. The chemotaxis movement takes place because of difference in gradient in medium.

While the soft agar plate method enables researchers to ascertain whether bacterial strains are resistant to antibiotics, chemotaxis enables germs to move toward or away from chemical gradients. The soft agar plate method was used in the above experiments to study the behavior of the whole population. Microscopy studies areused to observe the behavior of individual cells. Using the results, ImageJ softwarehas been used to find the expansion speed of the motile (wild type) cells and antibiotic susceptible cells. Expansion speed is used as a Parameter for studying the behavior of bacterial population. Capillary assay can also be used as a parameter for the study of bacterial population behavior. The adaptation time in the capillary assay may be shorter compared to the soft agar plate method because the cells are grown in a more controlled and defined environment. However, the soft agar plate method may require a longer adaptation time as the cells need to adjust to the new environment and establish cell-cell and cell-matrix interactions.

CONCLUSION and FUTURE PROSPECTS:

For the study of bacterial movement and chemotaxis, the soft agar plate method is frequently utilized. It gives bacteria a semi-solid surface that enables movement and growth while keeping their structure, making it a helpful tool for analyzing bacterial activity. The technique makes it possible to analyze how well bacteria can move toward or away from antibiotic gradients, which makes it particularly helpfulfor identifying antibiotic-resistant bacteria. With the advent of antibiotic-resistant types of bacteria, soft agar plate method is becoming more significant than ever in the area of microbiology.

The use of the soft agar plate method to identify and research antibiotic-resistant bacteria has the potential to be further developed as antibiotic resistance becomes agrowing concern in global health. Research can be done to examine how the technique can be utilized to screen possible novel antibiotics and find new targets for drug development. Additionally, the use of artificial intelligence and machine learning techniques can be incorporated to improve the accuracy and efficiency of the method. The soft agar plate approach has the

potential to develop into an even more effective weapon in the battle against antibiotic resistance with further study and development. The expansion speed of motile (wild type) and antibiotic resistance cells are found using ImageJ. The values will be integrated to a lab on chip device by developing an algorithm and feeding chemotactic velocity values inLab on Chip device. All the values obtained from the experiment are considered tobe as “Test values”. The device will be able to find about the antibiotic concentration and the type of cell based on the values fed to Lab on chip device through the algorithm. Based on the known antibiotic concentration, we can prescribe the medicine. For Samples like blood sample, algorithm will be used to find the result. Based on test values feed in to the Lab on chip device, algorithm is developed to predict other values.

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