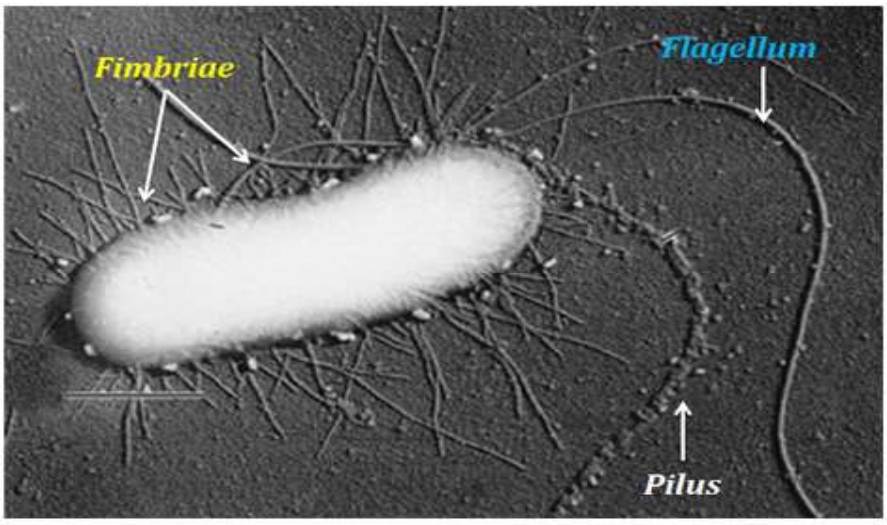
Pilli (Fimbriae): They are appendages found in many G-ve bacteria , shorter and finer than flagella , composed of protein subunits called pillin.



Pilli can be classified into 2 types:

1.Ordinary pilli : their role in adherence of symbiotic and pathogenic bacteria to host cells , which is called colonization Ag.

2.Sex pilli ; which are responsible for attachment of donor and recipient cells in bacterial conjugation.

Endospores :

1-The most common bacterial genera that form spores are Bacillus, Clostridium and the bacterial group Actiomycetes.

2-Endospores are forms of cellular differentiation undergo as a response to environmental conditions e.g. nutritional depletion.

3-The spore is a resting cell highly resistant to desiccation , heat and chemical agents.

4- At favorable environmental conditions, spores are activate and germinate producing single vegetative cells.

Structure of the spores :

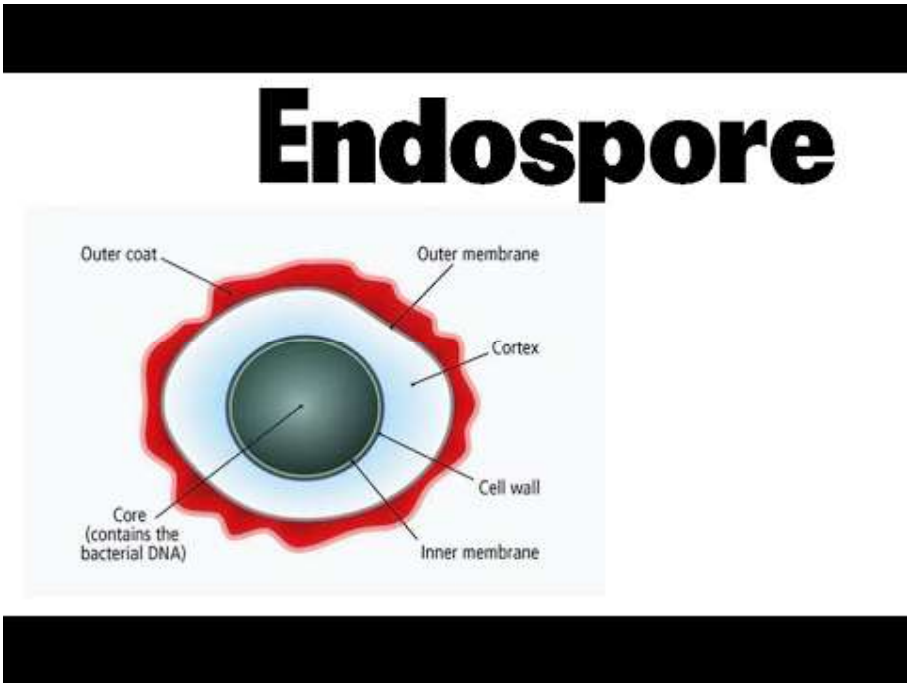
1.Core (spore protoplast) : contain DNA , proteins , enzymes of glycolysis , Ca-dipicolinate (which involves in spore heat resistance as enzyme stabilizers).

2.Spore wall ; consist mainly of peptidoglycan.

3.Cortex : represent the thickest layer of the spore envelope , composed of special type of peptidoglycan.

4.Coat : composed of keratin-like protein , pocessing high impermeability to antibacterial and chemical agents.

5.Exosporium : is a lipoprotein membrane with some carbohydrates.



Spore germination :

There are 3 main stages:

1.Activation : after resting period (days) , they can be activated by rich nutrient media, heat , abrasion , acidity or compounds containing free sulphydry groups .

2.Intiation : spores are containing receptors to recognize different effectors (signaling factors) , e.g L-alanine or adenosine (initiation triggers) .

3.Outgrowth : degradation of cortex and and outer layers releasing the protoplast with its cell wall as a new vegetative cell .

Biosynthesis period is started to build up the growing cell inside rich nutritional media to support cellular growth.

Bacterial staining:

- **Basic stains :** Consist of a colored cation and colorless anion , e.g. methylene blue+ & Cl^-.
- **Acidic stains:** Na+ and eosinate $^-$, e.g. safranine and carbol fuchsin.
- **Bacterial DNA :** are negatively charged → combine with basic dyes (positively charged).

Acidic dyes do not stain bacterial cells, and be used as a contrasting color as they stain background material .

A)The Gram´s stain : called as a differential stain (compound stain):

Procedure:

1.Crystal violet (1 min) → blue cells

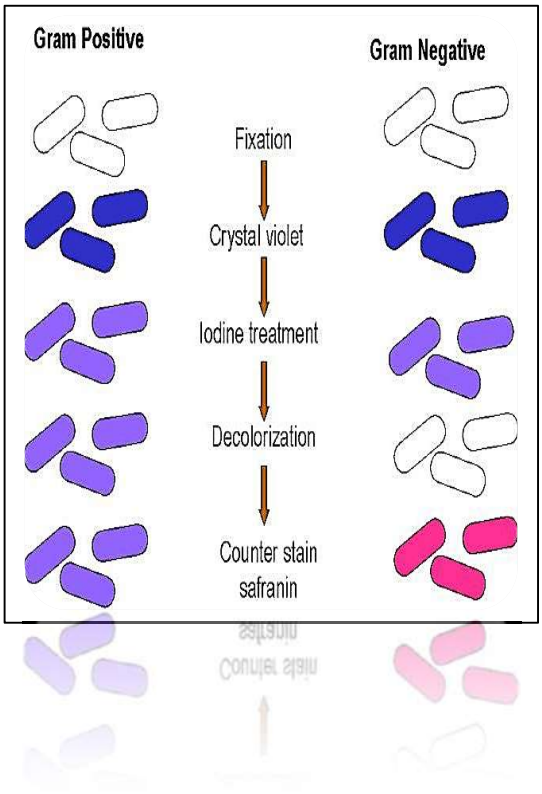
2-Iodine solution (2min) →mordant (fixative) blue cells.

3-Alcohol : (10-30 sec.)

→ Decolorize

blue cells= G+ve

→Colorless cells =G-ve



4-Counter stain (safranin) (1-2 min)

→ Bluish purple cells =G+ve

→Red cells = G-ve

5.Examining under the microscope

B)The Acid-fast stain: To stain and differentiate acid-fast bacteria , as these cells retain carbol fuchsin red dye after treatment with strong decolorizer (like acid alcohol).

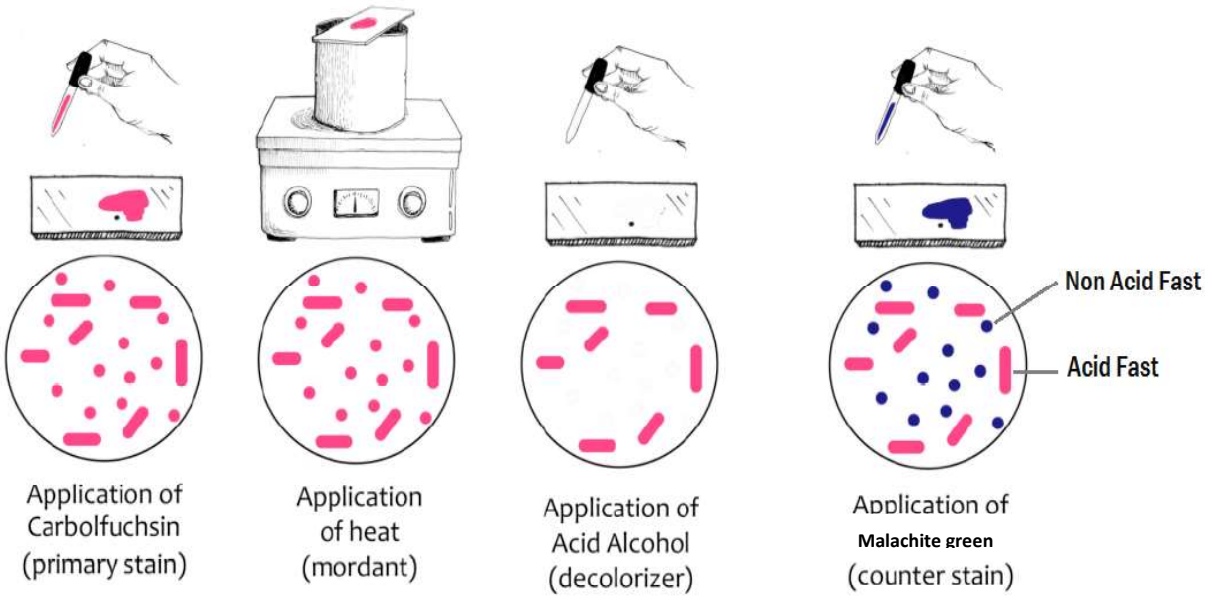
Procedure :

1.Carbol fuchsin (flooding smear) and steam bath (5 min.).

2.Acid- alcohol (decolorization) (15-20 sec.).

3.Malachite green (contrast) counter stain (1-2 min.).

- A-F bacteria (red) ;e.g. Mycobacteria and related Actinomycetes.
- Non A-F (green) … other bacteria.



c)Negative stain :staining the background with an acidic dye , leaving the cells contrastingly colorless .

Nigrosin black dye is commonly used (China ink) (India ink).