

**Biofilm Formation.****What is a Biofilm?**

It is widely known that bacteria form colonies. However, when found in certain numbers and densities, the bacterial colonies become a different class of colonization. A biofilm is an arrangement of bacteria cells attached to a surface through secretions which protect the cells within it. This density and arrangement actually protects the cells within the colony, or aggregate, and the surfaces they attach to supply nutrients needed for growth and cell function.

Bacterial biofilms can be found in many places on earth and in the human body. The leaf litter in forests like the Amazon, riverbeds , and hot springs in Yellowstone National Park are all habitat for bacterial and microbial biofilms. Biofilms can also be found in the Arctic and desert regions. Treated wood, plastics, the bottom of boats and trash could also be suitable surfaces for microbial biofilms. This is because all biofilms need are surfaces in which water and nutrient sources are found together.

This is why the mucous, teeth and skin of humans can also support biofilms. The thin coating of slime felt on teeth in the morning or the plaque which builds up over time are examples

of biofilms in humans. Recent studies have also shown biofilms can develop on the skin and are dependent on the environment a person lives in, as surfers have been shown to have completely different biofilms on their skin than non-surfers.

The slime seen on the surface of the water is actually what is known as a biofilm.

### **Biofilm Formation**

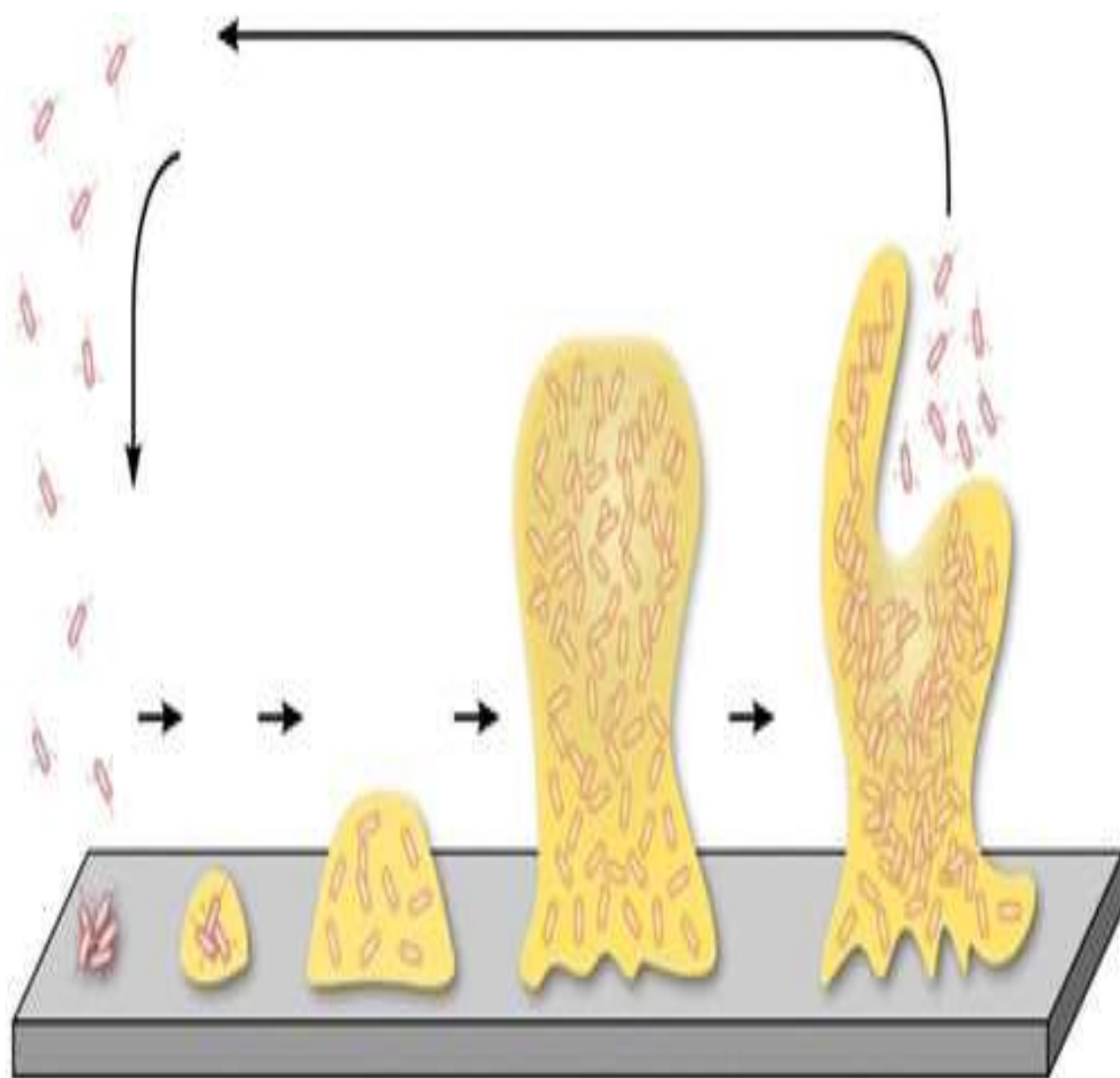
Biofilms form within a singular species, or by the cooperation of multiple species, and form a specialized matrix complex in which all bacterial and microbial cells work together. The formation of the biofilm is quite simple:

- 1- Free-floating bacteria land on a surface that has water and nutrients to promote reproduction and survival.
- 2- A sugary substance containing both polysaccharides and proteins which allow it to adhere to the surface, much like biological super glue. This substance is called an extracellular polymeric substance, or EPS, and is strands which help with the adhesion of the bacteria.
- 3- The EPS also forms channels in the matrix to allow nutrients and waste to pass through, as well as chemical signaling for communication.
- 4- As long as the surface remains hospitable, the biofilms will reproduce and grow, adding to the matrix.

**As stated before, biofilms are more complex than they appear. Communication signals pass from cells and as the biofilms have access to nutrients and space, they can become thick visible plates of slime, plaque or sheens on the soil, teeth or water, respectively. If given enough time and transplantation is available, biofilms can break off and transplant to other areas when given the means.**

### **Structure**

**Structurally, many biofilms are the same. Each is composed of colonies of cells which adhere to suitable surfaces through use of the EPS. These are made of polysaccharides, proteins, lipids and sometimes DNA. Biofilms are also, like much life on earth, mainly water.**



Stage 1. Adhesion, aggregation and biofilm formation

Stage 2. Growth and maturation

Stage 3. Dispersion

## **Biofilm in Infections**

Biofilm infections are more a part of everyday human life than originally thought. Some common biofilm infections which effect humans are:

\*Contact lens infections, from "coatings" which are actual biofilms

\*Gingivitis, common dental infection which includes bleeding gums

\*Inner ear infections

\*Urinary tract infections

Biofilms can also form on medical equipment if not properly sterilized, causing complications after surgery if used on a patient. They have also been known to grow in catheters and cause clogging and infection as well.

## **Why Are Biofilms Important?**

Biofilms may cause unwanted illness at times, but they are important parts of ecosystems and the microbes themselves. For the species living in the biofilm, these structures give:

- Communication
- Habitat
- Nutrition

- **Protection - both from other microbes and antibiotics**

**In the large ecology of an area, biofilms also remediate polluted soil and water. An important tool in cleaning oil spills has come from the use of biofilms which can break down the oil, therefore biologically removing it from the environment. This is known as bioremediation. Biofilms also help in nutrient recycling, as they are an important part of the decomposition process.**

### **What is a biofilm and how is it formed?**

**A biofilm is a community of bacteria colonizing a surface and acting as a multicellular organism. Biofilms are formed when a bacterial cell is able to attach to an appropriate surface and begins to reproduce. The reproduced bacterial cells help to create the more complex organism.**

### **What are biofilms in the body?**

**Biofilms in the human body can be both helpful and harmful. Common places to have biofilms are teeth (plaque build up), surface of the skin, and in mucus. Biofilms can be introduced during surgical implantation of objects, which is why surgery rooms and equipment are sterilized.**

## What is the purpose of biofilm?

Biofilms are created by bacterial cells and give protection against antimicrobial and immune responses. These complex matrixes allow bacterial colonies to live longer in place. Dental plaque is an example.

## The Detection of Bio-film formation

Biofilms, which are microbial communities encased in a self-produced matrix, can be detected using a variety of methods, broadly categorized as **direct** and **indirect**, and also including **microscopic** and **non-microscopic techniques**.

### Direct Detection Methods:

- **Microscopy:**
  - **Light Microscopy:** A basic and cost-effective method for visualizing biofilm structure and estimating microbial attachment.
  - **Fluorescence Microscopy:** Utilizes fluorescent dyes or stains to highlight specific biofilm components (e.g., extracellular matrix, bacteria) or to differentiate between live and dead cells.

- **Confocal Laser Scanning Microscopy (CLSM):** Provides high-resolution, three-dimensional images of biofilms, allowing for detailed analysis of their structure and composition.
- **Scanning Electron Microscopy (SEM):** Offers high magnification and depth of field, revealing fine details of the biofilm's surface and internal structure.
- **Atomic Force Microscopy (AFM):** Enables visualization of biofilm topography at the nanoscale, providing information about surface roughness and mechanical properties.

- **PCR Detection:**

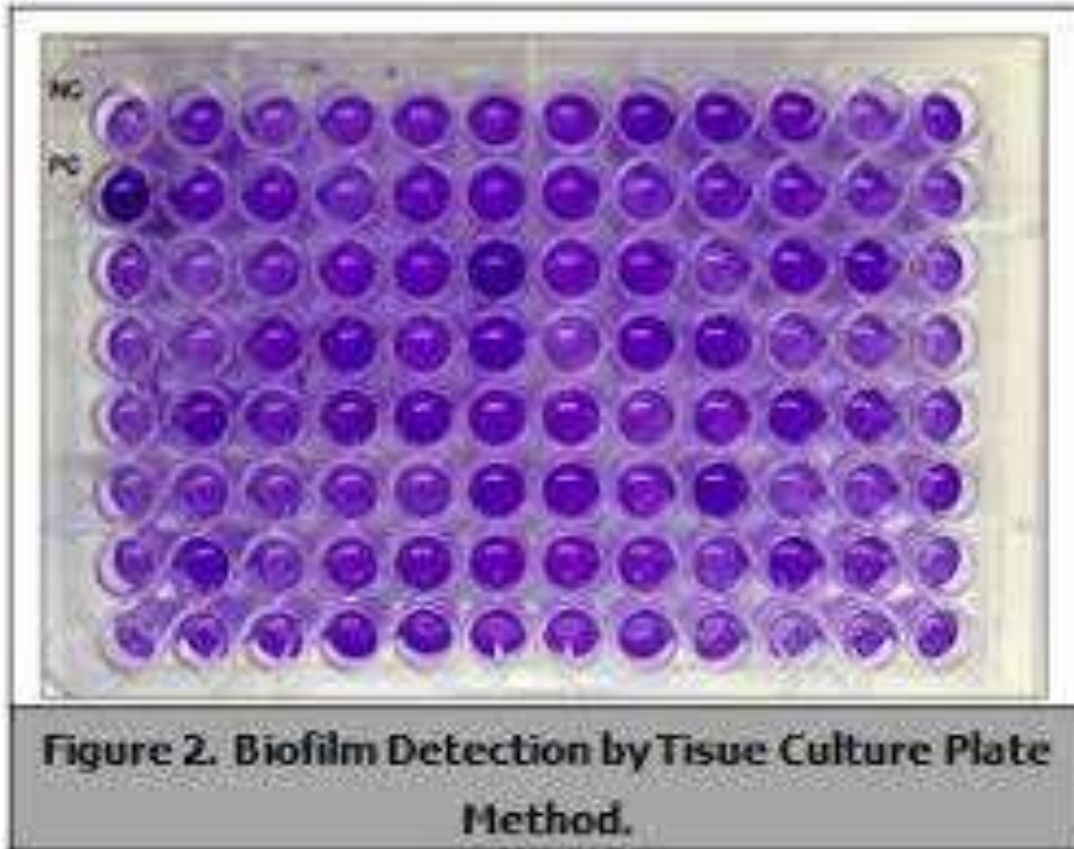
Can identify specific genes associated with biofilm formation, such as *icaA* and *icaD*.

- **Biofilm Matrix-Degrading Enzymes:**

Assaying for enzymes that break down the biofilm matrix can indicate the presence and activity of biofilms.

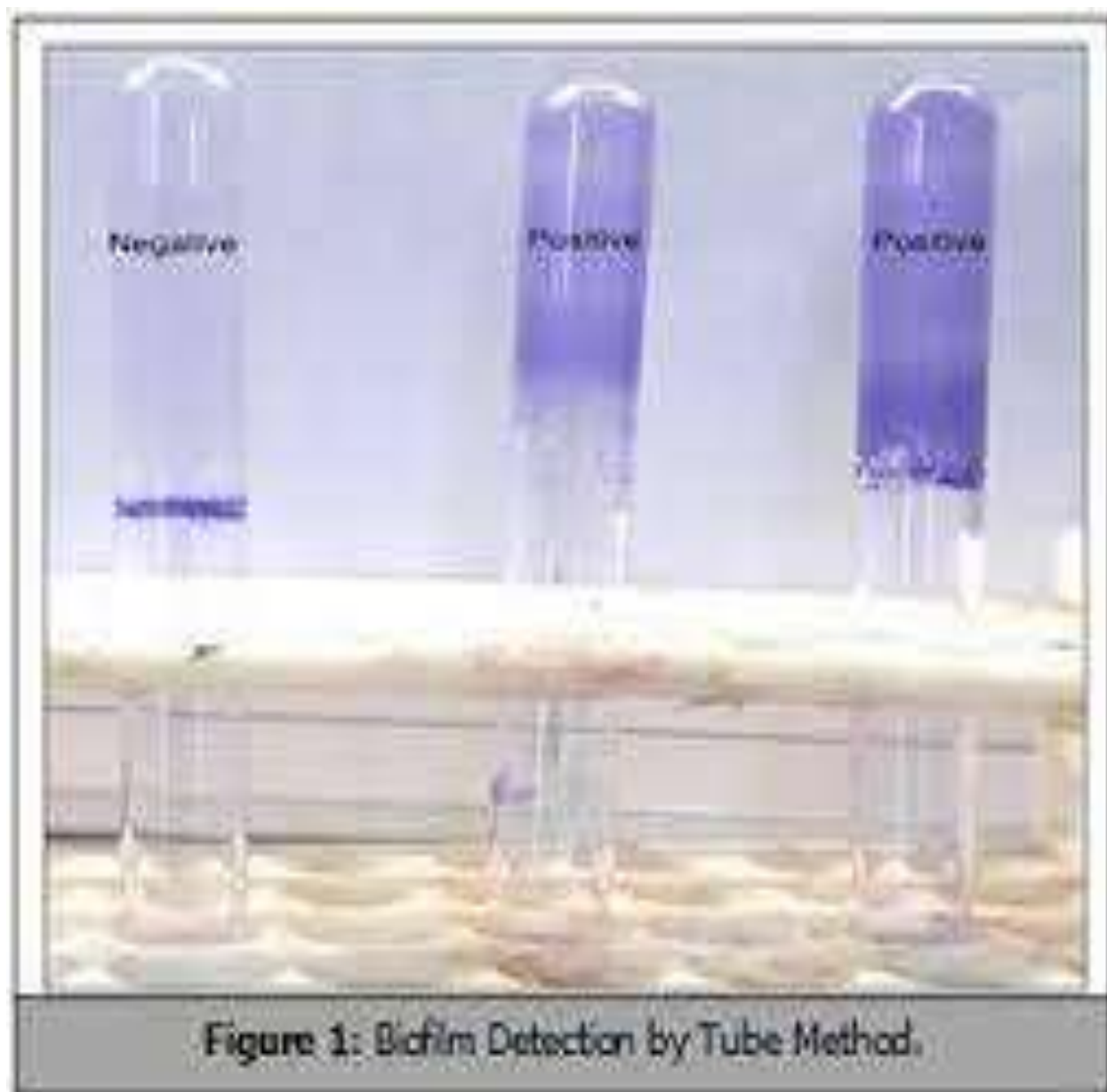
- **Tissue Culture Plate (TCP) Method:**

A widely used **quantitative method** where bacteria are grown in a microtiter plate, stained with crystal violet, and quantified by measuring the optical density.



- **Tube Method (TM):**

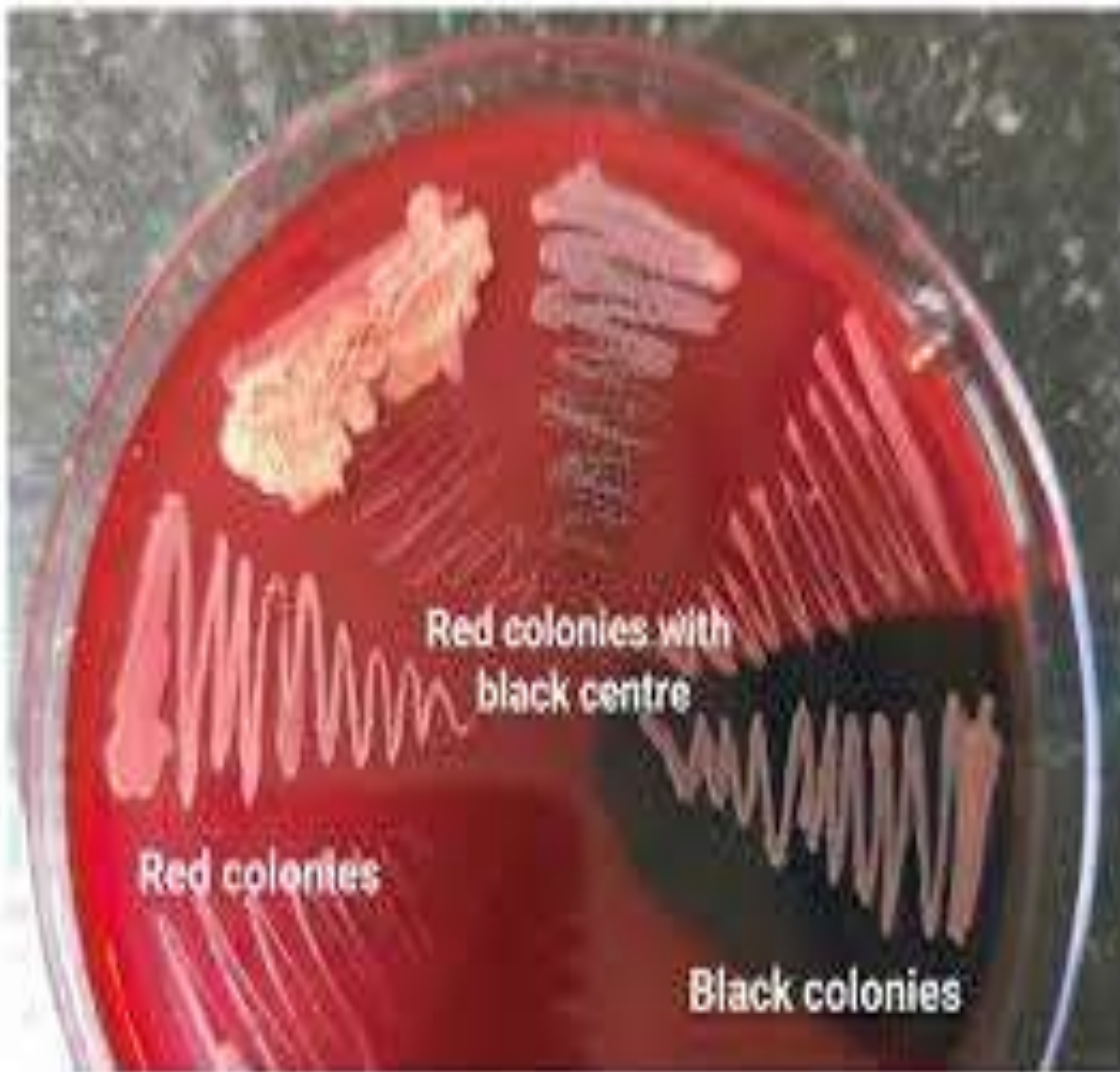
Another **quantitative method** where bacteria are grown in tubes, stained with crystal violet, and visually assessed for biofilm formation.

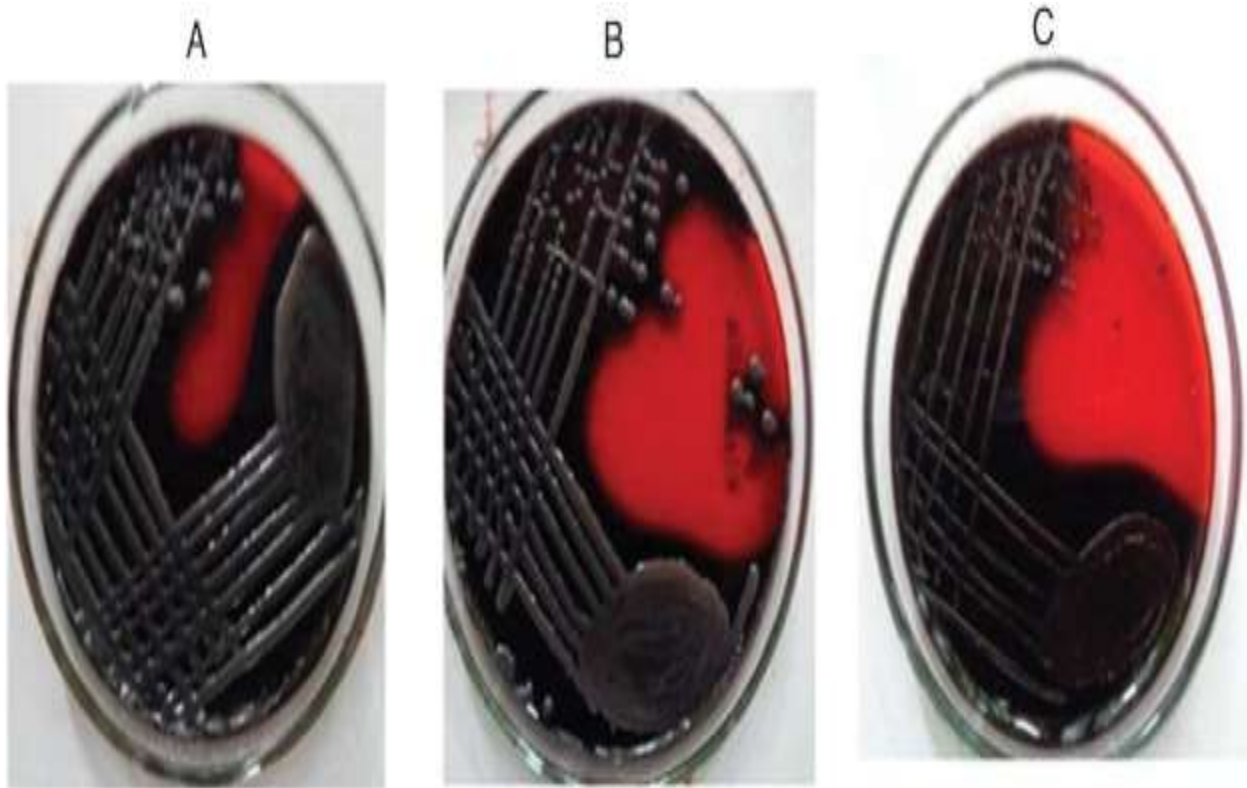


## Indirect Detection Methods:

- [Congo Red Agar \(CRA\) Method:](#)

A **qualitative method** that uses a specific agar medium to identify biofilm-producing bacteria based on colony morphology.





- **Antimicrobial Susceptibility Testing:**

Biofilms exhibit increased resistance to antimicrobial agents, so testing susceptibility can indirectly indicate biofilm formation.

- **Bioluminescent Assays:**

Measure bioluminescence produced by bacteria in a biofilm, allowing for quantification of biofilm formation.

- **Piezoelectric Sensors:**

Measure changes in electrical impedance caused by biofilm formation, providing real-time monitoring capabilities.

- **Flow Cells:**

Allow for the study of biofilm formation and behavior under dynamic flow conditions.

- **Rotating Biofilm Reactors:**

Used to simulate biofilm growth in various environments and assess the effectiveness of different treatments.

Other Considerations:

- **In clinical settings:**

Tissue biopsies can be used to detect biofilms in wounds, but their small size and uneven distribution can make them difficult to find.

- **Real-time detection:**

Techniques like optical, mechanical, and electrochemical methods can provide valuable insights into biofilm properties and behavior.

## **\*\*procedure**

### **The Congo Red Agar Test**

The obtained bacterial strains were inoculated into CRA and incubated for 24-48 hours at 37°C.

BHI broth was supplemented with 5% sucrose and Congo red stain to make CRA. BHI (37 g/L), Sucrose (50 g/L), Agar (10 g/L), and Congo red stain (0.8 g/L) medium Congo red was prepared as a concentrated aqueous solution and autoclaved separately from the other medium constituents at 121°C for 15 minutes before being added when the agar was cooled to 55°C. When black colonies with a dry crystalline consistency were present, isolates were considered strongly positive. A moderately positive biofilm producer was indicated by a darkening of the colonies in the absence of a dry crystalline colonial morphology. Non-biofilm producers were identified as colonies that remained pink. As a positive control, a lab-confirmed biofilm producer strain was used.