

# **Stains and Staining Reactions**

# Stains and objectives of staining

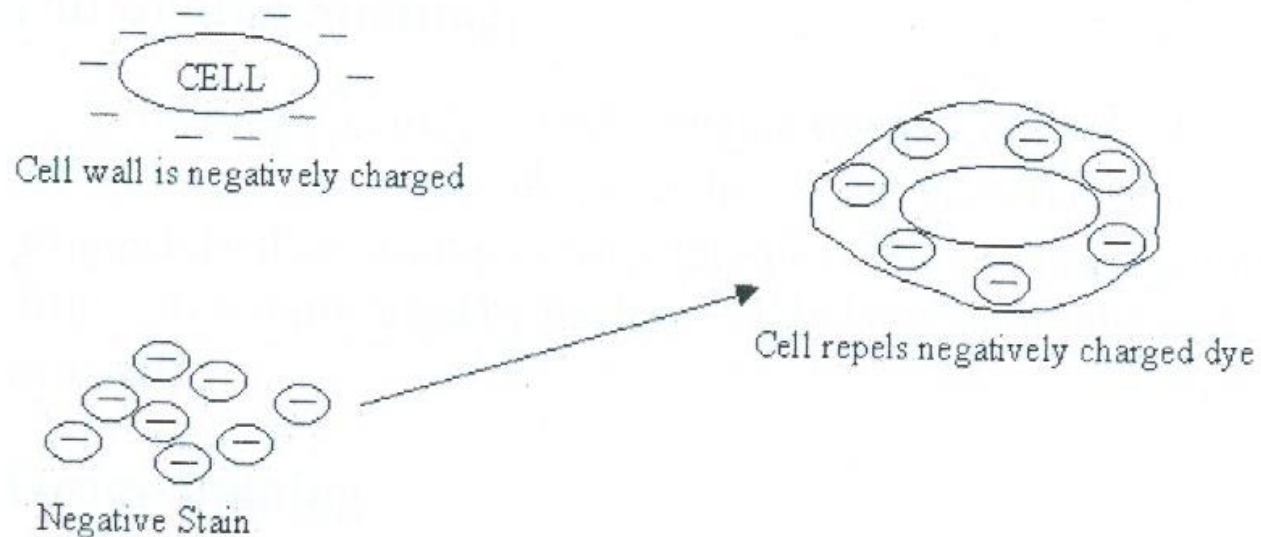
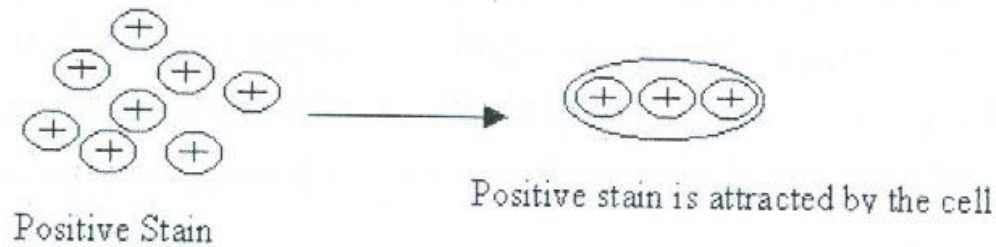
- Stains are useful for the following reasons
- It makes the microscopic semi-transparent objects visible
- To study the shape and size
- To reveal the presence of various internal and external structures
- To produce specific chemical and physical reaction
- A colouring agent that is used for general purposes is called a dye

- The one that is used for biological purposes is called a stain
- acidic, basic and neutral
- An acid (or anionic) dye has a negative charge. Eg., Eosin, Rose Bengal, Acid fuchsin
- Basic dye (or cationic) carries a positive charge. eg., Methylene Blue, basic fuchsin, crystal violet, malachite green, safranin
- Basic dyes bind to negatively charged molecules like nucleic acid and many proteins
- Bacterial cells surfaces are negatively charged
- Neutral dye is a complex salt of a dye acid with a dye base

# Dye Characteristics

- Dyes have two features in common
- chromophore groups bind with cells by ionic, covalent or hydrophobic bonding
- Positive staining procedure, a positively charged, chromophore is attracted to the negatively charged outer surface Methylene blue
- Negative staining procedures, negatively charged chromophore repelled by the negatively charged microorganisms
- Nigrosin and Indian ink

# Staining Principle



# Staining reactions

- When the pH of the surroundings of the microbial cells is either neutral or alkaline, all microbial cells have a negative charge on their surface, called the surface charge
- microbes from acidic environments stain poorly with basic dyes
- basic dyes are made up as alkaline solutions
- potassium hydroxide (OH) is added to solutions of methylene blue Loeffler's Methylene blue

- Some bacteria excrete alkaline materials during growth
- cell surface has a greater negative charge
- Basic dyes stain microorganisms better under neutral or alkaline conditions
- If the dye base molecule has a negative charge, it is repelled by the cell's negatively charged surface
- negatively charged dyes neither bind to the cell's surface nor are they able to penetrate into the cell
- acid dyes
- Negatively charged dyes are called negative stains

# Simple staining

- simple staining solution, contains only one stain
- microorganisms give the colour characteristic of the staining solution
- purpose of simple staining to reveal the size and shape
- simple stains, carbol fuchsin, crystal violet, methylene blue



# Differential Staining

- more than one dye is employed
- Differential staining divide the bacteria into separate groups
- Gram stain and Acid-fast stain

# Gram Staining

- In 1884
- Christian Gram discovered a new technique to differentiate the bacteria of similar morphology

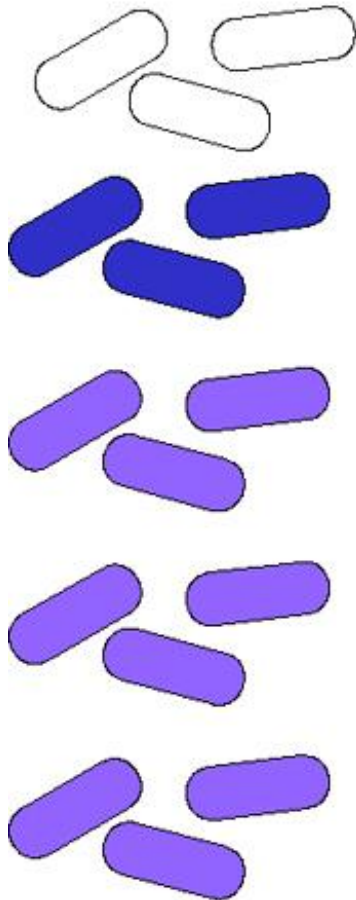
# Principle

- Gram – positive bacteria will retain the crystal violet and appear deep violet in colour
- Gram-negative bacteria lose the crystal violet on decolorization are counter stained by the safranin, appear red in colour
- reactions are associated with the structure and composition of the cell wall
- Gram-negative bacteria are thinner, contain a higher percentage of lipid content
- alcohol treatment extracts the lipid

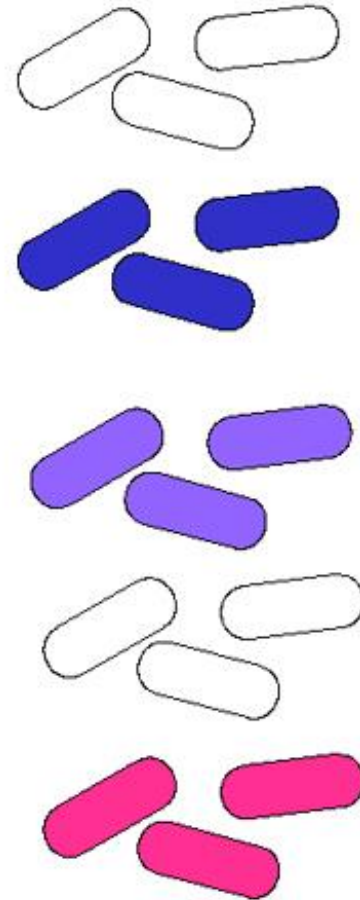
- results in increased porosity or permeability of the cell wall
- violet-iodine complex, can be extracted, decolorized
- take up the colour of the counter stain safranin
- Gram-positive bacteria lower lipid content dehydrated
- pore size decreased, permeability is reduced, CV-I complex can - not be extracted

# Gram staining

**Gram Positive**



**Gram Negative**



Fixation



Crystal violet



Iodine treatment



Decolorization



Counter stain  
safranin

# Endospore Staining

- Endospores strongly resist application of simple dyes, but once stained are quite resistant to decolorization
- simple stains are used, the body of the bacillus is deeply colored, whereas the spore is unstained and appears as a clear area in the organism
- By vigorous staining procedures the dye can be introduced into substance of the spore
- contrasting counter stain is usually applied in the ordinary fashion initial stain taken up by the spore, second stain appear in the cytoplasm