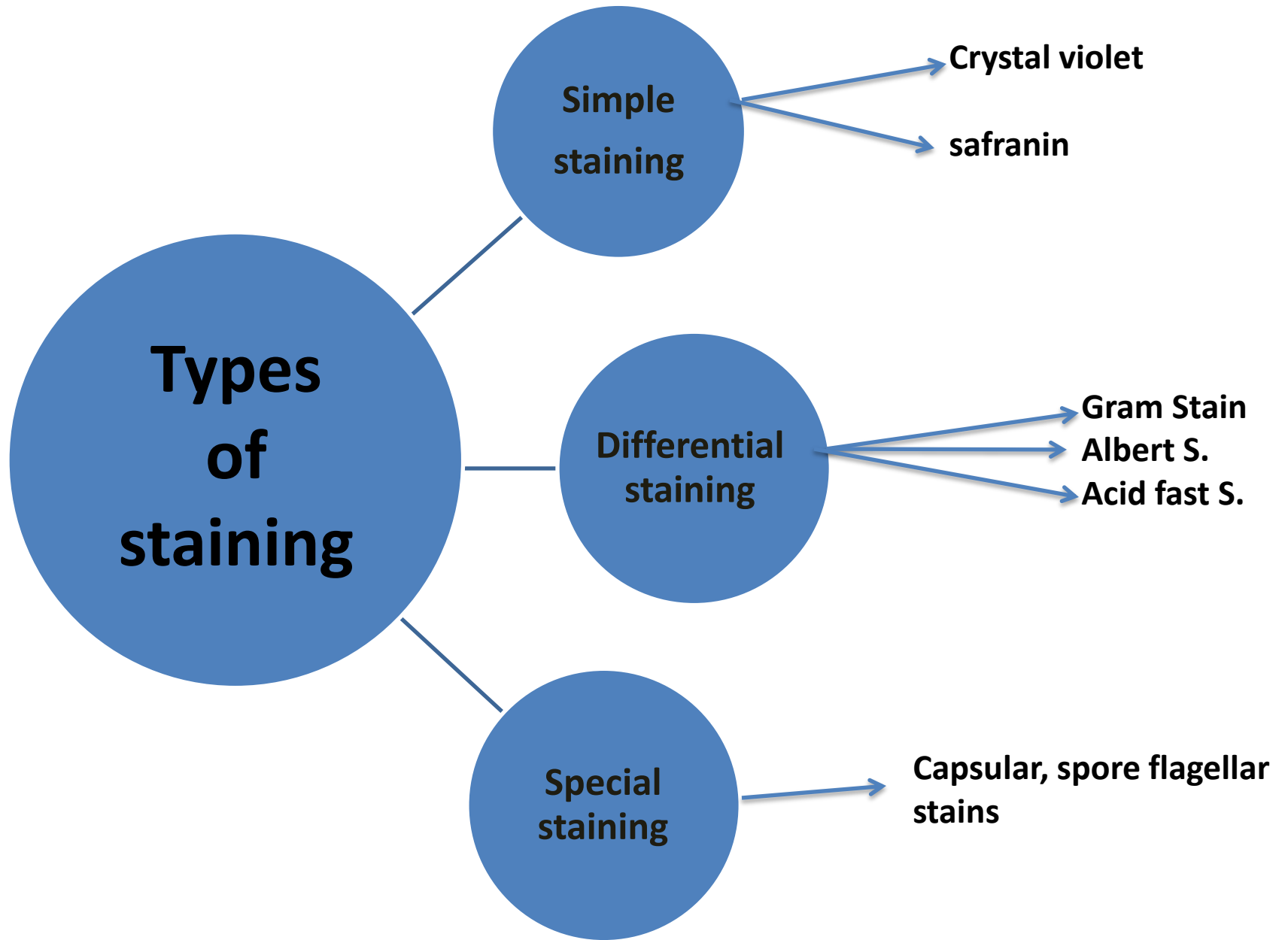


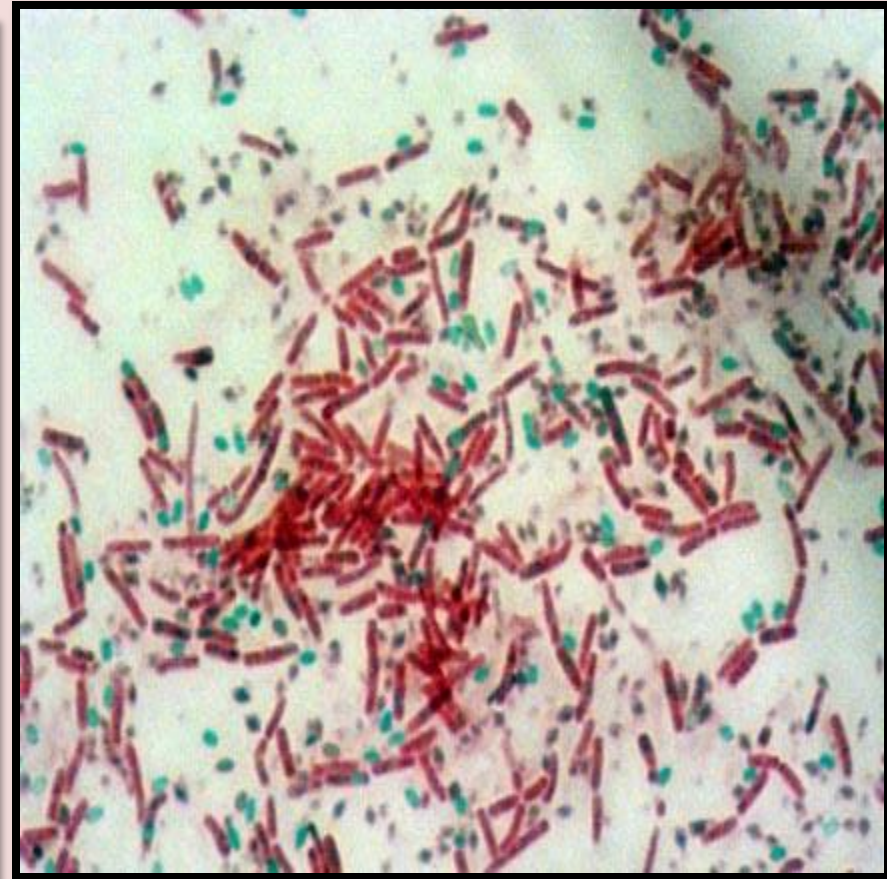
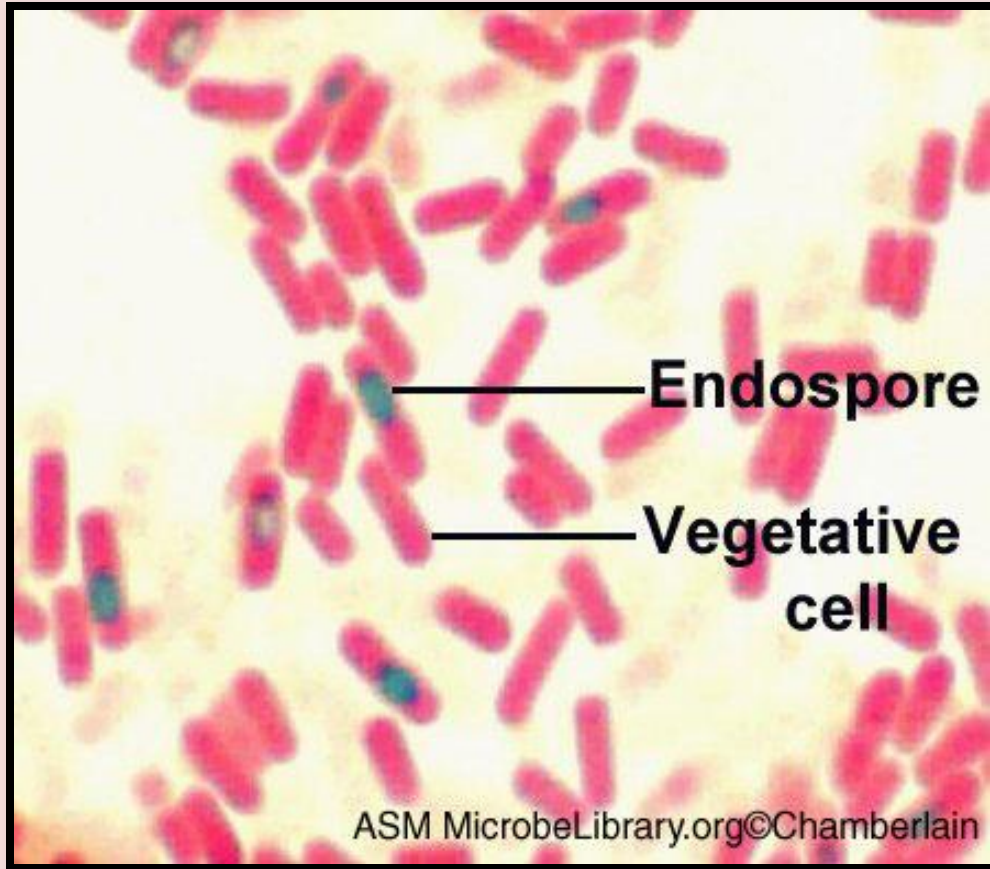
Bacterial Staining





Flagellar stain

Spore Stain



The Gram stain has four steps:

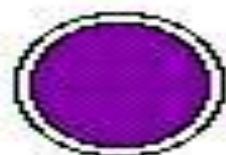
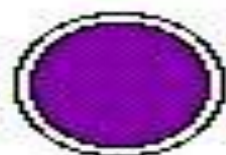
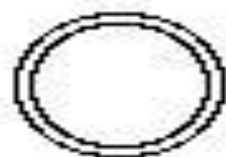
**1. crystal violet, the *primary stain*:
followed by**

**2. iodine, which acts by forming a crystal
violet-iodine complex, then**

3. alcohol, which *decolorizes*, followed by

4. safranin, the *counterstain*.

GRAM +



Fixation



Crystal
Violet



Iodine
treatment

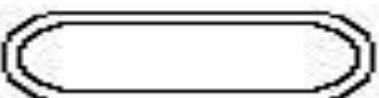
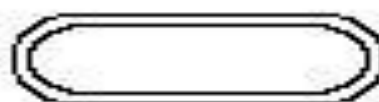


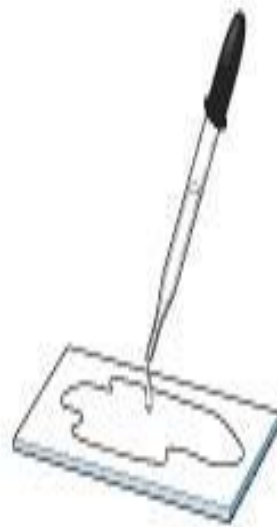
Decolorization



Counter stain
(safranin)

GRAM -





- Crystal violet
- Iodine
- Alcohol
- Safranin



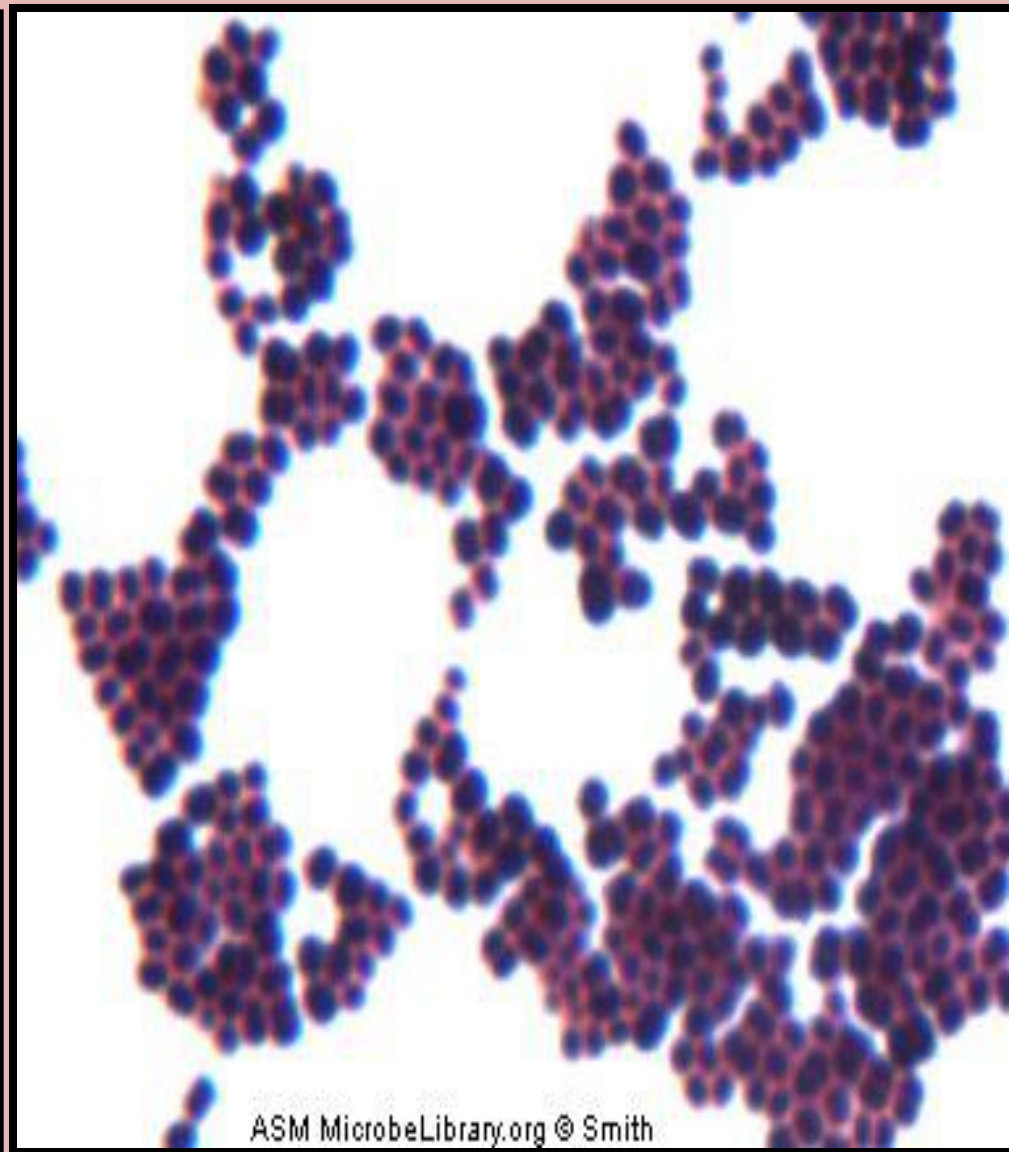
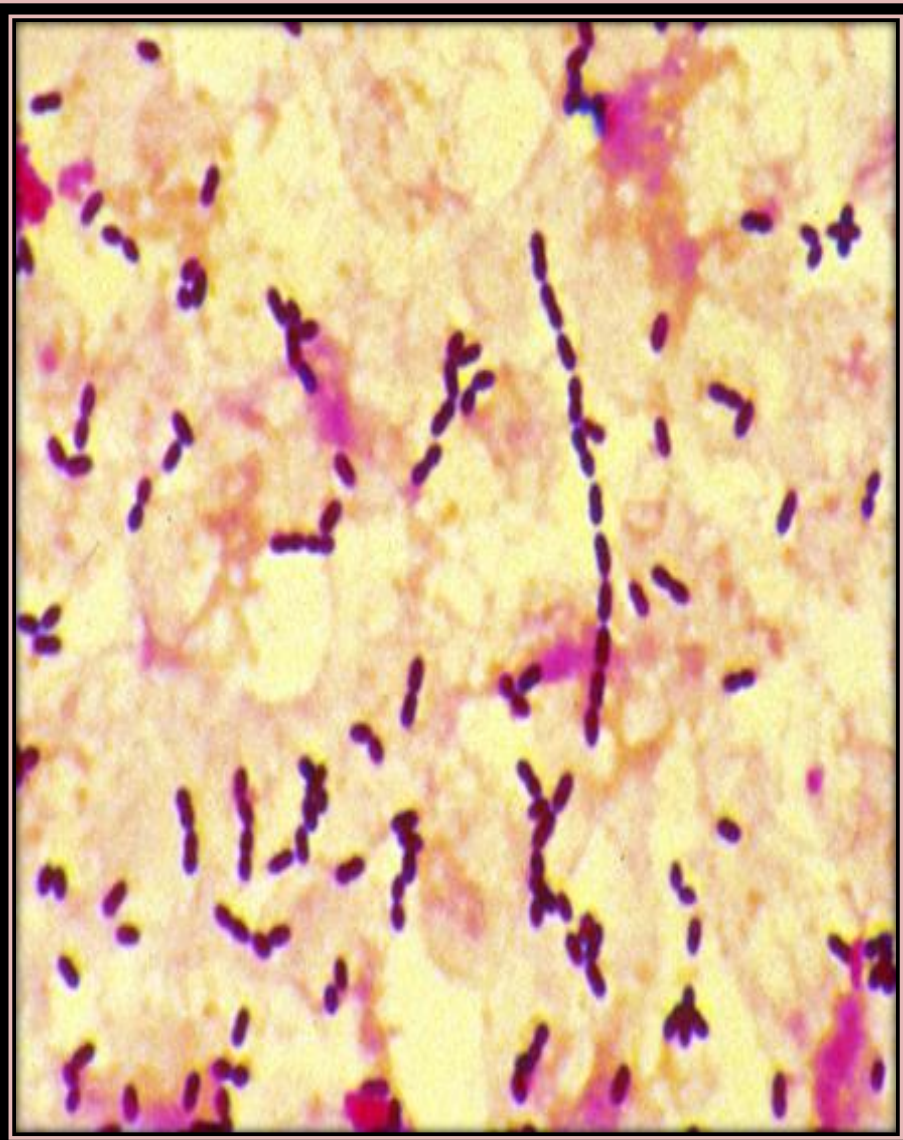
1 Application of crystal violet (purple dye)

2 Application of iodine (mordant)

3 Alcohol wash (decolorization)

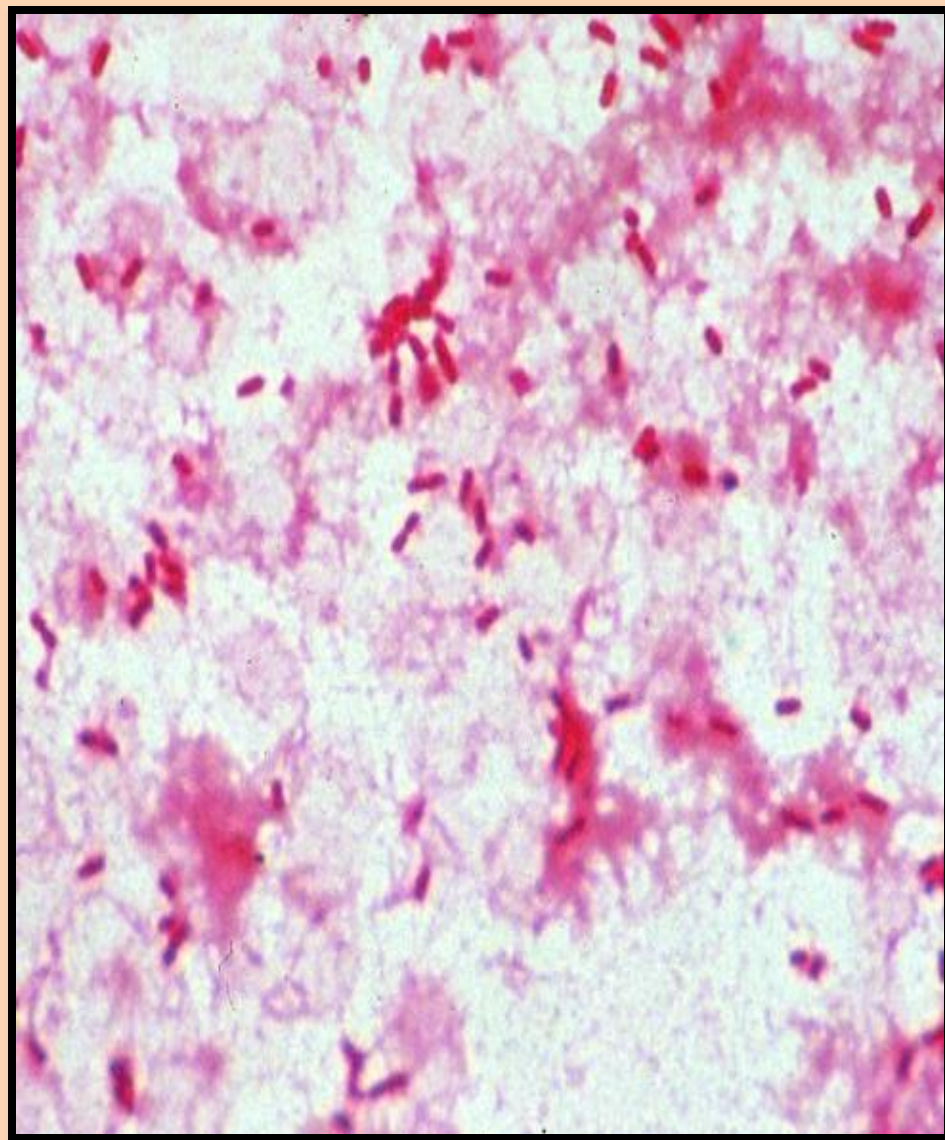
4 Application of safranin (counterstain)

(a)

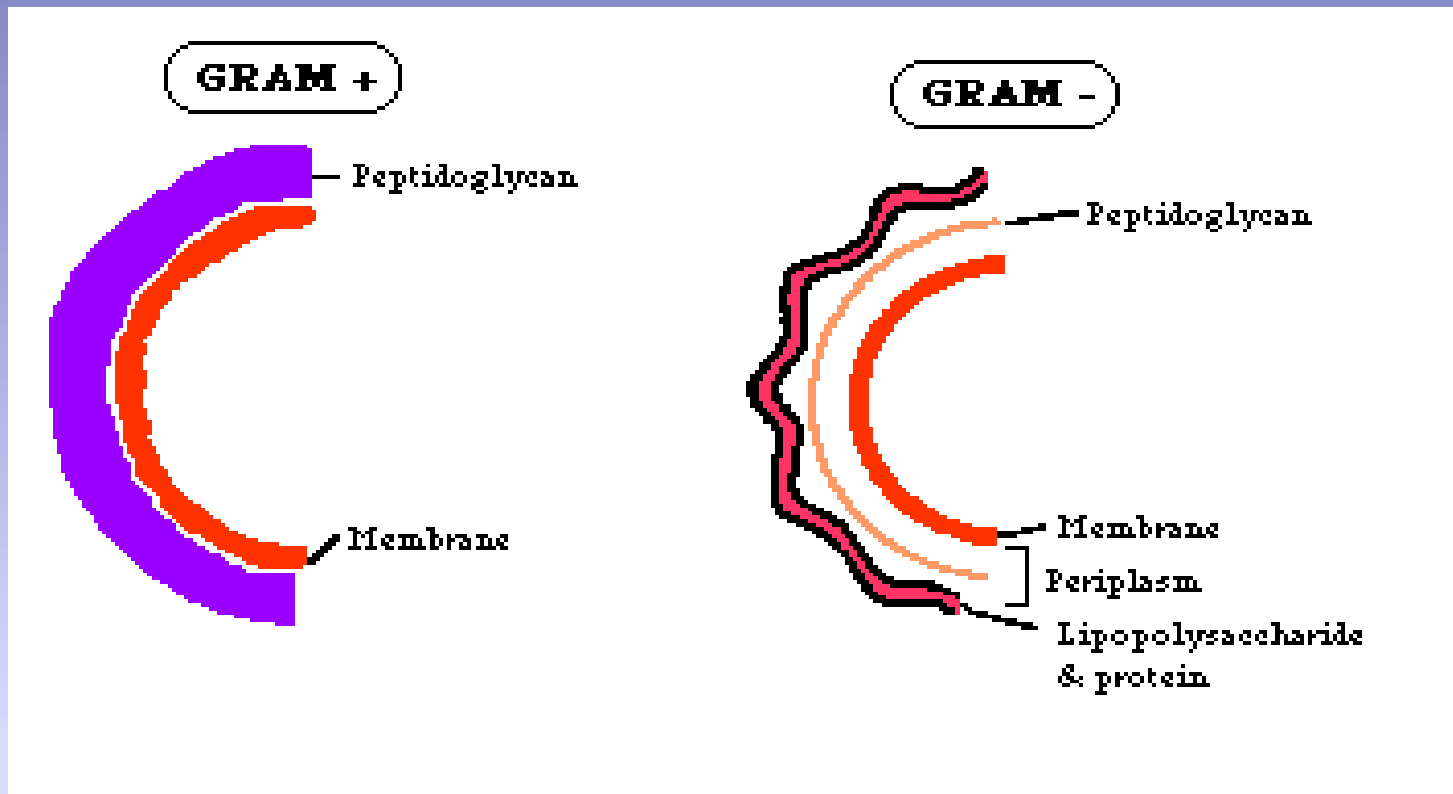


ASM MicrobeLibrary.org © Smith

gram positive bacteria.



gram negative bacteria.



In Gram-positive bacteria, the purple crystal violet stain is trapped by the layer of peptidoglycan which forms the outer layer of the cell. In Gram-negative bacteria, the outer membrane of lipopolysaccharides prevents the stain from reaching the peptidoglycan layer. The outer membrane is then permeabilized by acetone treatment, and the pink safranin counterstain is trapped by the peptidoglycan layer.