Bacterial Enemies of Human Health

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Edited by

Ajay Kumar Prajapati

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In Loving Memory of My Beloved Mother

Late Smt. Pyari Devi
(1st January, 1948 to 18th January, 2024)

Dr. Ajay Kumar Prajapati
Editor

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PREFACE

As we know knowledge in clinical microbiology is necessary to understand the pathogenesis of infectious diseases. Clinical microbiology is a vast subject. That includes bacteria, viruses, fungi, and Parasites. The present book is all about pathogenic bacteria. In this book, we have tried to explain the classification, habitat, pathogenesis, disease, diagnosis, and treatment of pathogenic bacteria. The book, "Bacterial Enemies of Human Health" is written by eminent professors, research scholars, and medical doctors. In this book, which consists of twenty-three chapters, the readers will obtain valuable information regarding pathogenic bacteria in Human beings. Dr. Jyotsana Singh discussed the identification of bacteria. Dr. Sheetal Goenka explained gram-negative bacteria. Kanchan Bahuguna explained about Escherichia coli. Jagriti Bansal discussed Pseudomonas. Varshni R.K., Dr. Maheswary Datchanamoorthy, Dr. Anusha Gopinathan, Pittala Kiranmai, and K.V. Leela explained about Klebsiella pneumonia. Dr. Sshrutkirti Gupta discussed about Proteus. Dr. Vidhi Arora explained about Serratia marcescens. Prof. (Dr.) Saba F. Hussain discussed about Neisseria gonorrhoeae. Dr. Maheswary Datchanamoorthy, Varshni R.K, and K.V Leela explained about Neisseria meningitidis. Neelofar Shaik explained about Acinetobacter baumanii. Rajal Pranav Dave discussed Haemophilus influenza. Rajal Pranav Dave and Ajay Kumar Prajapati explained about Gram Positive Bacteria. Dr. Saumya Singh discussed Streptococcus. Jagriti Bansal explained about Staphylococcus. Shahin Ansari discussed Enterococcus. Pittala Kiranmai, Anusha Gopinathan, Maheswary D, and Varshini RK explained Corynebacterium diphtheriae. Neha Pandey explained about Clostridium. Rajal Pranav Dave and Ajay Kumar Prajapati discussed Listeria. Mycobacterium tuberculosis-pathogenesis and recent advances in diagnosis of TB is discussed by Dr Roobhini Sri N S K. Dr. Sshrutkirti Gupta discussed Antibiotics. Antimicrobial Resistance is explained by Rajvinder Kaur. Future-Proofing Healthcare: Artificial Intelligence (AI) strategies for Antimicrobial Resistance (AMR) Management are discussed by Rajhi Hayfa, Vidhya K S, Achal Shetty, Dr. Harish Rangareddy, and Venkateshappa C. Antimicrobial Resistance and Hospital Infection and Control is discussed by Dr. Sonali Choudhari.

I hope readers will gain valuable knowledge.

Ajay Kumar Prajapati

ACKNOWLEDGMENTS

The successful development of the book *Bacterial Enemies of Human Health* is the result of the collective efforts of all the fellow contributors. I would like to express my deepest gratitude and appreciation to **Cambridge Scholars Publishing** for allowing me to publish.

First and foremost, I extend my heartfelt thanks to all the authors for their wonderful contributions. I am deeply grateful to our loved ones for their emotional support, which played a vital role in the success of this project. Finally, I want to acknowledge the incredible journey itself. Without the collective contributions of these remarkable individuals, this ambitious project would not have been possible.

Thanks to everyone who has been a part of this amazing journey.

Ajay Kumar Prajapati

CHAPTER 1

IDENTIFICATION OF BACTERIA

JYOTSANA SINGH

Introduction

Bacteria are microscopic, single-celled creatures. Nearly every place on Earth has bacteria, which are essential to the planet's ecosystems. Certain species can withstand extremely high and low temperatures and pressures. It's estimated that there are more bacterial cells in the human body than human cells, although the body is full of germs. Most microorganisms in the body are benign, and some may even be beneficial. Only a tiny percentage of species are known to cause illness. Microorganisms with a variety of forms are called bacteria. They may take the form of spirals, spheres, or rods. While some bacteria are harmful and classified as pathogenic, other beneficial bacteria do not cause disease. For example, the bacteria in our stomach and digestive tract are essential to maintaining proper bodily functions. The intriguing thing about bacteria is that they make up 10 times more cells in our body than human cells do. In biotechnology, bacteria are also significant. They are also important because, once more, they will support the body's natural health maintenance.

Importance of Identification Bacteria

For accurate disease diagnosis, effective infection treatment, and the tracking down of disease outbreaks linked to microbial infections, precise and conclusive microorganism identification, including bacterial identification and pathogen detection, is crucial. Bacterial identification applications include environmental research, criminal investigations, microbiological forensics, and bioterrorism threats. The clinical diagnosis process necessitates the identification of a bacterial pathogen. After the bacterium or causal agent has been identified, treatment can only be

started. The morphology, physiology, and biochemistry of different bacterial species vary. Many morphological, physiological, and biochemical tests are therefore carried out to identify the specimen.

Conventional techniques for bacterial identification

1. **Macroscopic Identification:** The shape, size, colour, and smell of a microbe are all considered to be part of its macroscopic features, or the aspects that are visible to the unaided eye.

A. Examining Agar Cultures

- The texture of bacteria can range from slimy to dry, and they frequently form discrete colonies that are occasionally smaller than fungal colonies. They are coloured in shades ranging from white to vivid crimson.
- Unlike filamentous fungi, which might have an earthy or odourless scent, bacteria can have a strong odour.
- Filamentous fungi and molds develop into "hairy," irregularly shaped colonies and frequently release visible spores that might have a dusty or powdery appearance. Fungal colonies can have many colours; typically, the darker colour is concentrated in the centre, which is frequently elevated, and the lighter colour is diffused outward. The youngest filamentous fungi grow outward from the centre of an agar plate, while the older, darker material inside that is rich in spores is the result of radial growth. It is also possible for filamentous fungi to grow as a monochromatic, fuzzy mat devoid of any visible spores.

These are some common types of culture media used in modern microbiology laboratories:

a) **Nutrient Agar media** – This kind of nutrient medium is the most widely used; it is non-selective and supports the growth of many different species. These include aerobic, anaerobic, and microaerophilic microorganisms.
b) **Selective Agar**: This particular variety of agar selectively grows gram-positive bacteria only, preventing the growth of other bacteria. An example of this would be mannitol salt agar.
c) **Differential Culture Media** – This type of medium is employed to distinguish between different bacteria. For instance, blood agar is frequently used to identify bacteria, such as *Streptococci*, that cause blood haemolysis.

B. Microscopic Features

With the ability to examine the bacteria under a microscope, you can recognize them by a broad range of physical traits. The primary method for doing this is examining their size and form. Cocci, bacilli, and spiral are the three primary forms, though there are many more variations.

- **Cocci –** The most prevalent kind of bacteria are these. They are called cocci because of their spherical shape, despite frequently occurring in groups. As an example, diplococci are cocci that are paired, *Streptococci* are cocci that are in chains, and *Staphylococci* are cocci that are in clusters.
- **Bacilli** – Similar to cocci, these rod-shaped bacteria can be found alone or in groups. For instance, two bacilli close to one another are called diplobacilli, whereas chains of bacilli are referred to as *Streptobacilli.*
- **Spiral** – These are just microorganisms with a spiral form. Examples are spirochetes, which are thin, flexible spirals, and spirillium, which are thick, resilient spirals.

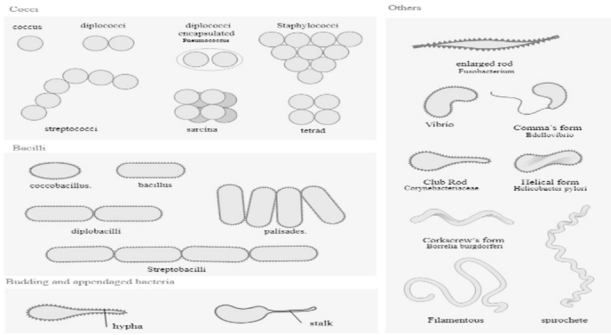


Fig1: Bacteria Morphology

C. Staining Identification

In the field of microbiology, staining is a method used to highlight and draw attention to a biological material at the microscopic level. For histological investigations and diagnostic purposes, the specimen is highlighted at the microscopic level using dyes and stains, allowing for higher-magnification research. The staining technique is one of the methods available for identifying microorganisms.

I. **Simple Staining:** By measuring the size, shape, and arrangement of the cells, the simple stain can be used to quickly and easily identify bacteria. As its name implies, the simple stain is applied very simply and requires only one stain solution. Basic dyes used to dye bacteria include methylene blue, safranin, and crystal violet. Hydroxide- or hydrogen-bearing stains easily release or take up hydroxide or hydrogen ions, leaving behind positively charged stains. Because the cytoplasm and majority of bacterial cells are negatively charged, these positively charged stains stick to them easily. After staining, the bacterial cells' morphology that is, their arrangement and shape will be examined.

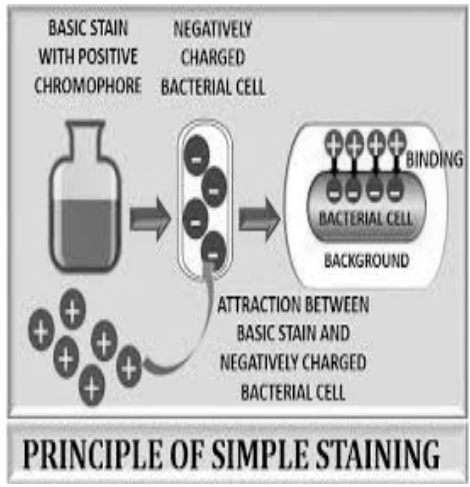


Fig 2: Simple Staining

II. **Gram staining:** Physician Hans Christian Gram began researching the causes of respiratory illnesses like pneumonia in 1884. Using a staining technique he invented, he was able to identify a bacterium in lung tissue removed from patients who had passed away as the causative agent of a deadly form of pneumonia. At an autopsy, the Gram's stain procedure greatly simplified the process of determining the cause of death, even though it offered minimal treatment for the disease. Gram's staining techniques are still used today to help identify bacteria. First, the bacteria are classified into two groups based on their initial characteristics: gram-positive or gram-negative. The ability of certain bacterial cells to withstand a decolorization procedure and preserve a primary stain (crystal violet) accounts for

the differential nature of Gram's stain. The process of Gram's staining has four steps. Cells are first stained with crystal violet, and then iodine is added as a stain-setting agent. After that, the stain is only selectively removed from the gram-negative cells by using alcohol. The decolorized cells are then counterstained pink with the addition of safranin, a secondary stain. Their cell walls are the primary distinction between these two kinds of bacterial cells, even though Gramme was unaware of it at the time. An exterior membrane known as the envelope is present on gram-negative cell walls, and it dissolves during the alcohol wash. As a result, the crystal violet dye can escape. The pink dye safranin is only taken up by the decolorized cells, which explains the color difference between the two cell types. Gram-positive cells show up purple after the Gram staining process, while Gram-negative cells show up pink.

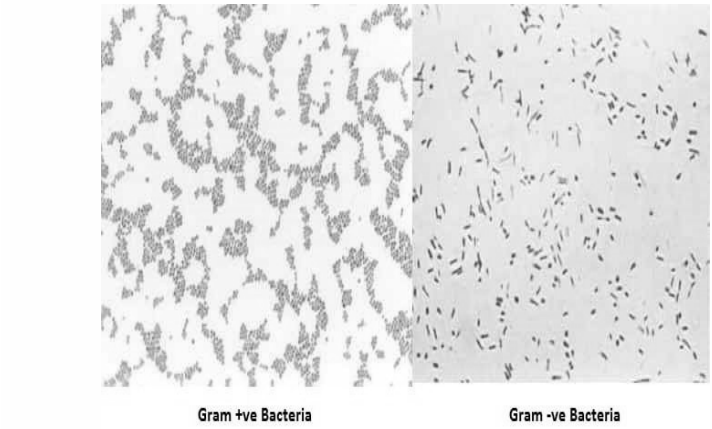


Fig 3: Gram staining

III. Capsule staining - To differentiate capsular material from the bacterial cell is the primary goal of capsule staining. A bacterial cell's outer covering, known as a capsule, is made of gelatine and clings to the cell wall. Although polysaccharides make up the majority of capsules, polypeptides can also be found in some. The capsule is a distinct layer outside the cell wall that is thick and noticeable, unlike the slime layer that most bacterial cells produce. The capsule stain uses a basic stain and an acidic stain to identify the formation of capsules. Using simple staining chemicals on capsules yields very poor results, and the term "capsule stain" can be misleading depending on the technique employed because the capsule may or

may not be stained**.** Negative staining techniques produce a contrast between stained cells and an unstained capsule on a translucent, darker-colored background. Using India ink, nigrosine, or Congo red, the background is created. These days, it's hard to find Indian ink, but it's easy to get nigrosin. A mordant that precipitates the capsule is needed for a positive capsule stain. When counterstains such as methylene blue or crystal violet are used, the dye is absorbed by the bacterial cell wall. When their pigmented cells are set against a dark backdrop, capsules appear colorless. Because they are delicate, capsules can be reduced in size, dried out, warped, or even destroyed by heat. To increase the capsule's size and facilitate easier observation with a standard compound light microscope, a drop of serum can be added during the smearing process.

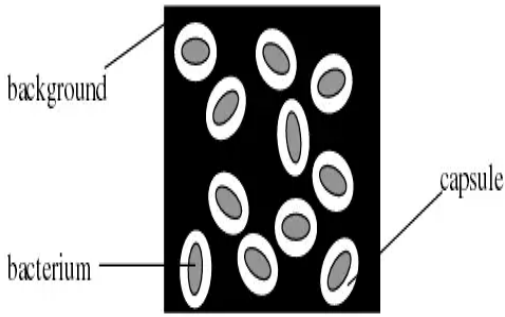


Fig 4: Capsule Staining (*microbugz)*

IV. **Endospore Staining:** Scientists Cohn and Koch conducted the first study on endospores in 1876 (Gould, G. W., 2006). It was discovered that basic stains like methylene blue, safranin, and carbol fuchsin could not stain endospores. These scientists and a few others determined that spores were resistant to heat and dormant. Researchers were looking for several ways to improve the sickness and infection caused by these endospores in the early 1900s (Gould, G. W., 2006). In bacteriology, endospore staining is a method used to determine whether endospores are present in a bacterial sample (Microbiology an Introduction, Tenth Edition; Tortora Funke Case) Endospores are protective structures found in bacteria that let them withstand harsh environments, such as high temperatures and strong chemical resistance (Leboffe and Michael, 2015). Endospores may be quiescent because they contain little to no ATP. Endospores are shielded from nucleic DNA and other modifications by their hard

outer layer, which is composed of keratin. Because endospores can regerminate into vegetative cells, they have a protective quality that makes it challenging to stain them with standard methods like Gram's and simple staining. The Moeller stain and the Schaeffer-Fulton stain are two unique methods for endospore staining. By heating the bacteria, a primary stain containing malachite green is pushed into the spore in the Schaeffer-Fulton staining technique. To stain the spores with malachite green, leave them on the slide for at least fifteen minutes. Because of the spores' bulk, heat serves as a mordant in this differential stain because it takes a long time for them to stain.

Since malachite green is soluble in water, it can be used to decolorize vegetative cells and spore mother cells. Then, they can be counterstained with 0.5% safranin ("UW System Authentication Redirector". wayf.wisconsin.edu. 2021-11-11). Ultimately, an accurate smear would display the endospore as a green dot inside a cell that is either pink or red (Leboffe, Michael, 2015).

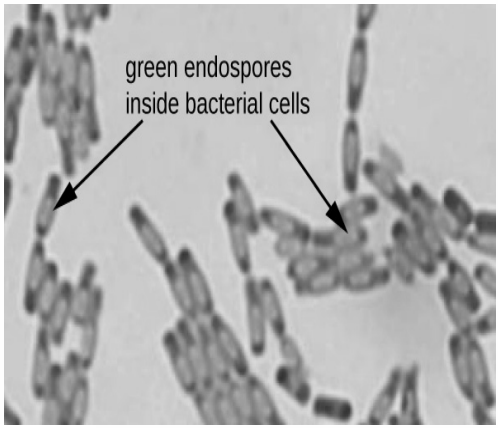


Fig5: Endospore Staining

V. **Acid-Fast Staining** A differential stain, known as an acid-fast stain, is used to identify acid-fast organisms, such as those in the genus *Mycobacterium*. Acid-fast microorganisms have practically impermeable, wax-like cell walls; they also have a high concentration of complex lipids, fatty acids, and waxes, as well as mycolic acid. Since this kind of cell wall is resistant to most substances, acid-fast microorganisms need a unique staining method. The bacterium is said to be acid-fast when it can withstand

being decolored by acid-alcohol. The main genus *Mycobacterium* has large amounts of the lipid mycolic acid in its cell walls. Even though they are tough to stain, once the stain penetrates the cell wall, the cell will resist easy decolorization or staining. The bacteria are considered acid-fast when they can withstand decolorization by acid-alcohol. It is made easier for the dye to enter the bacterium's waxy wall by the phenol in carbol fuchsin. Acid-fast bacteria exhibit weak Gram-positive or Gram-variable staining when subjected to the Gram staining protocol. Most often, the acid-fast staining method is used to characterize them. To obtain the principal carbol fuchsin dye within the cell wall, steam is utilized. When it gets in, it stays there; but because the primary dye does not attach tightly to the cell wall, the acid-alcohol decolorizer will remove it from the non-acid-fast cell walls. Carol fuchsine will also be absorbed by non-acid fast bacteria, but since the main dye does not attach firmly to the cell wall, the acid alcohol decolorizer will remove it from the wall. Carbol fuchsin, the main stain used in acid-fast staining, is lipid-soluble and contains phenol, which aids in the stain's penetration of the cell wall. Additionally, helping with this is the infusion of heat in the form of steam. The main stain is more likely to enter the cell when steam is used to break away the waxy coating. After the smear has been rinsed, the stain is removed from all non-acid-fast cells but remains outside the cell walls of acid-fast organisms thanks to the use of an extremely potent decolorizer (acid-alcohol). Methylene blue is the counterstain that is subsequently taken up by the decolorized, non-acid-fast cells. The rare quality that the genera Nocardia and Mycobacterium share—that of being somewhat acid-fast—is acid-fastness. Due to this characteristic, the stain is very useful for identifying diseases, especially leprosy and tuberculosis, that are caused by bacteria that ferment quickly. In patients receiving antibiotic medication, the stain is also used to detect the presence of acid-fast bacteria in lung tissue.

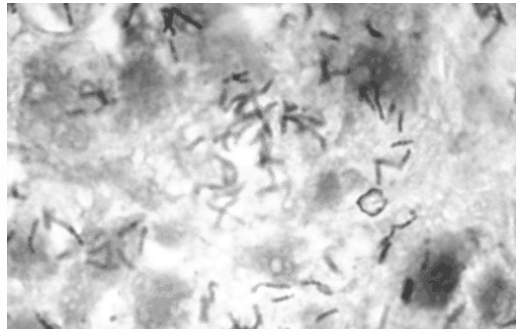


Fig 6: Acid-Fast Staining

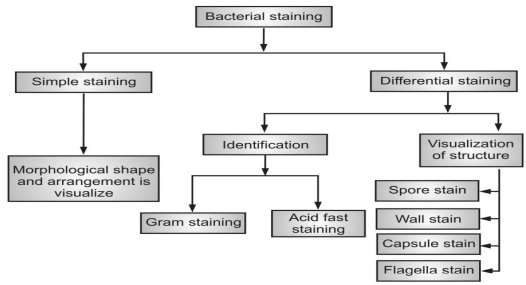


Fig7: Different Staining Methods for Bacterial Identification

2. **Biochemical Identification -** Tests known as "biochemical tests" are those that are carried out on various bacteria to identify them based on their biochemical reactions to various biochemical chemicals.

- Phenotypic identification is typically used in conjunction with biochemical tests, which are one of the more established techniques for identifying microorganisms.
- These techniques have been widely employed for a long time and are still used today, particularly in some laboratory procedures where a specific type of microorganism needs to be quickly recognized.
- Microbes' ability to exploit specific biomolecules and produce organic chemicals that are valuable to them is the foundation of many biochemical tests.
- One of the more conventional techniques that is frequently employed is the straightforward visual identification of the organism's growth in the presence of vital nutrients using an increase in turbidity in the liquid medium.

- In contrast, in other tests, the outcomes are determined by observing how the medium's colour changes in response to variations in its pH.
- Microorganisms can be classified into different groups based on their reactions to such tests. Some tests even allow the distinction of microorganisms at the species level.
- Hence, because biochemical tests are affordable and rather easy to conduct, they are crucial.
- It is possible to differentiate microorganisms based on their differences in physiology, such as bacteria and other types of microbes.
- Nonetheless, there are certain drawbacks to biochemical testing. These procedures are time-consuming and labour-intensive, and results are not visible for many days, even though they are affordable and allow for both quantitative and qualitative information about the diversity of microorganisms present in a sample.
- False positives can occur occasionally, particularly when identical microbial species are taken into account.

The identification of distinct bacterial species is accomplished by a variety of biochemical tests, including the following types:

A) Haemolysis: Hemolysin enzymes, which lyse erythrocytes, are produced by certain types of pathogenic bacteria (RBCS). In vitro, this can be seen on blood agar plates. Hemolysis comes in three different forms:

a) α - hemolysis: *Staphylococcus aureus* and *Streptococcus pyogenes*, for example, have a completely clean circular zone surrounding their colonies as a result of the red blood cell lysis.

b) β- haemolysis: occurs surrounding the colonies of certain bacteria, such as *Streptococcus viridians*, as a greenish zone caused by partial haemolysis of RBCs.

c) γ-haemolysis: There are no visible alterations near the *Enterococcus faecalis* colonies (no haemolysis).

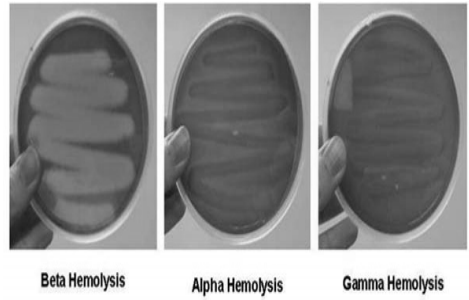


Fig 8: Different haemolysis

B) Catalase production test: Certain aerobic bacteria possess the ability to generate the catalase enzyme, which is responsible for catalysing hydrogen peroxide (H2O) and releasing both oxygen and H2O.

$2O_2 + 2H$ — catalase —> $O_2 + 2H_2O$

Procedure: Using a stick or glass rod, remove a small amount of the bacterial culture to be tested from the nutrient agar and place it on the surface of a clean slide, to which a drop of 3% H2O has been added. The appearance of gas bubbles signifies a successful outcome. If the iron loop is utilized or if the culture medium (Blood agar) contains catalase, a false-positive reaction could result.

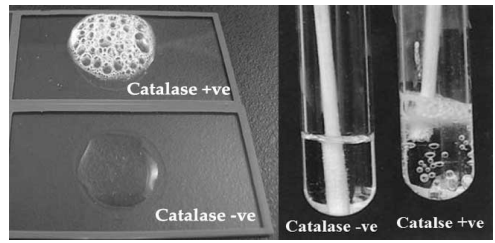


Fig 9: Catalase Test

C) Coagulase production: A coagulase enzyme produced by certain bacteria causes the coagulation of plasma, which is the transformation of soluble fibrinogen protein into insoluble fibrin protein. Coagulase is one of *Staphylococcus aureus*'s virulence factors. An infection brought on by these bacteria will be shielded from phagocytosis by the creation of a clot around it.

a) **Bound coagulase (**detected in the **Slide method):** On a sterile slide, a drop of saline is used to create a homogenous solution of the test organism. This is followed by a drop of pure human or rabbit plasma. As the enzyme will precipitate the fibrin in the plasma on the cell surface, examine it under a microscope and check for clumping, which is a favorable outcome.
b) **Tube method (**detected in **Free coagulase):** This is accomplished by diluting 1 millilitre (mL) of human or rabbit plasma (l:6) in sterile saline with 5 drops of an overnight broth culture of the test organism. The tubes are tilted every hour to check for the formation of clots during the four hours while they are incubated at 37 °C in the water bath. Either the clot will float in the liquid, or the entire plasma will gel.

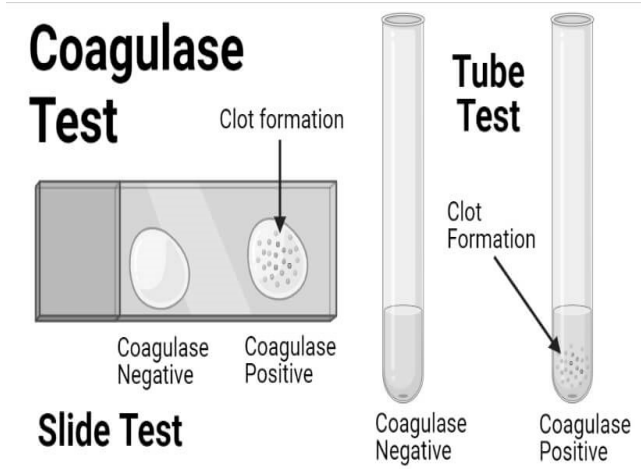


Fig 10: Coagulation Production (https://microbenotes.com/)

D) **Oxidase test**: used to identify the production of cytochrome oxidase, which is produced by exclusively aerobic bacteria such as *Pseudomonas* and *Neisseria* and is associated with the respiratory electron transport chain.

Procedure: The bacterial colony to be tested is removed from the agar using a stick or glass rod, and it is placed on a small section of filter paper that has been soaked with a freshly made 1% oxidase reagent (Tetramethyl-p-phenylene Diamine Dihydrochloride). The creation of a rich purple hue as a result of the dye's degradation by the oxidase enzyme indicates a successful outcome.

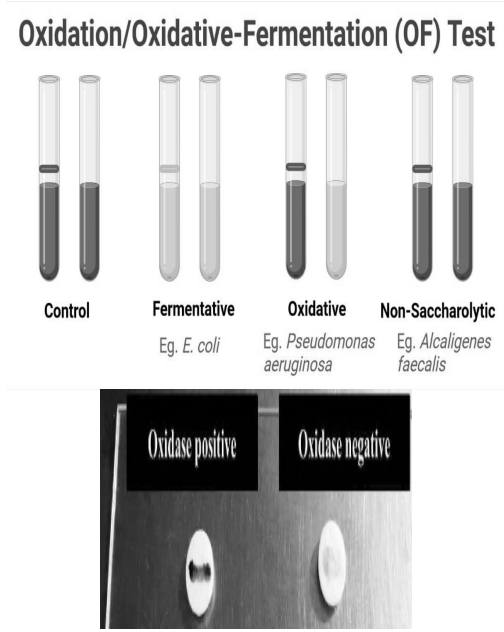


Fig 11: Oxidation Test (https://microbenotes.com/)

E) Carbohydrate utilization (Fermentation): Numerous species of bacteria are capable of breaking down mono-, di-, and even polysaccharides into acid or gas. Depending on how the pH indicator's colour changes as a result of acid generation, media containing phenol red (a reliable pH indicator) and appropriate sugar can be used to validate fermentation. A Durham tube, a tiny inverted tube inserted into the liquid medium to gather gas bubbles, can be used to identify the presence of gas generation.

- **A positive result for acid production** is a transition from red to yellow.
- **A positive result for gas production:** the Durham tube has a bubble. A negative outcome has no bubbles, no color shift, and no reddish tint.

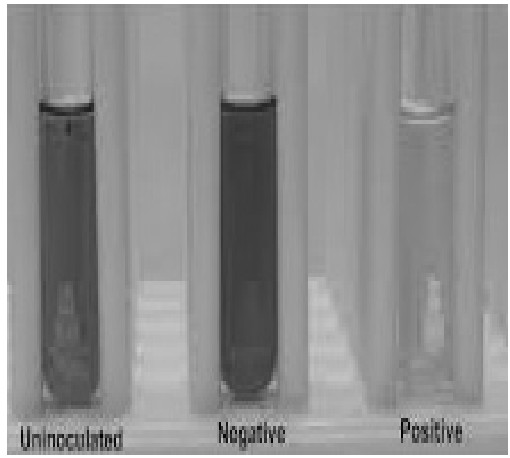


Fig 12: Carbohydrate Fermentation Test

F) Triple sugar iron (TSI) and Kligler's iron agar (KIA):

- **TSI medium contains**:
  - Sugars: glucose, lactose, and sucrose (KIA contains only glucose and lactose).
  - pH indicator: phenol red (red in alkaline pH and yellow in acidic pH).
  - Ferrous sulphate can be used as an indicator of H2S production.

  To identify and categorize enteric Gram-negative bacilli (Enterobacteriaceae), these media are used to measure the ability of bacteria to ferment certain sugars. There are three discernible criteria:

  i. The bacteria's capacity to ferment sugar and release gas. As a result, the media either pushes up or breaks down.

  ii. The formation of black precipitate at the media's bottom indicates the presence of H2S gas. Black ferrous sulfide is created in the butt as a result of H_2S's reaction with iron in the medium.

  iii. The capacity to ferment sugars, which is shown by a change in colour from red to yellow. The location of the color shift distinguishes the acidic products of lactose or sucrose fermentation from the acid generation associated with glucose fermentation. The pH indicator turns yellow in the butt, but not in the slant, due to the acid produced by bacteria that digest glucose. However, fomenters of lactose

or sucrose release more acid, which causes both the butt and the slant to turn yellow.

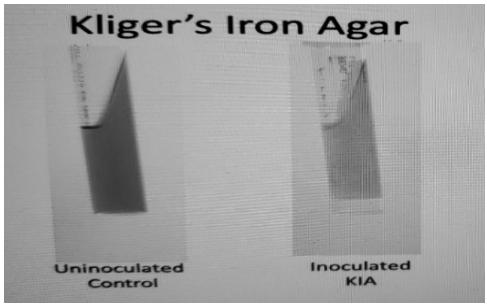
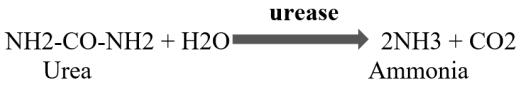


Fig 13: Kliger's Iron Agar

G) Urease test: By employing the urease enzyme to hydrolyze urea and produce carbon dioxide and ammonia, bacteria that are capable of undertaking this test are identified. Phenol red, a pH indicator, causes the medium to turn reddish-pink from yellow as urea hydrolysis raises the pH to over 7.0.



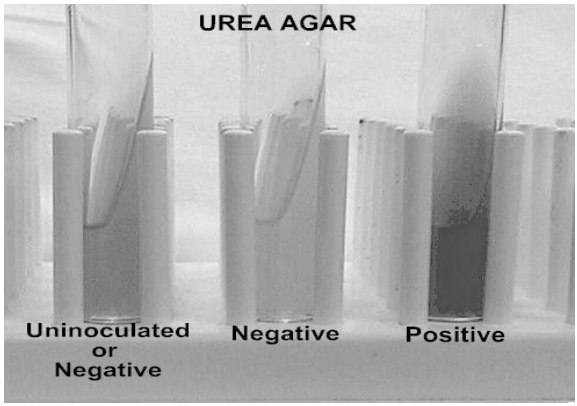


Fig 14: Urease Production Test

Ex: of urease producers are *Helicobacter pylori and V. cholerae, Klebsiella & Proteus*.

H) IMViC: A set of biochemical tests known as enteric G-ve bacilli (Enterobacteriaceae) assays are used to identify and distinguish between one another.

i. **Indole production test**: It evaluates the bacteria's capacity to make indole. The amino acid tryptophan is broken down by the enzyme tryptophanase in bacteria to produce pyruvic acid, ammonia, and indole.

Tryptophan — **Tryptophanase** —> Indole + ammonia + pyruvic acid

The examined bacteria are injected into a peptone liquid medium containing tryptophan, and the mixture is then cultured for 24 hours at 37 °C. To the bacterial growth, a few drops of Kovac's reagent are introduced. A positive outcome of indole production, such as E. coli, is shown by the presence of red rig in the medium's surface layer. *Klebsiella* is one example of a negative outcome, as shown by the yellow ring.

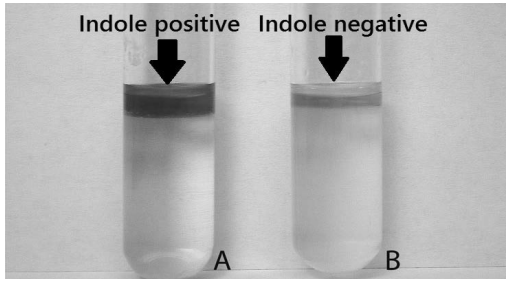


Fig 15: Indole Production Test

i. **Methyl red/Voges-** Depending on the kind of enzyme that the bacteria contain, the MR and VP tests are used to identify the end products that are produced when the examined organism breaks down glucose (for energy production). Acid is an end product of fully fermented glucose, is detected by MR-VP, and is used to measure the synthesis of acetoin (acetyl methyl carbinol) during the partial fermentation of glucose. For both tests, glucose phosphate peptone water medium is used; the test bacteria are added to it, and it is then incubated for 24 hours at 37°C:

ii. **Proskauer tests**: Five drops of methyl red indicator are applied to the MR sample. When the medium changes colour, it becomes red when results are positive, like *E. Coli*, and yellow when results are negative, like *Klebsiella*.

- The medium is mixed with VP, Voges Proskauer reagent (Barritt reagent). Reagent A (5% **α-**naphthol) and Reagent B (80% KOH) make up this reagent. Within 20 to 30 minutes, the positive reaction can be identified by the development of a pink-burgundy colour. For example, *Enterobacter aerogenes* and *Klebsiella* are shown as +ve results, whereas *E. coli* is shown as -ve.

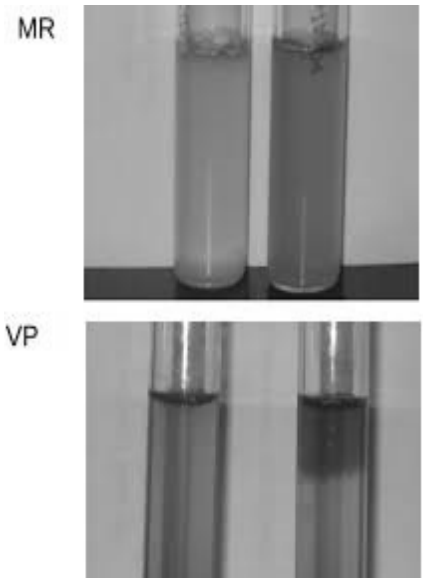


Fig 16: Methyl Red/Voges-Proskauer Test

iii. **Citrate utilization**: It is employed to assess a bacteria's capacity to use citrate as its only carbon source. Bromothymol blue can be used as a pH indicator in conjunction with Simmon's citrate agar. After inoculation, the tubes will be incubated by stabbing; if the +ve result is blue, it indicates that the bacteria (such as *Enterobacter* and *Klebsiella*) have metabolized citrate, and if the – ve result is green, it indicates that *E. coli* has not.

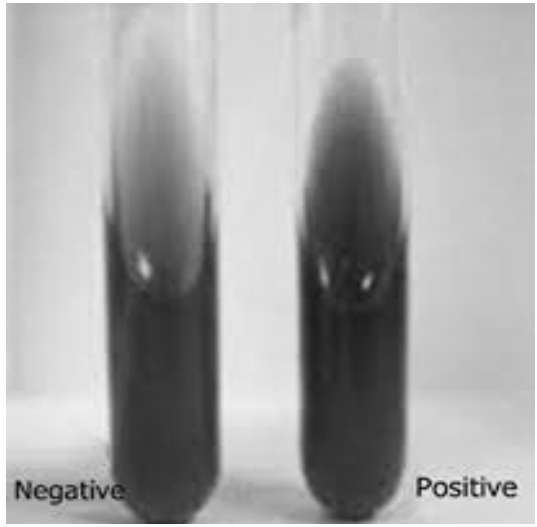
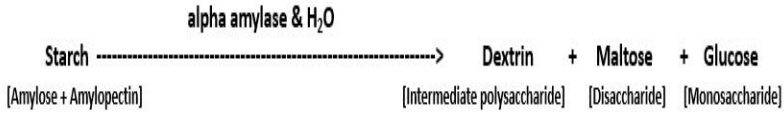


Fig 17: Citrate Utilization Test

I) Starch test: Enzymes referred to as hydrolases are produced by many microorganisms, according to the starch test. When organic molecules come into contact with water, hydrolases catalyse their breakup into smaller molecules. This activity will demonstrate the breakdown of carbohydrate starch. A big branched polymer called amylopectin and an unbranched glucose polymer called amylose, which has 200–300 units, make up the starch molecule. Using their amylases, some bacteria quickly hydrolyze both amylopectin and amylose to produce dextrins, maltose, and glucose, like below:



To detect the presence of starch, use Gram's iodine. It transforms from blue to brown when it comes into contact with starch. Starch that has been hydrolyzed does not alter colour. In a medium containing starch and bacterial growth, if Gram's iodine is added, a clear region will show that the bacteria have created amylase. Hydrolyzed starch has not occurred if there is no clearance.