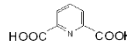
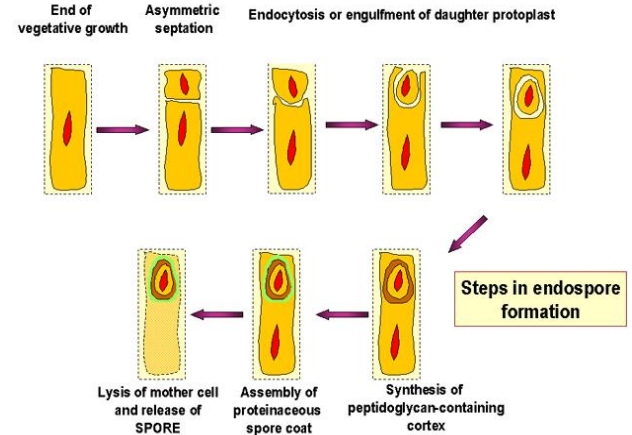


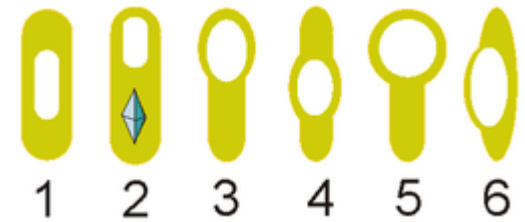
Spores - endospores



- Some pathogenic Gram positive bacteria from genus **Bacillus** and **Clostridium** - can form spores. Their localisation is diagnostic
- **Sporulation** - In unfavorable conditions the vegetative cell forms one spore (dormant phase of life cycle) – formation of multiple layers – coat – dipicolin acid and Ca

Resistance to environmental extreme conditions

- Endospores can survive environmental assaults that would normally kill the bacterium. These stresses include high temperature, high UV irradiation, desiccation, chemical damage and enzymatic destruction.
- **Germination** – a reverse process transform the spore into a vegetative cell.
- return to vegetative phase of life cycle – breaking of coat, water intake
- Activation – 1st step of germination – changes in environmental condition – mild temperature, alanin



Variations in endospore morphology:
 (1, 4) central endospore;
 (2, 3, 5) terminal endospore;
 (6) lateral endospore



Cell wall of vegetative cell

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Thin outer spore coat

Immature endospore

Sporangium (mother cell)

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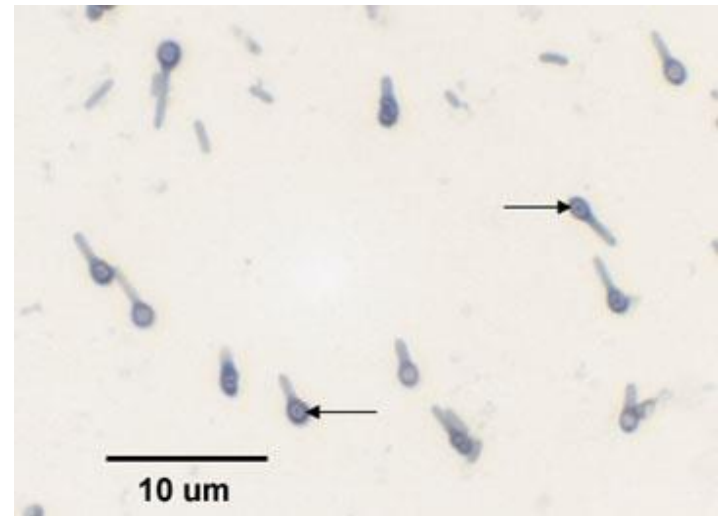
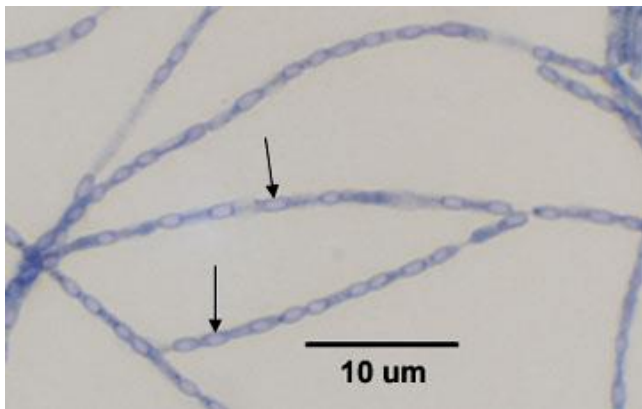
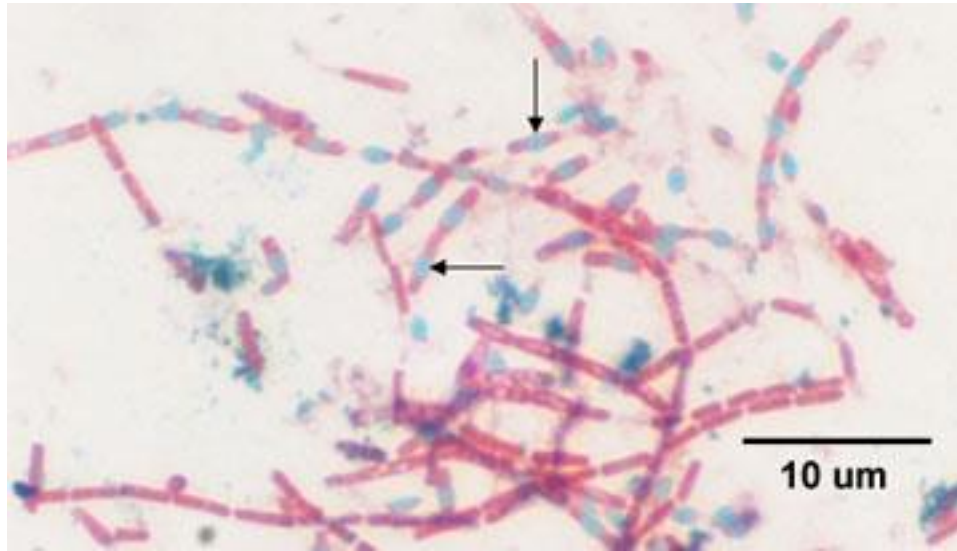
Thick outer spore coat

Sporangium (mother cell)

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Wirtz Conklin

- Differential staining form spores
 - Spore forming bacteria – red
 - Spores – green
1. Prepare fixed smear: suspension dried on the air, heat fixed
 2. colorization: 5% malachit green - heating untill evaporation 3times (3-6 minutes).
 3. Rinse under water flow
 4. add 0,5% water solution of carbol fuchsin - counterstaining (60 sec.)
 5. Rinse with water, dry and read



Metachromatic staining - principle

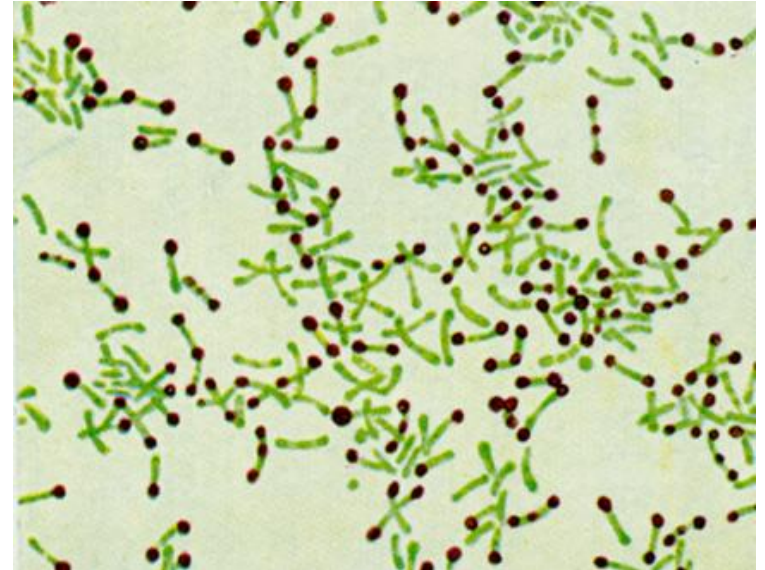
Metachromatic granule

- granular cell inclusion that stains a color different from that of the dye used.
- they are composed of complex polyphosphate, lipid, and nucleoprotein molecules (volutin) and serve as an intracellular phosphate reserve.
- called also Babès-Ernst body or volutin granule (nucleoprotein complex) . storage granules

Differentiation of *Corynebacterium diphtheriae*
from saprophytic corynebacteria.

Albert's staining

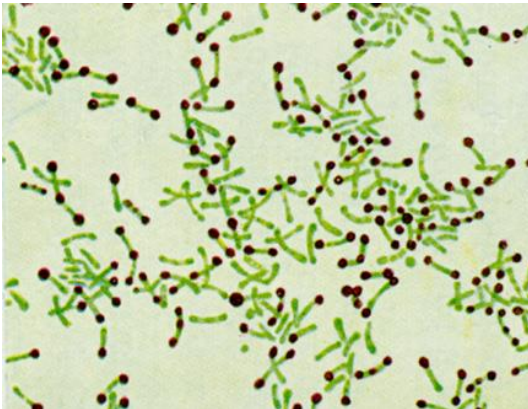
1. Fixed smear – *C. diphtheriae*
 2. Apply Albert's solution I. - allow it to act for 5-7 min
 3. Pour off the stain
 4. Add Albert solution II. (Lugol solution) 2 min.
 5. Rinse with water
- Bacteria are green (malachit green)
granules are dark blue (bluish black)



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