Bacterial structure and classification 2

Dr. Hala Altarawneh

Outline

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   - Taxonomy as a science
   - Bacterial nomenclature
2. Approaches for Bacterial Classification
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      - Morphological Classification
      - Classification by Staining
   B. Modern Molecular Methods for Bacterial Classification

Importance of Bacterial Classification

"Scientists estimate there may be up to **a trillion species** of microbes on Earth, only a fraction have been discovered and described. Around **30,000 to 43,000**"

Importance:

- Organizes vast microbial diversity into manageable groups
- Facilitates accurate communication among scientists & clinicians
- Accurate classification is essential for correct diagnosis and appropriate antimicrobial treatment.
- Facilitates identification of pathogens and prediction of pathogenic properties

Principles of Bacterial Taxonomy

Taxonomy = science of classification and naming organisms

Taxonomy has three interrelated components:

1. **Classification:**

   Bacterial taxonomy organizes bacteria into hierarchical groups, based on shared characteristics like phylogenetic relationships (determined by gene sequences like 16S rRNA), morphology, metabolic properties, and genetic makeup.

Principles of Bacterial Taxonomy

2. **Nomenclature:** once classified, organisms need names.
3. **Identification:** Identification involves using the rules of classification and nomenclature to recognize an unknown organism.

Unknown Bacterium → Apply Classification Rules → Apply Nomenclature → Identification Complete (E.g. *E. coli*)

Bacterial Taxonomy- Example 1: *S. aureus*

Dear

Philip

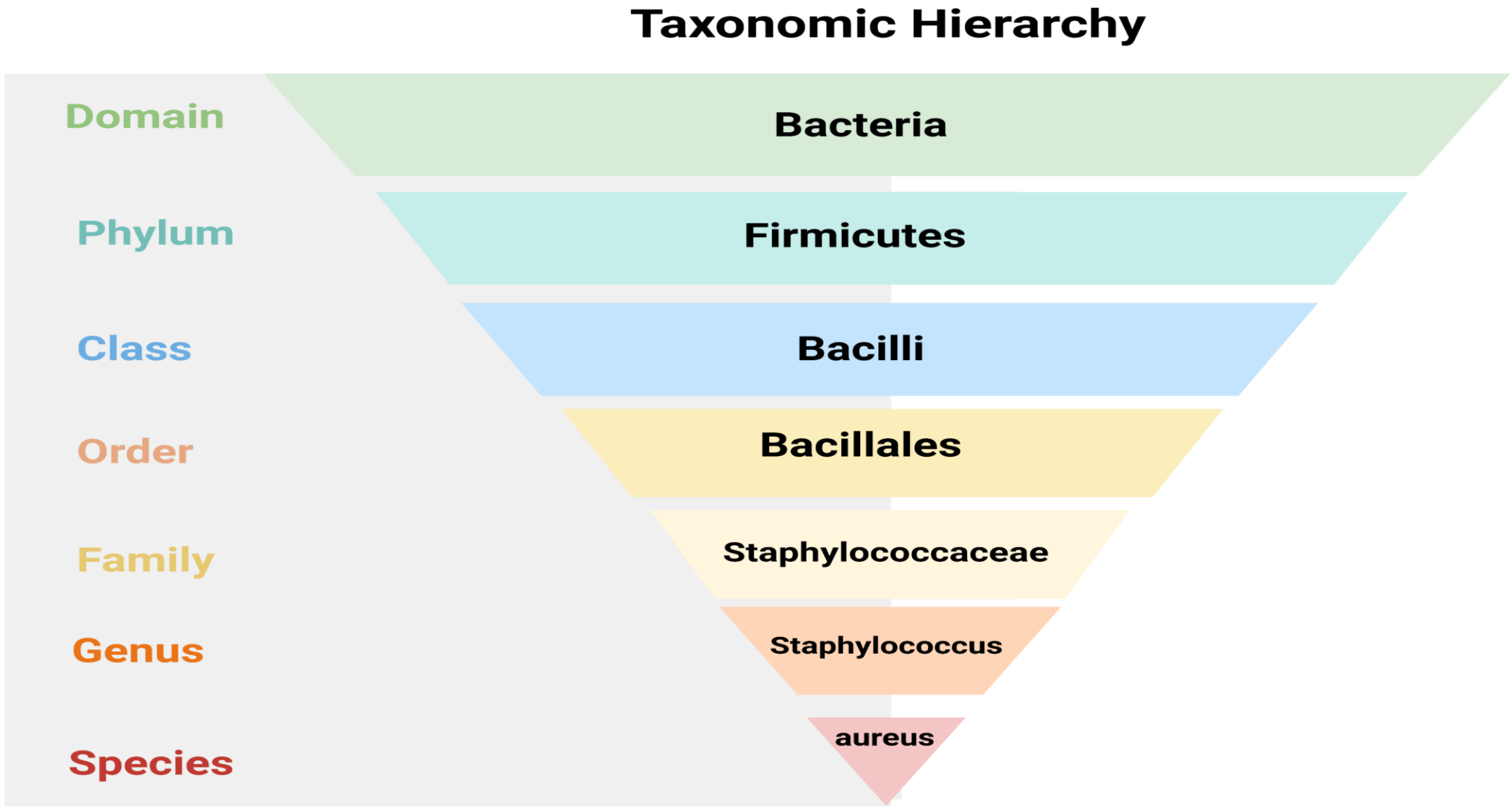
Came

Over

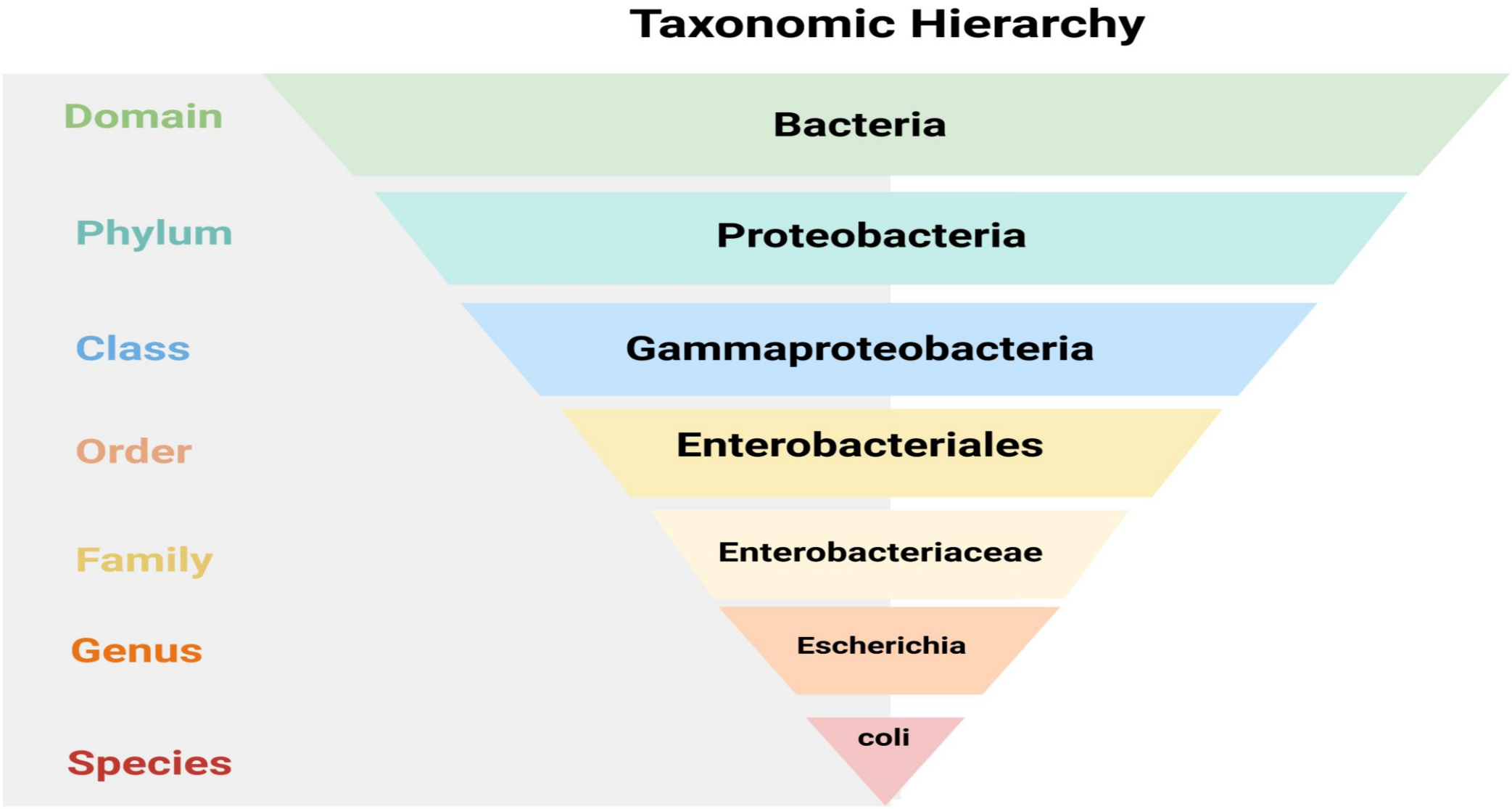
For

Good

Soup



Bacterial Taxonomy- Example 2: *E. coli*



Bacterial Nomenclature

- Bacteria are named using two parts:
  - Genus (group name)
  - Species (specific name)

- Rules of bacterial naming:
  - ✓Genus is capitalized
  - ✓Species is lowercase
  - ✓Both are italicized
  - ✓Handwritten names should be <u>underlined</u>
  - ✓After first mention, genus can be abbreviated

Bacterial Nomenclature

Examples:

Escherichia coli

Genus name **Species name**
Capitalized **lowercase**

E. coli → **genus abbreviation**
Both genus and species are italicized

- *E. Coli*
  - ❌ WRONG (species should not be capitalized)
- Escherichia coli
  - ❌ WRONG (not italicized)
- *e. coli*
  - ❌ WRONG (genus must be capitalized)
- *E. coli*
  - ✅ CORRECT

Bacterial Nomenclature: Knowledge Check

Which is the **correct** formatting of the species name?

A. *staphylococcus Aureus*

B. Staphylococcus Aureus

C. *Staphylococcus aureus*

D. staphylococcus aureus

Approaches to Bacterial Classification

Traditional/Phenotypic Methods:

- **Morphology (shape)**
- **Staining: Gram stain, Acid-fast stain**
- Culture requirements: oxygen requirements, temperature, pH
- Biochemical characteristics: Sugar fermentation tests

Modern (Genotypic & Molecular) Approaches

- DNA G+C content
- 16S rRNA sequencing
- Whole genome sequencing, phylogenetics
- MALDI-TOF

Traditional Approaches to Bacterial Classification: Morphology

The morphology of bacteria describes **the external appearance** of bacterial cells including shape, arrangement, and size.

Basic Bacterial Shapes:

- **Cocci:** Spherical in shape.
- **Bacilli:** Rod-shaped.
- **Spiral forms:**
  - **Vibrio:** A slightly curved rod or comma-shaped.
  - **Spirilla:** Helical-shaped, rigid, and twisted.

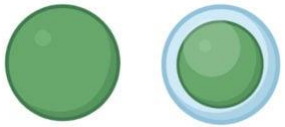
  - **Spirochetes:** Tightly coiled, flexible, and often slender spiral cells.

- **Unusual shapes:** Some bacteria have unique forms, such as branched filamentous structures (like Actinomyces) or even square or star-shaped cells.

Traditional Approaches to Bacterial Classification: Morphology- Arrangements of Cocci

Cocci bacteria can be arranged:

- **Coccus:** present as a single cell.

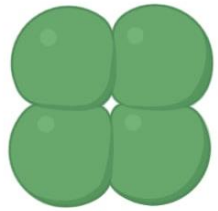


- **Diplococci:** two bacterial cells appear as a pair (joined together).

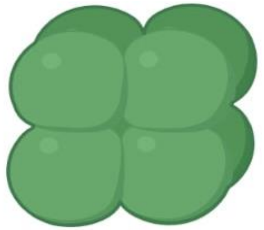
- **Streptococci:** bacteria are arranged in long chains.

- **Staphylococci:** bacteria that are arranged in grape-like clusters.

- **Tetrad:** bacteria are arranged in a group of four cells

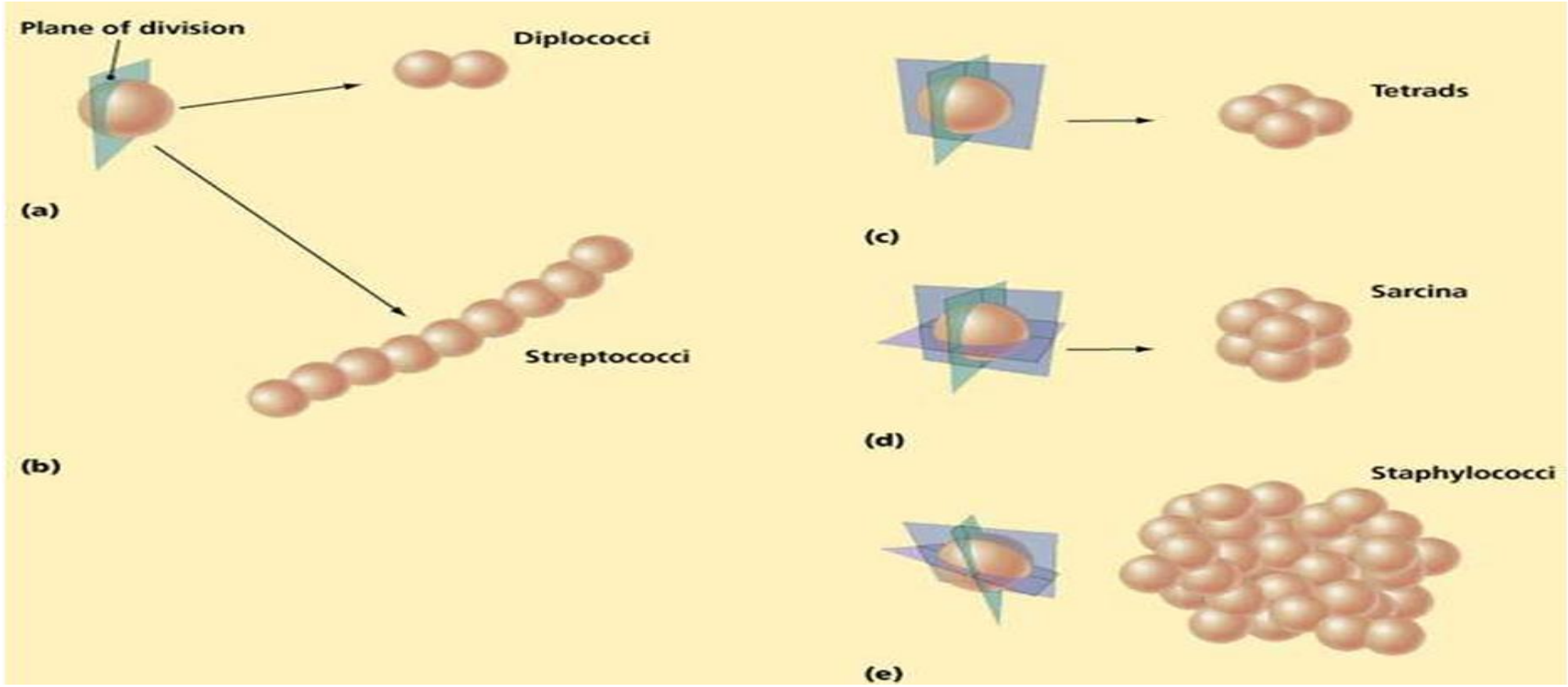


- **Sarcina:** bacterial cells form a group of eight cells.



Traditional Approaches to Bacterial Classification: Morphology- Arrangements of Cocci

Why do bacterial cells have different arrangement?

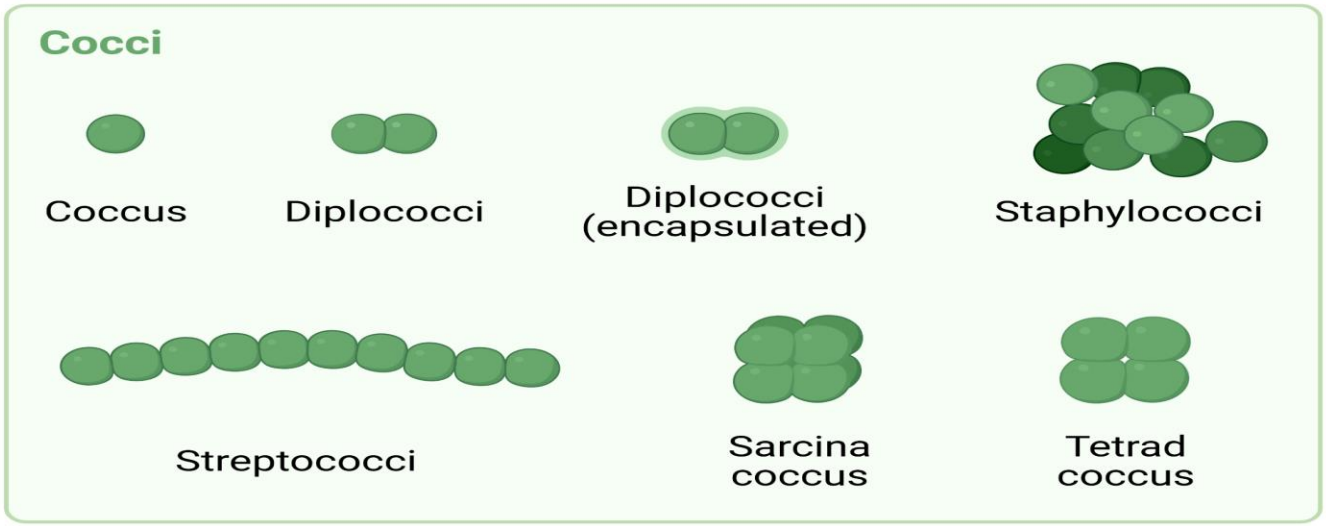


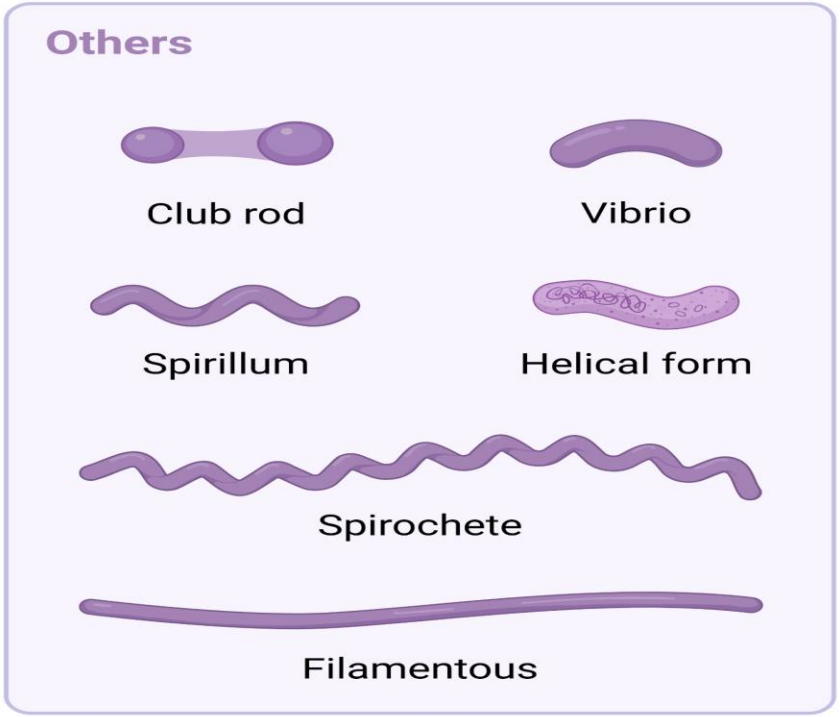
Traditional Approaches to Bacterial Classification:
Morphology- Arrangement of Bacilli

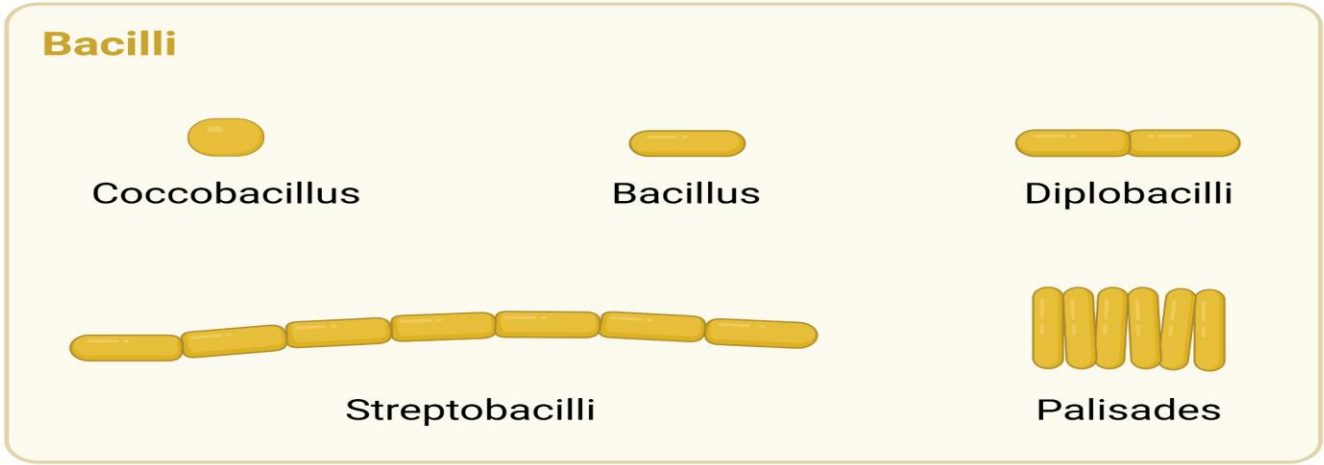
Bacilli bacteria can be arranged:

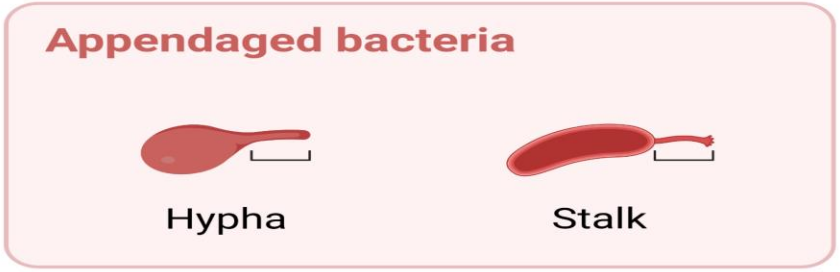
- **Bacillus:** present as single cells.
- **Coccobacilli:** coccobacilli resemble both cocci as well as bacilli.
- **Diplobacilli:** exists in pairs.
- **Streptobacilli:** bacteria are arranged in chains.
- **Palisades:** align side by side in a parallel or picket fence-like pattern.

Bacterial Shapes and Arrangements









Traditional Approaches to Bacterial Classification: Staining

Why Bacterial Staining?

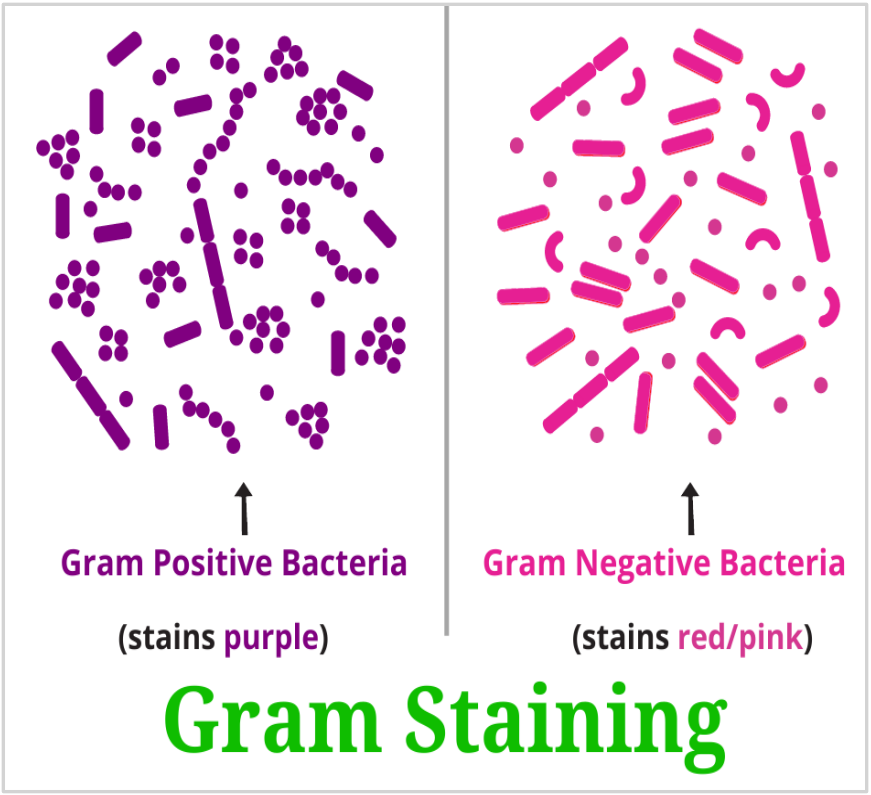
- Bacteria are colourless and transparent - invisible under light microscopy without staining
- Staining allows visualization, identification, and classification
- **Different staining properties** reflect **fundamental structural differences**

Traditional Approaches to Bacterial Classification: Gram Stain

- The Gram Stain developed by **Hans Gram** (in 1884)
- The most important staining technique in clinical microbiology
- **Differential stain** - divides bacteria into two major groups based on their reaction to the staining procedure
- **Two groups:**

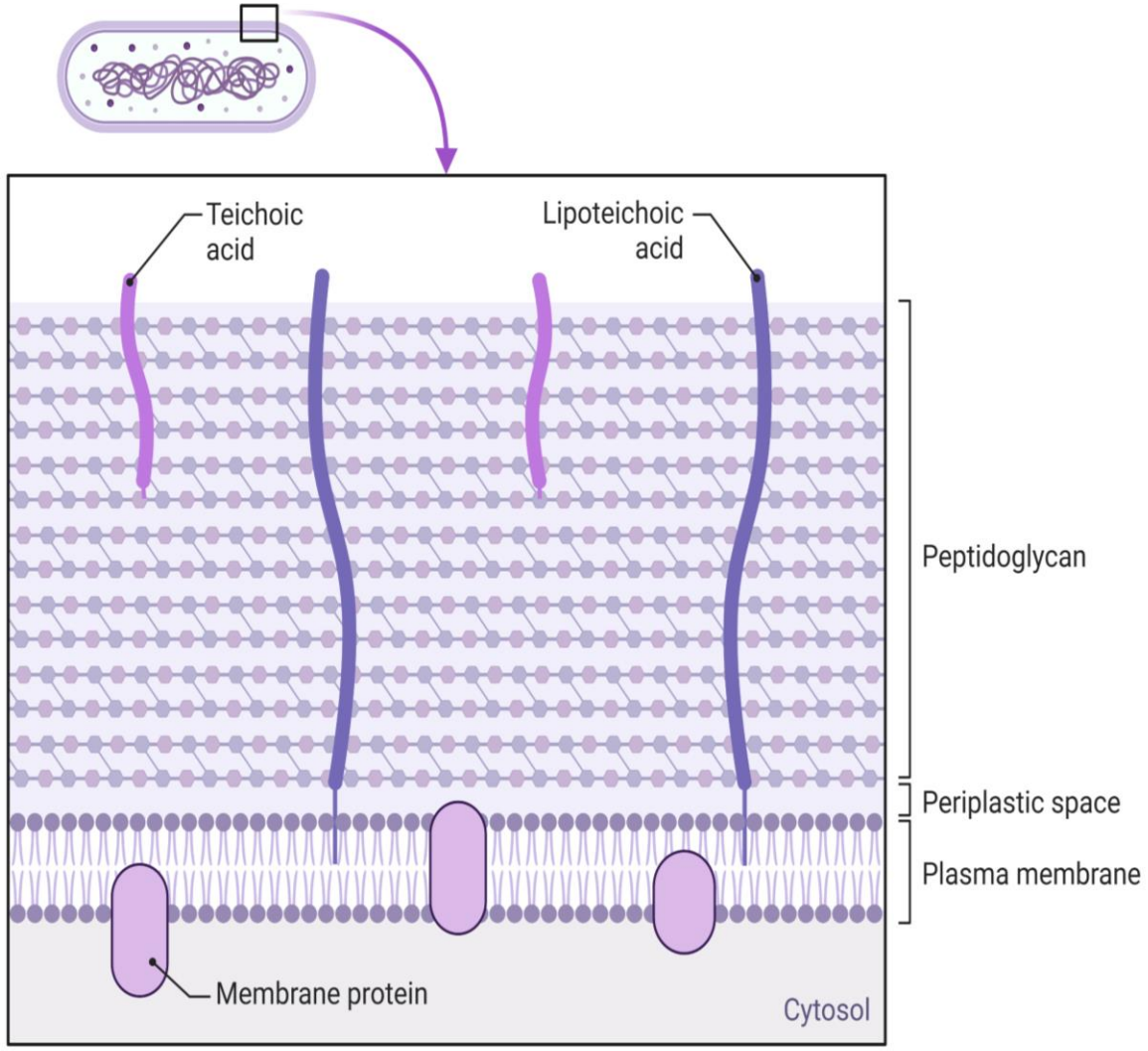
  Gram-positive bacteria → retain primary stain → appear **Purple/Blue**

  Gram-negative bacteria → lose primary stain → appear **Pink/Red**



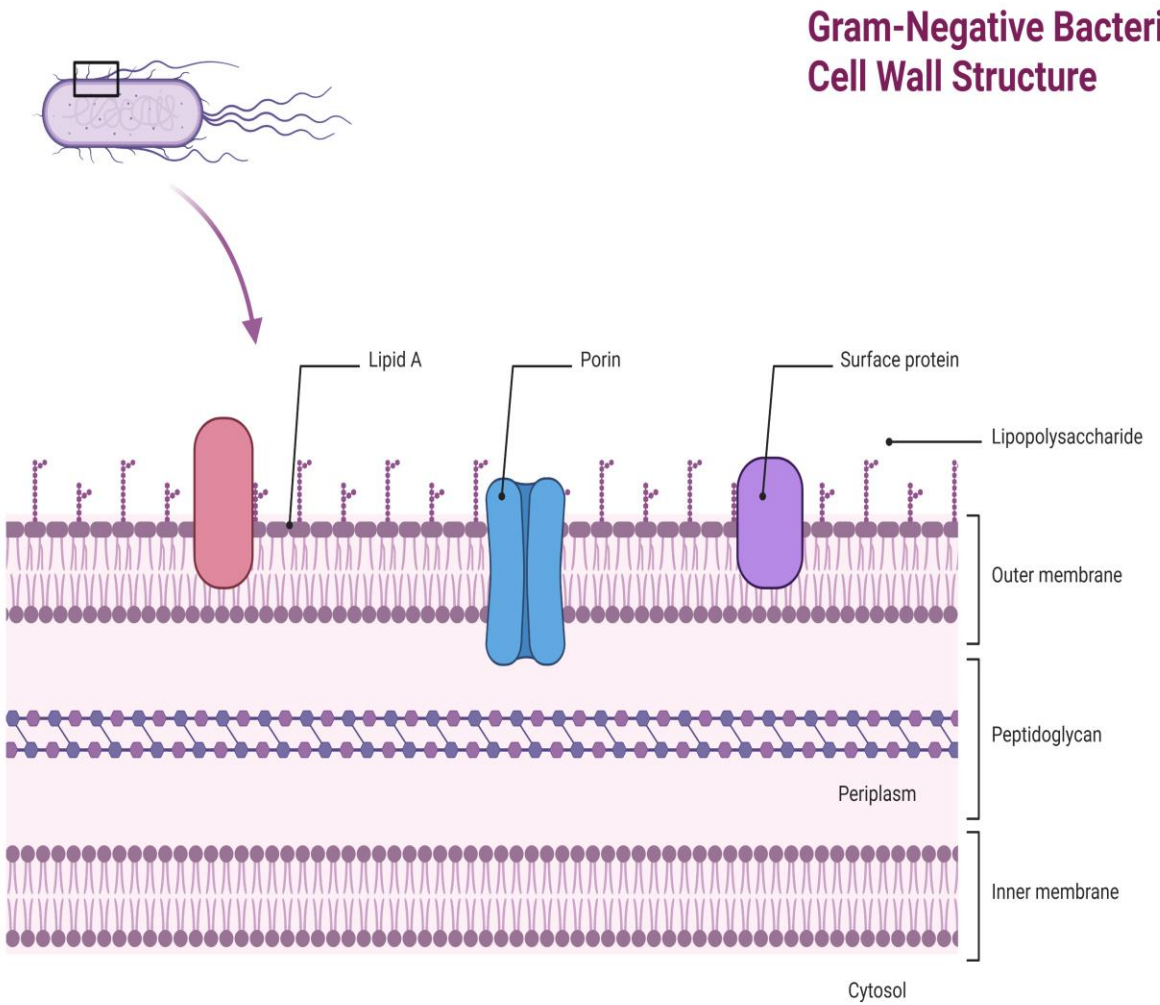
Traditional Approaches to Bacterial Classification: Gram Stain- Gram-Positive Bacteria

- **Thick peptidoglycan layer** (20-80 nm, multiple layers)
  - Makes up 90% of the cell wall
  - Like a thick protective wall
- **No outer membrane**
- Contains **teichoic acids** and **lipoteichoic acids**

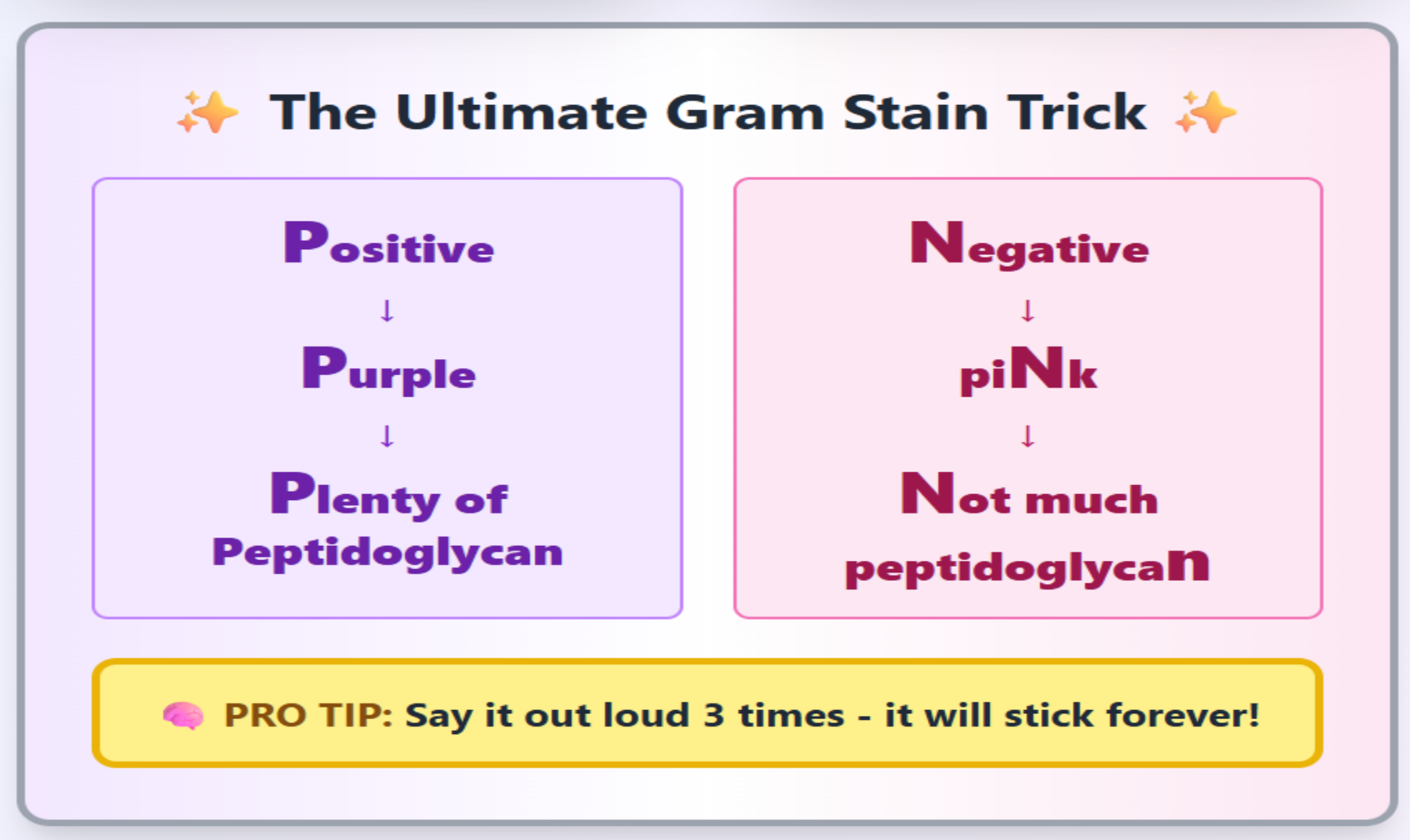


Traditional Approaches to Bacterial Classification: Gram Stain- Gram-Negative Bacteria

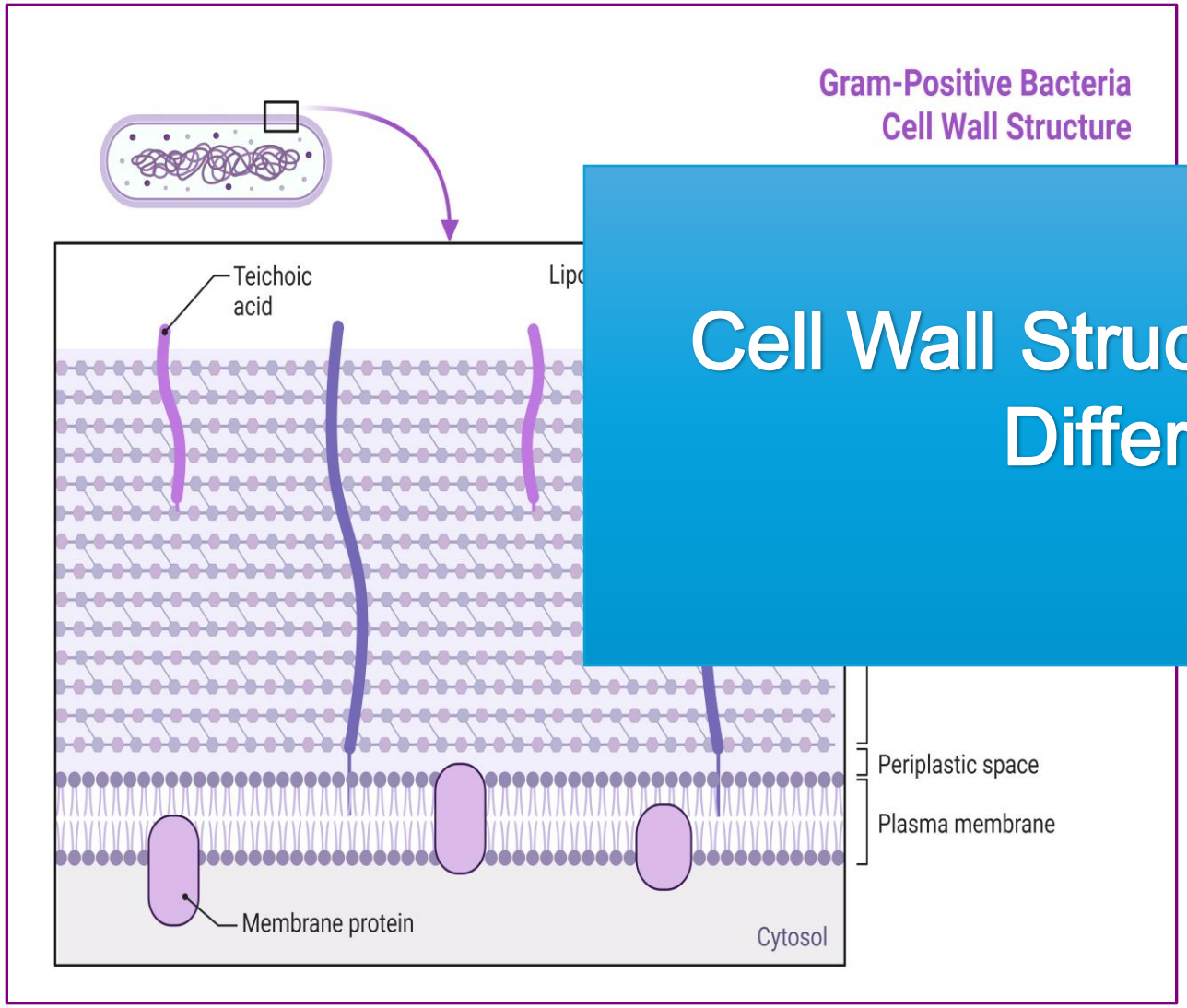
- **Thin peptidoglycan layer** (2-7 nm, single layer)
  - Only 10% of cell wall
  - Like a thin framework
- **Has outer membrane** (unique feature!)
  - Contains **lipopolysaccharide (LPS/endotoxin)**
  - Acts as permeability barrier

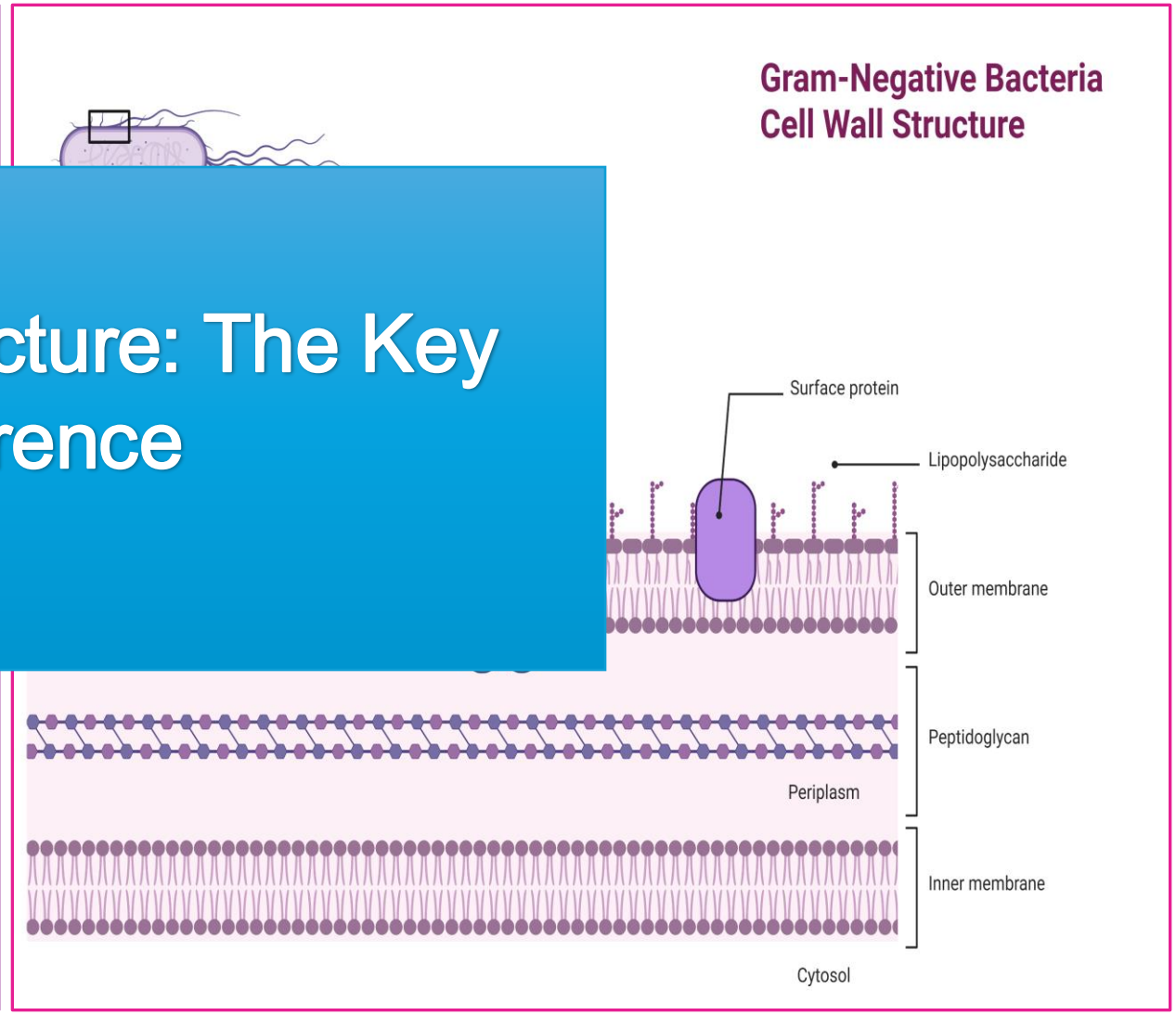


Traditional Approaches to Bacterial Classification: Gram Stain



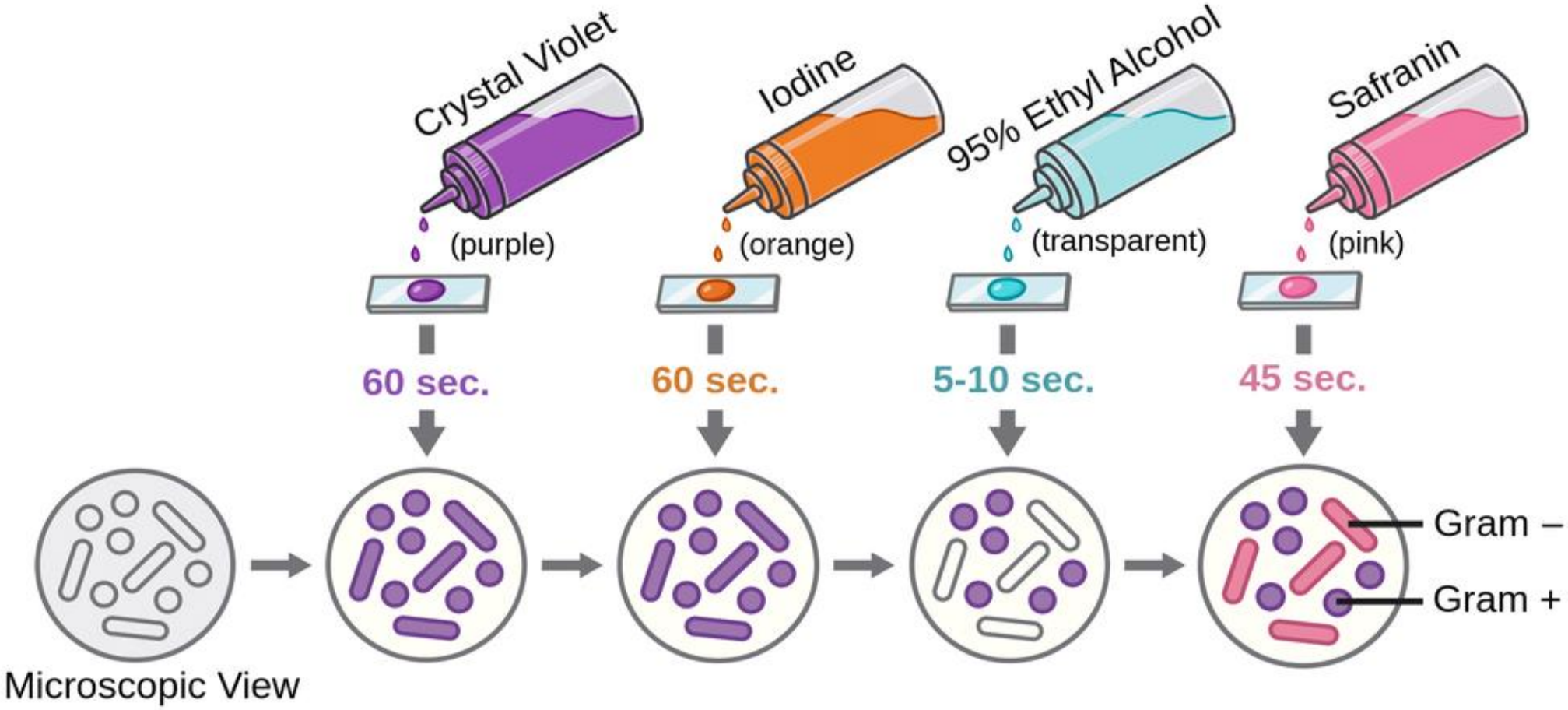
Gram-Positive vs Gram-Negative: Cell Wall Structure





Cell Wall Structure: The Key Difference

Traditional Approaches to Bacterial Classification: Gram Stain



Gram-Positive vs Gram-Negative: Cell Wall Structure

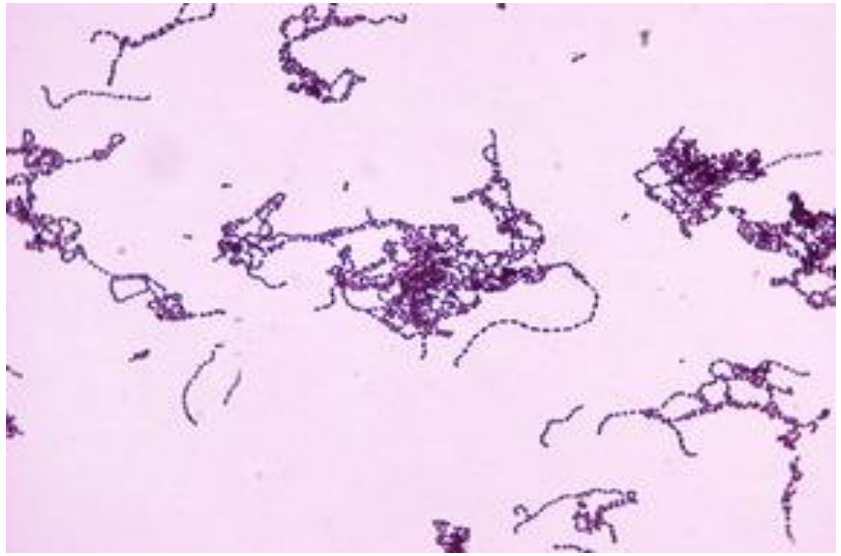
Feature	Gram-Positive	Gram-Negative
Peptidoglycan layer	Thick (90% of wall)	Thin (10% of wall)
Outer membrane	Absent	Present
Decolorization	Resists (traps dye)	Easily decolorized
Colour	Purple/Blue	Pink/Red
Teichoic acids	Present	Absent
LPS (endotoxin)	Absent	Present
Clinical examples	Staphylococcus	*E. coli*

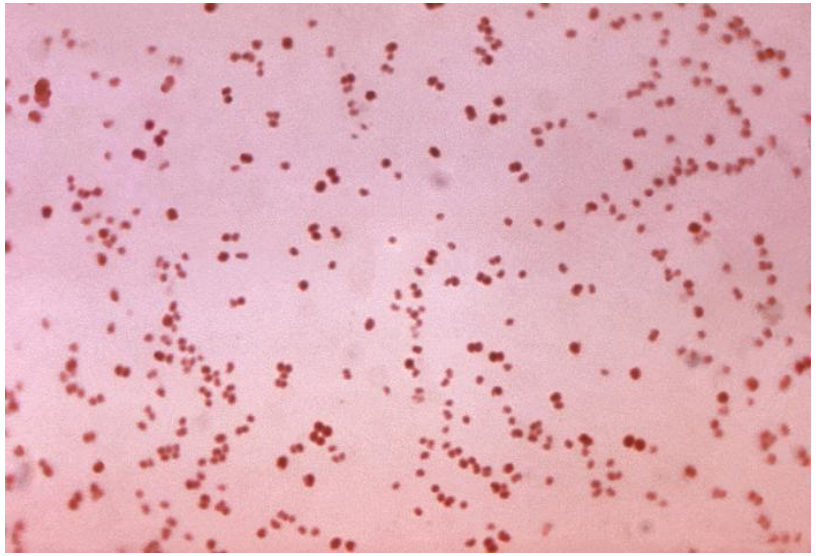
Gram Stain Results: Microscopic Appearance

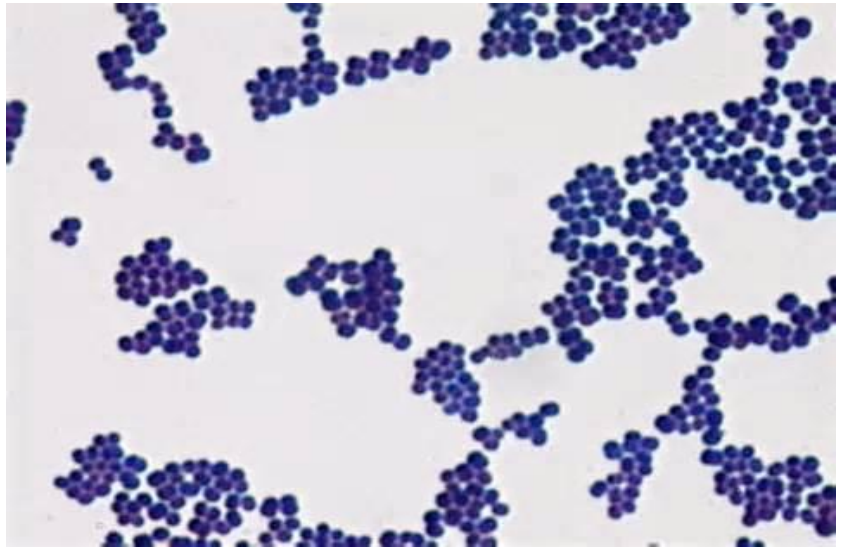
Q1: Is this Gram-positive or Gram-negative?

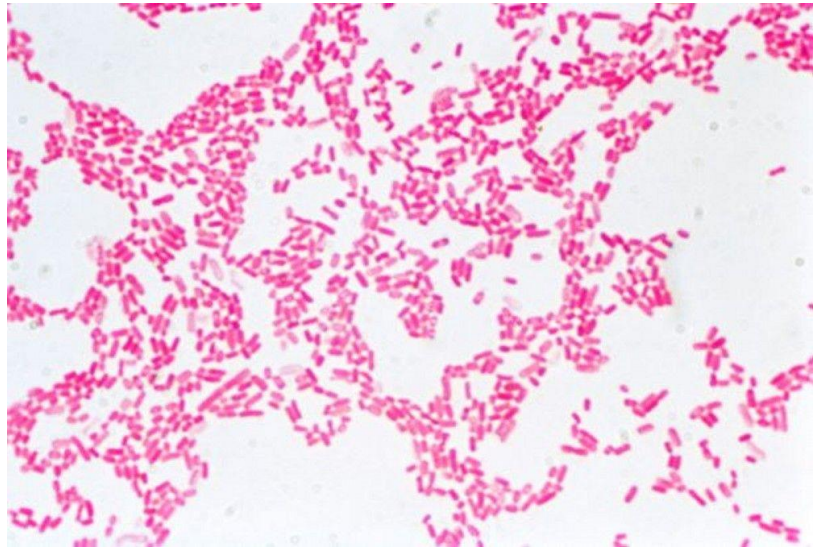
Q2: What is the shape of the bacteria?

Q3: What is the arrangement?









Acid-Fast Stain - Special Stain for Special Bacteria

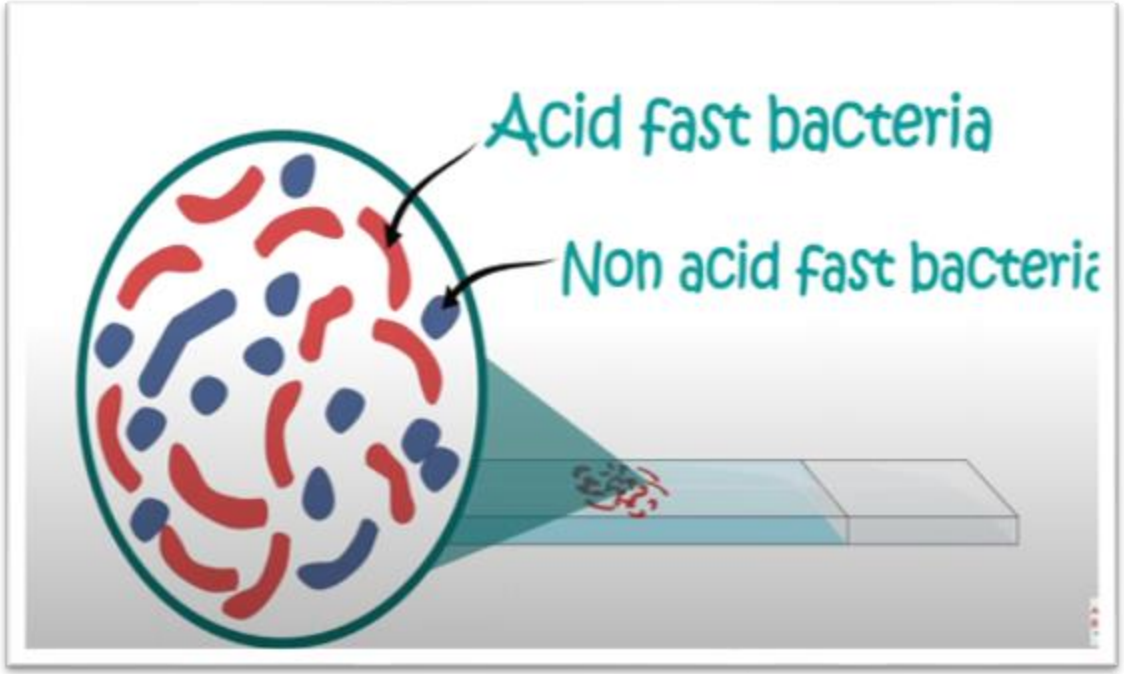
Why is it needed?

- Some bacteria have **waxy, lipid-rich cell walls** (mycolic acids)
- These bacteria **CANNOT be stained by Gram stain**
- Need special staining technique

- **Principle:**
  - Uses **strong staining** (heat or detergent) to penetrate waxy cell wall
  - Once stained, cells resist decolorization by acid-alcohol
  - Hence: "**Acid-fast**" = resist acid decolorization

Acid-Fast Stain - Special Stain for Special Bacteria

- **Two methods:**
  - **Ziehl-Neelsen stain** (hot stain - uses heat)
  - **Kinyoun stain** (cold stain - uses stronger detergent)
- **Results:**
  - **Acid-fast bacteria** = Red/Pink
  - **Non-acid-fast bacteria** = Blue

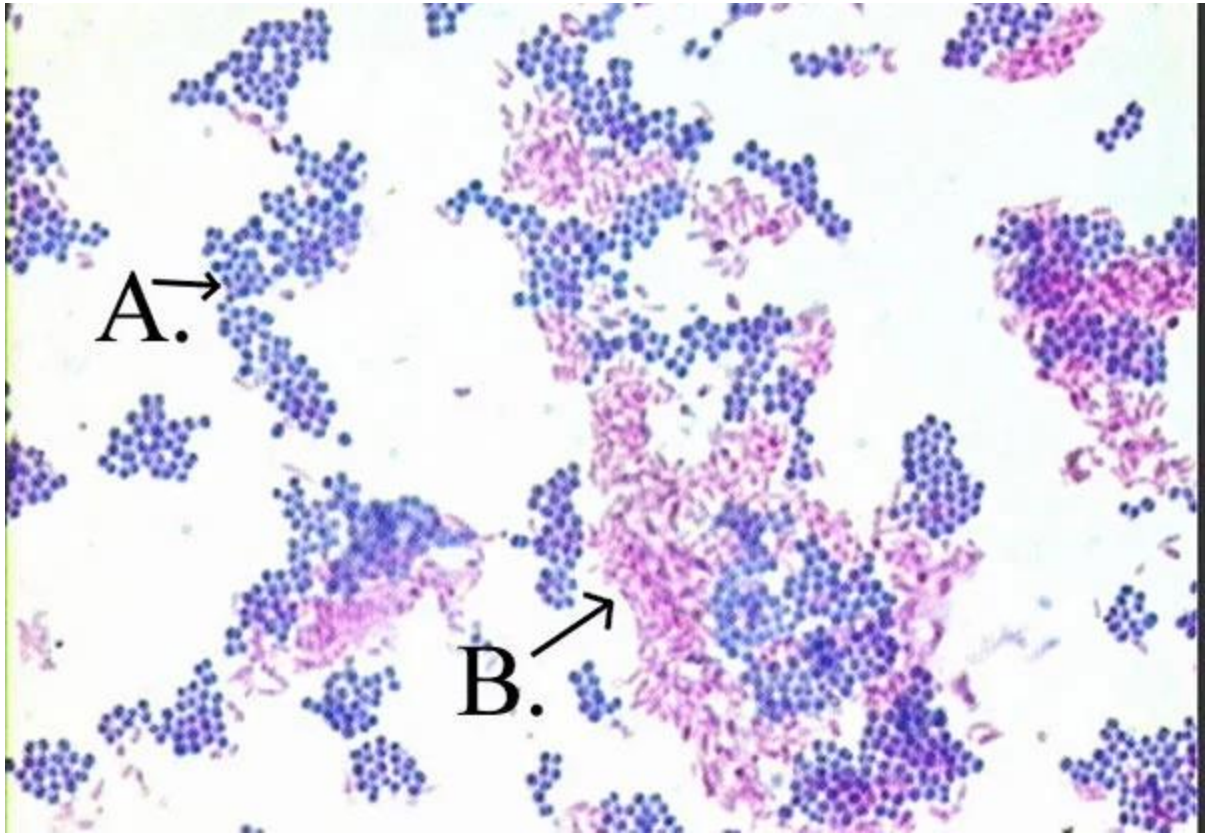


Acid-Fast Stain Results: Microscopic Appearance

Q1: Is this Acid-Fast-positive or -negative?

Q2: What is the shape of the bacteria?

Q3: What is the arrangement?



Traditional vs Modern Classification

- **Traditional (Phenotypic) Methods:**
- Based on **observable characteristics**
- **Advantages:** Fast, cheap, practical for most clinical situations
- **Limitations:**
  - Subjective interpretation
  - Limited discriminatory power (can't distinguish closely related species)
  - Some bacteria look/behave similarly but are genetically different
  - Slow-growing organisms (Mycobacteria) take weeks
  - Cannot identify unculturable bacteria

Traditional vs Modern Classification

- **Modern (Genotypic/Molecular) Methods:**
- Based on **genetic and molecular characteristics**
- Direct analysis of DNA, RNA, or proteins
- **Advantages:**
  - More accurate and objective
  - Can identify unculturable organisms
  - Better for epidemiology and outbreak tracking
  - Reveals evolutionary relationships
- **The Reality:** Both approaches are complementary!
- Traditional methods: Still essential, first-line in most labs
- Modern methods: For complex cases, research, epidemiology

Modern Molecular Methods for Bacterial Classification: DNA-Based Methods (Genotypic)

1. **DNA G+C Content**
   - Percentage of guanine and cytosine in bacterial genome
   - Closely related bacteria have similar G+C ratios

2. **16S rRNA Gene Sequencing**
   - **Gold standard for bacterial identification**
   - 16S rRNA gene present in ALL bacteria
   - Variable regions allow species identification
   - Used to identify unknown/unculturable bacteria
   - Created new bacterial phylogenetic tree

Modern Molecular Methods for Bacterial Classification: DNA-Based Methods (Genotypic)

3. Whole Genome Sequencing (WGS)

- Sequence entire bacterial genome
- Ultimate method for classification and typing
- Used for: outbreak investigation, antimicrobial resistance detection

Modern Molecular Methods for Bacterial Classification: Protein-Based Methods

MALDI-TOF Mass Spectrometry

- **Revolutionizing clinical microbiology labs**
- Identifies bacteria in minutes based on protein profile
- Fast, accurate, cost-effective
- Replacing many biochemical tests in modern labs

Thank You For Your Attention