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Gram-Positive and Gram-Negative Bacteria: Classification, Characteristics, and Staining Techniques

Shalini Pandey[1], Hansmeet Kour[2], Uma Kant Verma[3], Swarup Debroy[4], R.P. Diwakar[5]

[1]Assistant Professor, Department of Veterinary Microbiology, Covas, Buat, Banda.
[2]Assistant Professor, Department of Veterinary Microbiology, Covas, Buat, Banda.
[3]Assistant Professor, Department of Animal Genetics and Breeding, Covas, Buat, Banda.
[4]Assistant Professor, Department of Veterinary Anatomy, Covas, Buat, Banda
[5]Assistant Professor, Department of Veterinary Microbiology, Covas, Nduat, Ayodhya

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*Corresponding Author
Shalini Pandey*

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INTRODUCTION

Bacteria are classified into Gram-positive and Gram-negative groups based on the structure of their cell wall and their response to Gram staining. Staining techniques are important because bacteria are colorless and cannot be seen clearly under a microscope without staining. Different staining methods help in identifying bacteria and their special structures, which is essential for disease diagnosis and microbiological research.

Gram-positive and Gram-negative Bacteria

Bacteria are divided into Gram-positive and Gram-negative based on the Gram staining test, which shows the structure of their cell wall.

1. Gram-positive bacteria

Gram-positive bacteria have a thick peptidoglycan cell wall. During Gram staining, they retain the crystal violet stain and appear purple or blue under the microscope.
Examples:

- Staphylococcus aureus
- Streptococcus spp.
- Bacillus anthracis
- Clostridium spp.
- Listeria monocytogenes

2. Gram-negative bacteria

Gram-negative bacteria have a thin peptidoglycan layer and an outer membrane. They do not retain the violet stain and appear pink or red after the counterstain.
Examples**:**

- Escherichia coli (E. coli)
- Salmonella spp.
- Shigella spp.
- Klebsiella pneumoniae
- Pseudomonas aeruginosa
- Proteus spp.

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Lactose Fermenting and Non-Lactose Fermenting Bacteria

These bacteria are classified based on whether they can ferment (break down) lactose, a type of sugar. This is commonly tested on MacConkey Agar.

1. Lactose fermenting bacteria (LF)

These bacteria can ferment lactose and produce acid. On MacConkey agar, they form pink or red colonies.

Examples:

- Escherichia coli (E. coli)
- Klebsiella pneumoniae
- Enterobacter spp.
- Citrobacter spp. (some species)

2. Non-lactose fermenting bacteria (NLF)

These bacteria cannot ferment lactose. On MacConkey agar, they produce colorless or pale colonies.

Examples:

- Salmonella spp.
- Shigella spp.
- Proteus spp.
- Pseudomonas aeruginosa
- Yersinia spp.

Difference between Lactose Fermenters and Non-Lactose Fermenters

Feature	Lactose Fermenters (LF)	Non-Lactose Fermenters (NLF)
Ability to ferment lactose	Yes	No
Acid production	Produces acid	Does not produce acid from lactose
Colony color on MacConkey agar	Pink or red	Colorless or pale
Common examples	E. coli, Klebsiella, Enterobacter	Salmonella, Shigella, Proteus, Pseudomonas

Difference between Gram-positive and Gram-negative Bacteria

Feature	Gram-positive	Gram-negative
Cell wall	Thick peptidoglycan layer	Thin peptidoglycan layer with outer membrane
Gram stain color	Purple/Blue	Pink/Red
Outer membrane	Absent	Present
Endotoxin	Absent	Present (lipopolysaccharide in outer membrane)
Techoic acids	Present	Absent
Periplasmic space	Absent	Present
Lipid and lipoprotein content	Low (acid-fast bacteria have lipids linked to peptidoglycan)	High (due to presence of outer membrane)
Flagellar structure	2 rings in basal body	4 rings in basal body
Resistant to physical disruption	High	Low
Examples	Staphylococcus, Streptococcus, Bacillus	E. coli, Salmonella, Shigella, Pseudomonas

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Easy way to remember

- Gram-positive = Thick wall = Purple
- Gram-negative = Thin wall + Outer membrane = Pink
- Lactose fermenters = Pink colonies on MacConkey agar
- Non-lactose fermenters = Colorless colonies on MacConkey agar

Gram Staining Procedure

Gram staining is a common laboratory method used to identify bacteria based on the structure of their cell wall. It helps to separate bacteria into Gram-positive and Gram-negative groups.

Steps of Gram Staining:

1. **Preparation of smear:** A small amount of bacterial culture is placed on a clean glass slide and mixed with a drop of water. The smear is spread into a thin layer and allowed to dry. After drying, the smear is gently heated to fix the bacteria on the slide.
2. **Primary staining (Crystal violet):** The smear is covered with crystal violet stain for about one minute. This stains all bacteria purple.
3. **Mordant treatment (Gram's iodine):** Gram's iodine is added, which forms a complex with crystal violet and helps the stain stick inside the bacterial cell.
4. **Decolorization:** The slide is treated with alcohol or acetone. This is the most important step. Gram-positive bacteria retain the purple color because of their thick cell wall, while Gram-negative bacteria lose the purple stain.
5. **Counterstaining (Safranin):** The slide is then stained with safranin, which gives a pink color to Gram-negative bacteria. Gram-positive bacteria remain purple because the crystal violet stain is still present.
6. **Observation under microscope:** The stained slide is examined under an oil immersion lens (100× objective).
   - Gram-positive bacteria appear purple/violet.
   - Gram-negative bacteria appear pink/red.

In simple words:

Gram staining works by staining all bacteria purple first, removing the color from some bacteria, and then applying a second stain. Bacteria with a thick cell wall remain purple (Gram-positive), while bacteria with a thin cell wall become pink (Gram-negative)

Different types of Bacterial Staining Techniques

1. Simple (Plain) Staining

Simple staining is the most basic staining technique in microbiology. It uses a single dye to stain bacterial cells, making it easier to observe their size, shape, and arrangement under a microscope. Commonly used stains include methylene blue, crystal violet, safranin, and carbol fuchsin. Although this method does not differentiate between bacterial species, it is useful for routine microscopic examination.

2. Differential Staining

Differential staining employs two or more stains to distinguish between different groups of bacteria based on their structural or chemical characteristics. It is one of the most important techniques in diagnostic microbiology. The commonly used differential staining methods include:

Gram Staining: Differentiates bacteria into Gram-positive and Gram-negative groups according to the composition of their cell walls.

Ziehl–Neelsen (Acid-Fast) Staining: Used to identify acid-fast bacteria, particularly Mycobacterium tuberculosis, by distinguishing them from non-acid-fast organisms.

Modified Ziehl–Neelsen (MZN) Staining: A modified acid-fast staining technique commonly used for detecting

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organisms such as Brucella abortus and other weakly acid-fast bacteria.

3. Negative Staining

Negative staining stains the background instead of the bacterial cells, leaving the organisms unstained and clearly visible against a dark background. This technique is especially useful for demonstrating bacterial capsules. India ink and Nigrosin are the stains most frequently used for this purpose.

4. Special Staining

Special staining techniques are designed to demonstrate specific bacterial structures that are not easily visualized by routine staining methods. Some important examples are:

Wirtz–Conklin (Schaeffer–Fulton) Staining: Used for the detection of bacterial endospores.

Albert's Staining: Demonstrates metachromatic granules, particularly in Corynebacterium diphtheriae.

Leifson's Flagella Staining: Used to visualize bacterial flagella.

Hiss Method**:** Applied for the demonstration of bacterial capsules.

CONCLUSION

Staining is an important technique in microbiology that helps in the identification and study of microorganisms. Gram staining divides bacteria into Gram-positive and Gram-negative groups based on differences in their cell wall structure. Other staining methods, such as acid-fast staining, capsule staining, endospore staining, flagella staining, and negative staining**,** help to observe specific bacterial structures and characteristics. The study of lactose fermenting and non-lactose fermenting bacteria helps in the identification of intestinal bacteria and disease-causing organisms. Overall, staining methods are essential tools in microbiology for the proper identification, diagnosis, and study of bacteria.