

Diagnostic Microbiology



➤ Isolation of pure culture from specimen

✓ why?

➤ Culture media

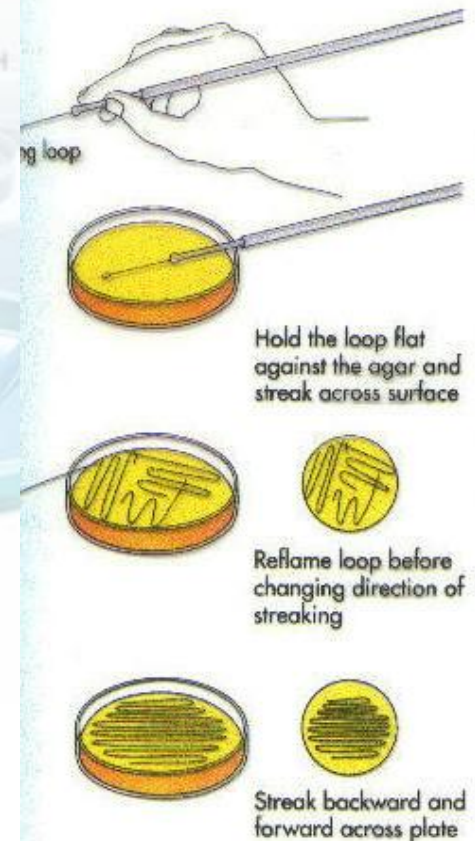
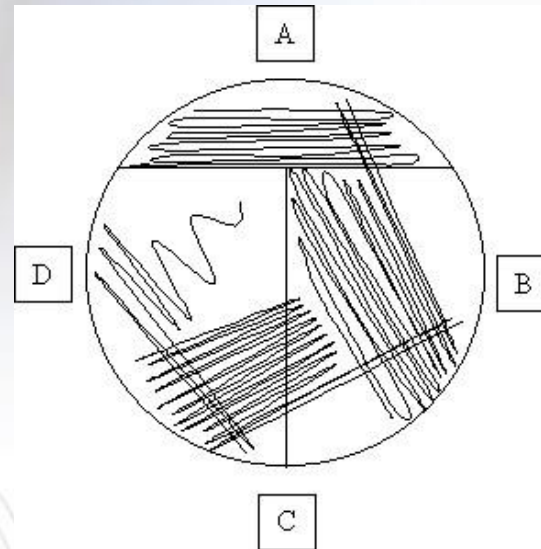
✓ why?

✓ what?

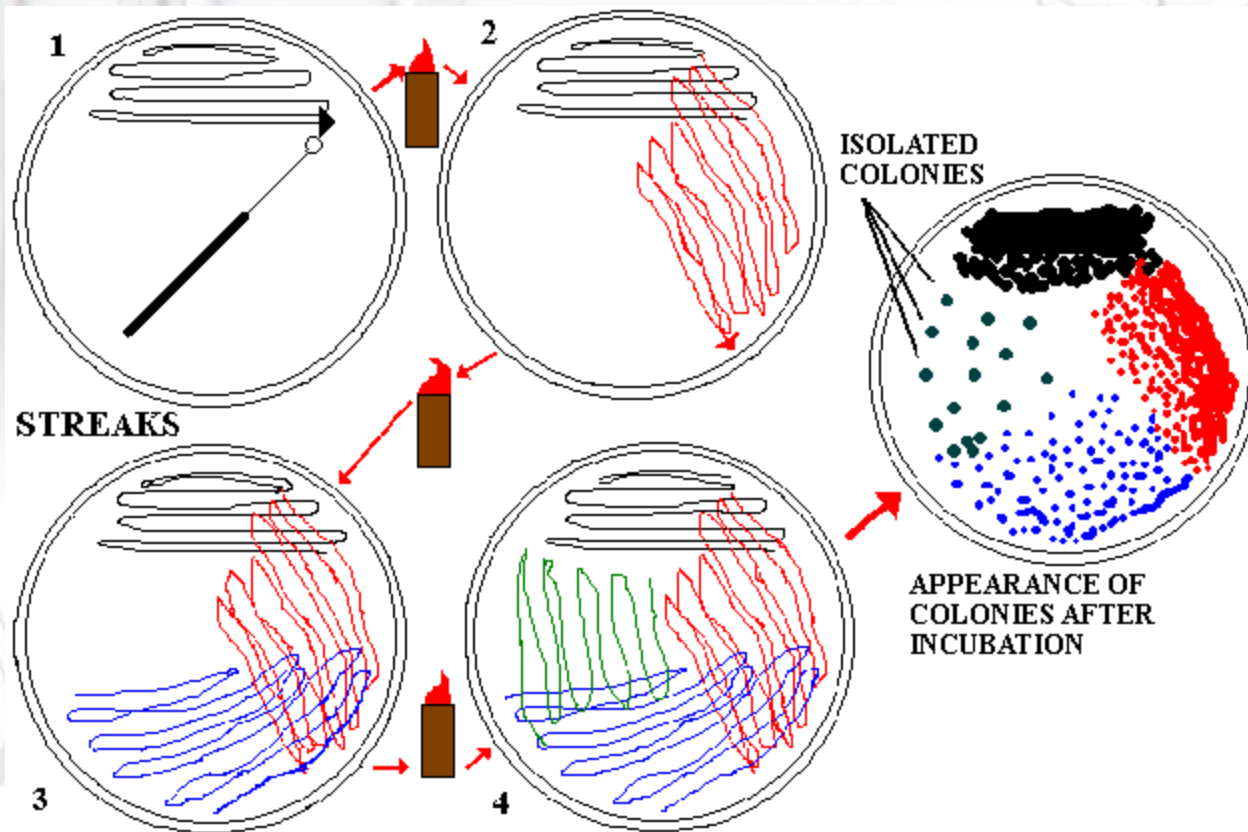
✓ who?

➤ Inoculation methods

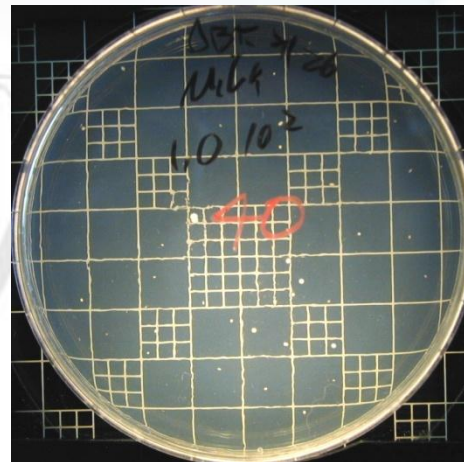
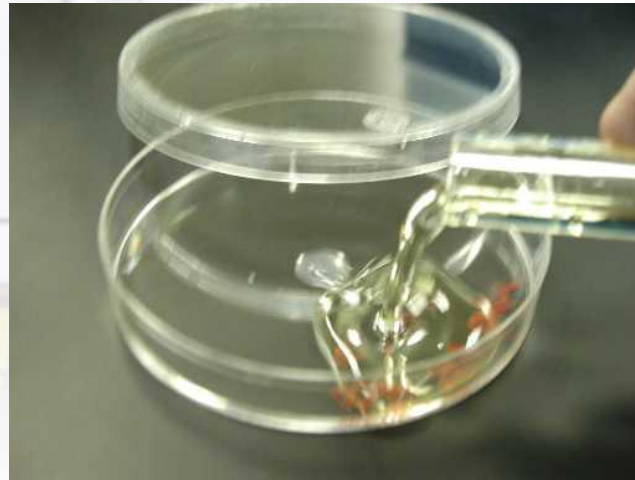
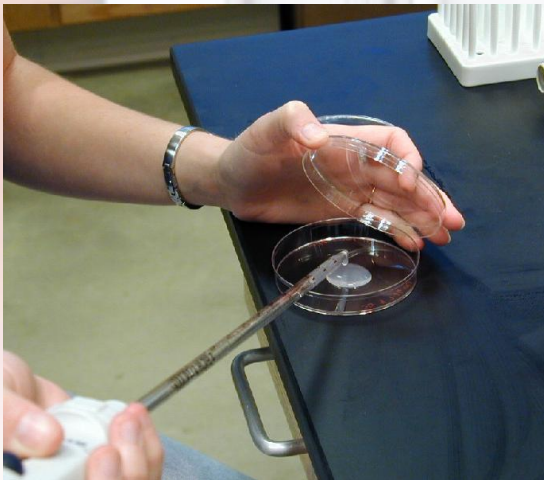
✓ streak, spread, or pour



Streaking a plate for isolated colonies

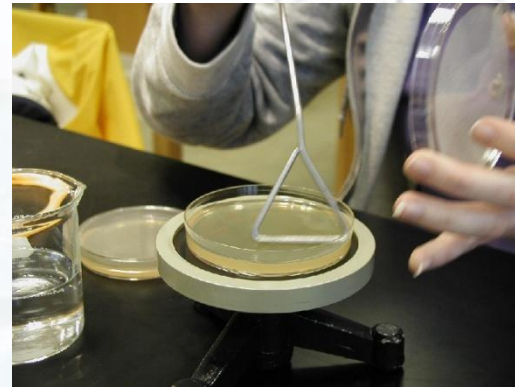


Pour plates



- Bacteria grow inside agar
- Used for enumeration of bacteria

Spread plates



Preservation of Cultures



- pure cultures of bacteria are stored:
- freeze-dried (lyophilized)
- frozen at -80°C



lyophilizer



Ultra-low freezer

Preservation of pure cultures



- Why would we want to keep a “copy” of a bacteria we isolated from a patient?
- Short term versus long-term
 - ✓ liquid nitrogen (-195°C)
 - ✓ freezers
 - ✓ lyophilization (freeze-drying)

Identification



- Now that you have a pure culture...
 - ✓ colony morphology
 - ✓ cellular morphology
- The microscope is your friend
 - ✓ resolving power (resolution) = ability to distinguish two closely located objects as separate, distinct entities



Colonial morphology



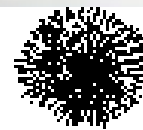
FORM



Punctiform



Circular



Filamentous



Irregular



Rhizoid



Spindle (lens)

ELEVATION



Flat



Raised



Convex

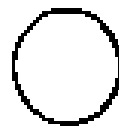


Pulvinate



Umbonate

MARGIN



Entire
(even)



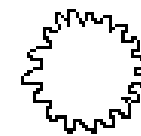
Undulate
(wavy)



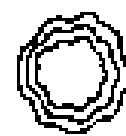
Filamentous



Lobate
(lobes)



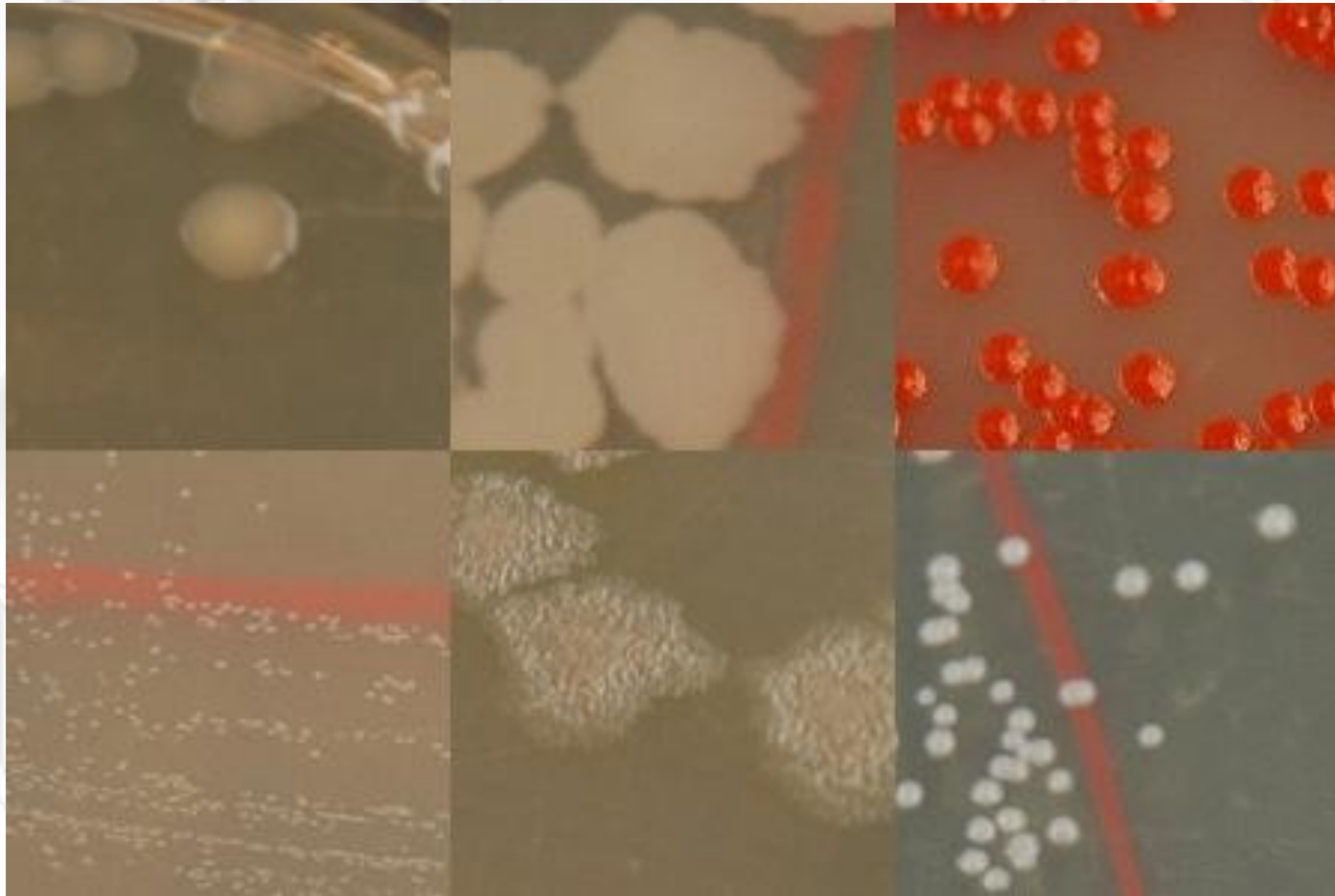
Erose
(serrated)



Curled



Examples of colonial morphologies



Identification

- Staining Techniques -



- Generally, three steps:
1. Make a smear
 2. Fix dried smear by heat
 3. Stain with desired dye



Simple vs. Differential staining



- Simple stain
 - ✓ single dye normally used
 - ✓ all organisms same colour
 - ✓ size, shape, number, arrangement, etc.
- Differential stain
 - ✓ two or more dyes
 - ✓ differences between microorganisms or parts of cells
 - ✓ acid fast, **Gram**



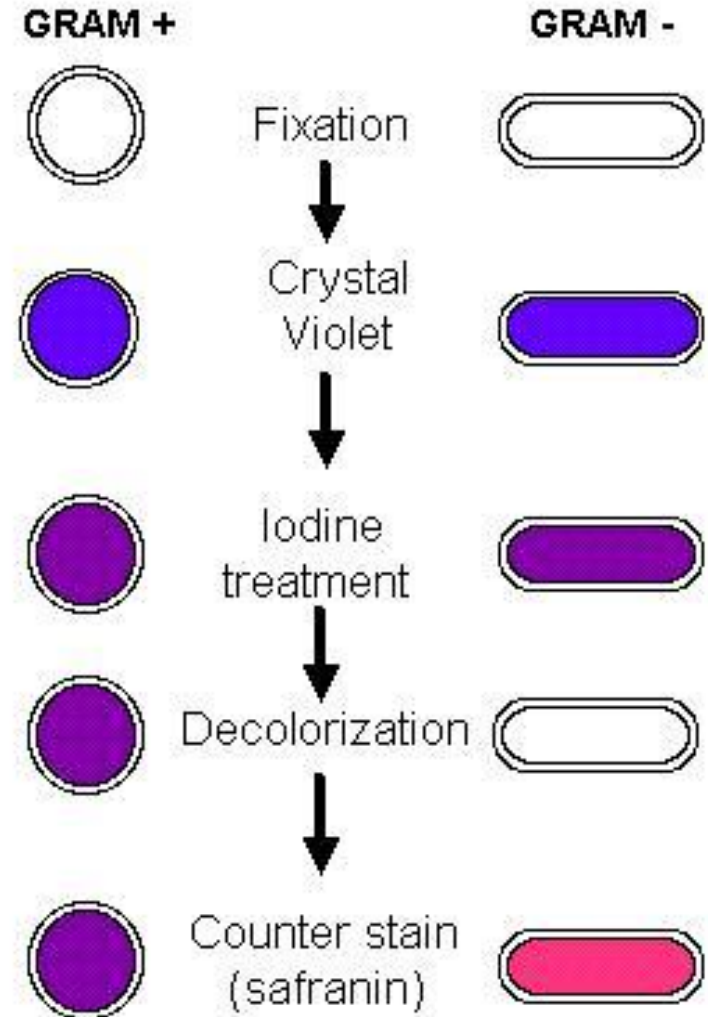
The Gram Stain (Hans Christian Gram)



1. Flood slide with crystal (or gentian) violet. (Wash with running tap water).
2. Flood with Gram's iodine. (Wash with water).
3. Carefully decolorize with 95% ethanol. (Wash with water).

This third step is the most critical and also the one most affected by technical variations in timing and reagents.

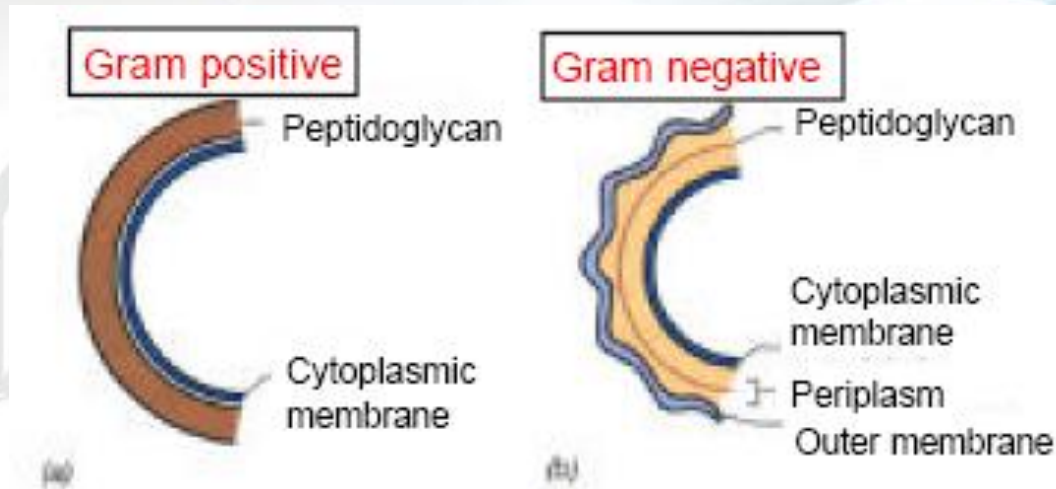
4. Flood with safranin (pink color). (Wash with water). Air dry, or blot with absorbent paper



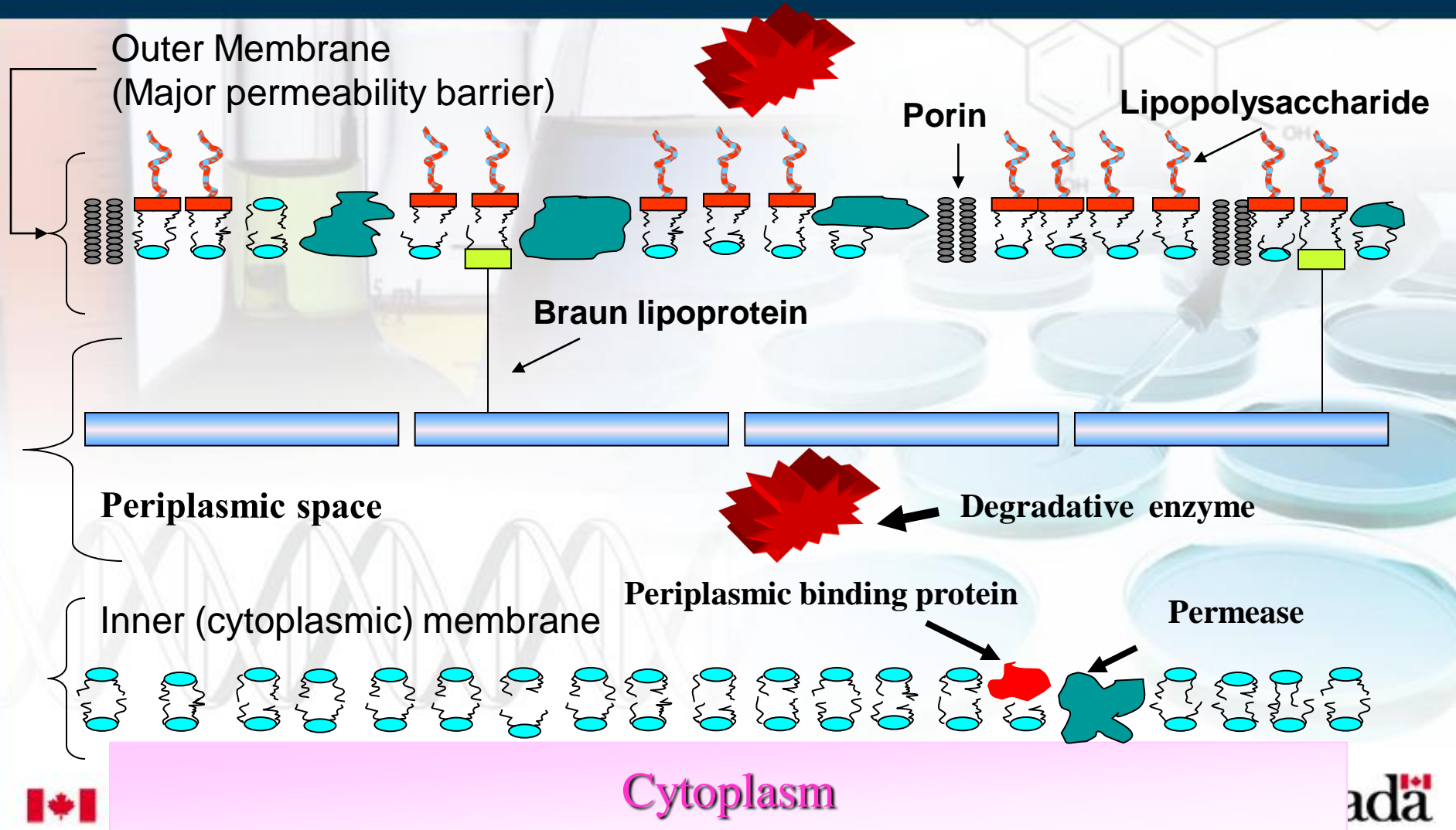
Cell wall is the key!



- Essential for cell growth and division
- Shape of bacteria related to peptidoglycan layer
- Gram negative usually thinner than Gram positive



GRAM NEGATIVE CELL ENVELOPE



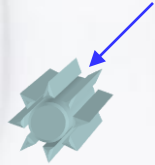
Cytoplasm

ada

GRAM POSITIVE CELL ENVELOPE

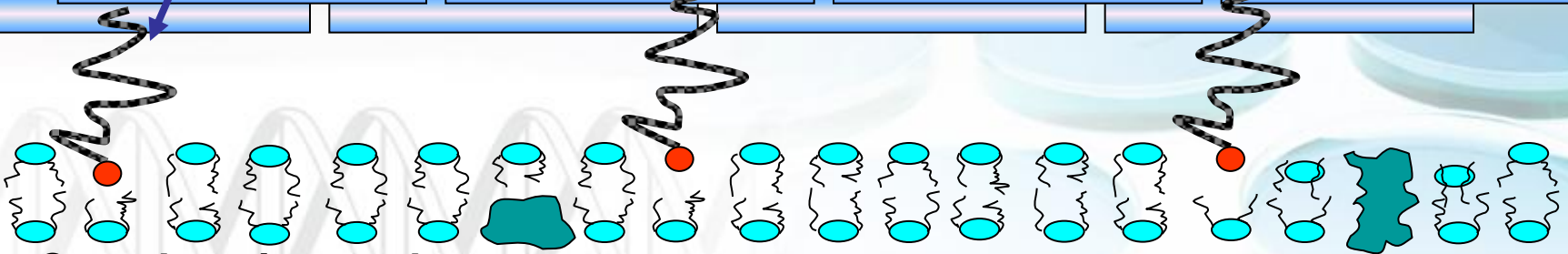
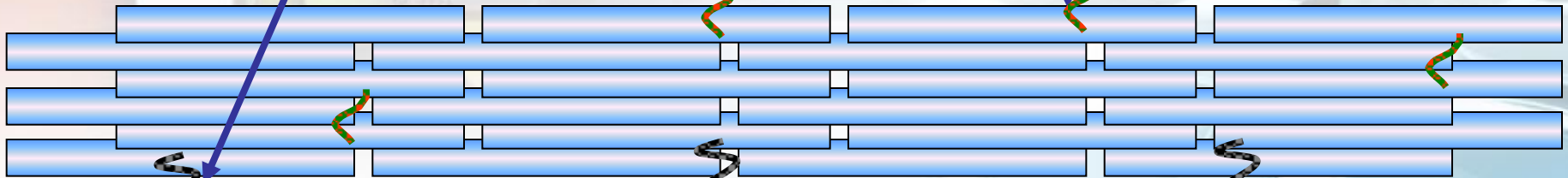


Degradative enzyme



Lipoteichoic acid

Peptidoglycan-teichoic acid



Cytoplasmic membrane

Cytoplasm



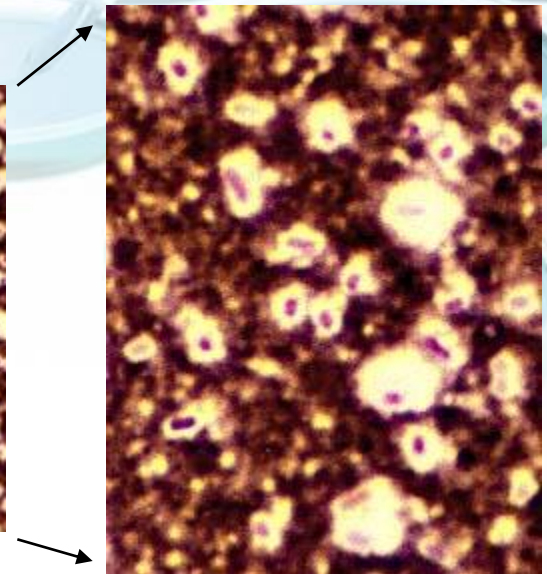
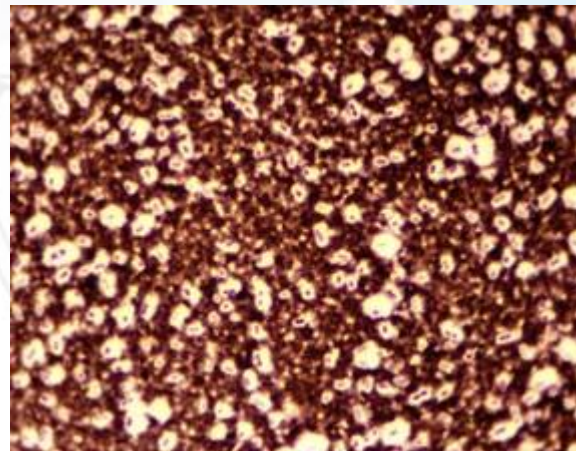
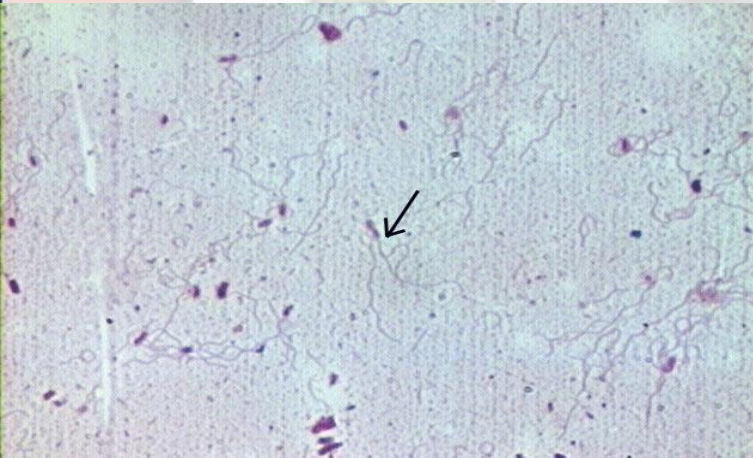
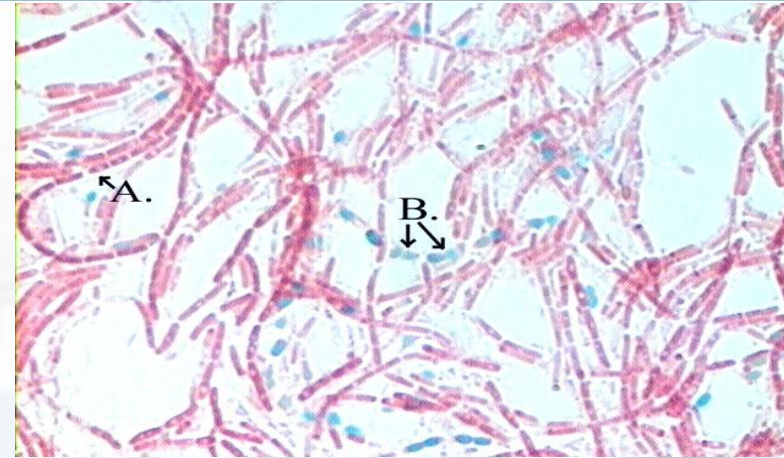
Health Canada
Santé Canada

Canada

Other stains



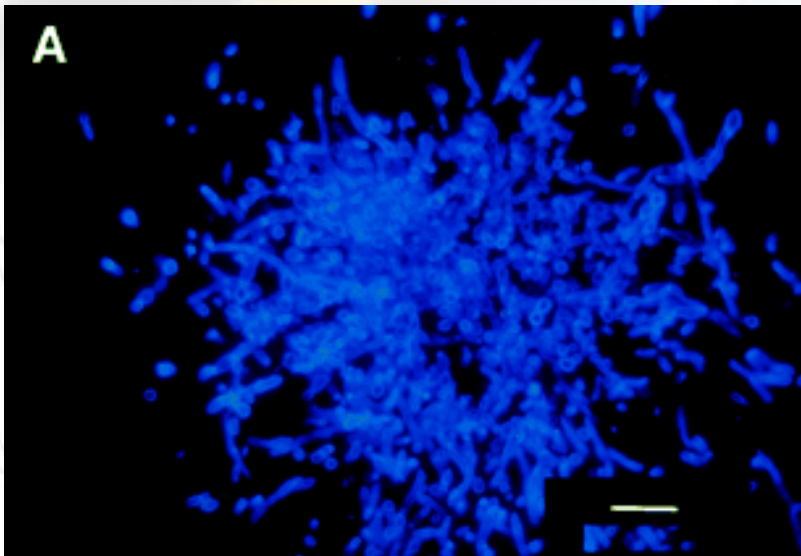
- Endospore
- Capsule
- Flagella



Fluorescence microscopy



- dye fluoresces at specific wavelength
- antibodies tagged with dyes are common (immunofluorescence microscopy)

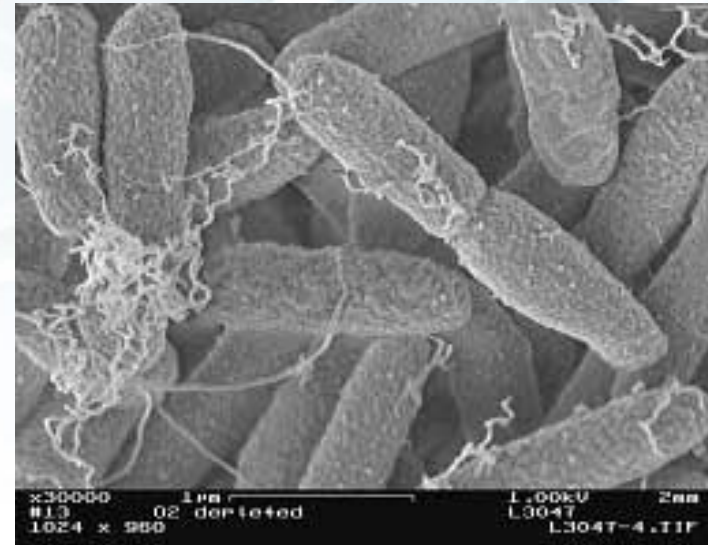


A section of the liver of a leukemic patient who had succumbed to culture-proven *C. albicans* mycosis. (staining done with Blankophor)

Electron microscopy



- Electron beam (instead of light)
- Million times magnification possible ($0.003 \mu\text{m}$)
- ✓ TEM (stain with heavy metals)
- ✓ SEM (3-D image of cell surface)



Electron microscopy



Scanning electron microscope (SEM)



Transmission electron microscope (TEM)

So what's the bottom line?



- Morphology helps to classify and identify
 - ✓ Gram stain
- Gives clues to how they behave in environment
 - ✓ capsules, endospores

Characteristics of bacteria



- Small (0.75 – 1.25 μm in diameter/width)
- Higher surface area / volume ratio
 - ✓ higher metabolism
 - ✓ faster growth
 - ✓ replication rate (~20 minutes)

Shapes and sizes of bacteria

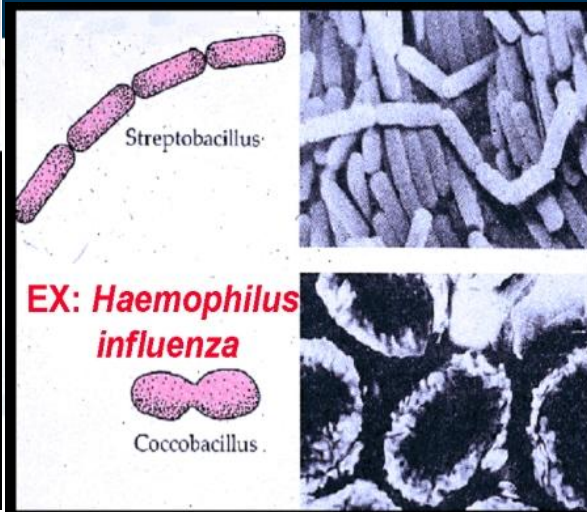
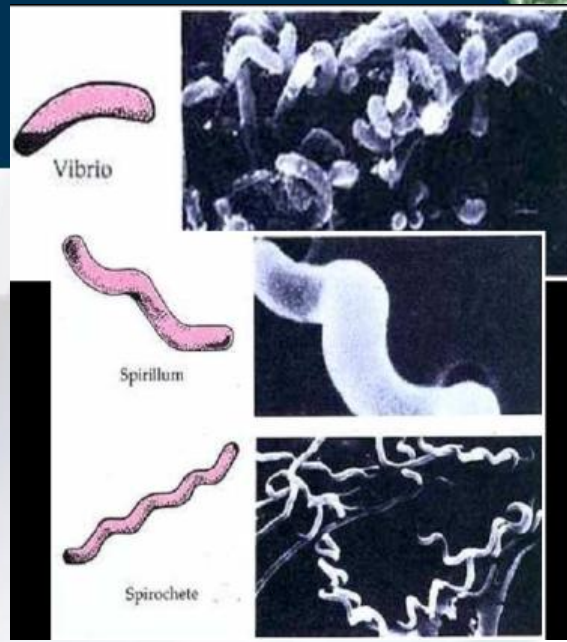
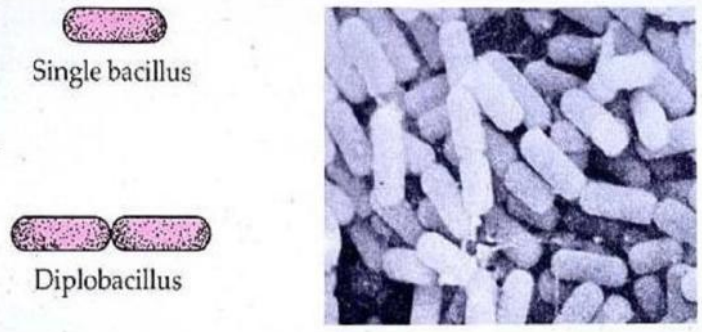


- Bacteria are usually arranged in specific patterns:
 - ✓ single cells (spiral and/or rod shaped)
 - ✓ diplococci (pairs) – single plane
 - ✓ chain (divide in one plane and remain attached)
 - ✓ tetrads (cocci dividing at right angle to first plane of division)
 - ✓ division in three planes (grapelike clusters)
 - ✓ cubical packet of 8 cells (sarcinae)

Shapes and sizes of bacteria

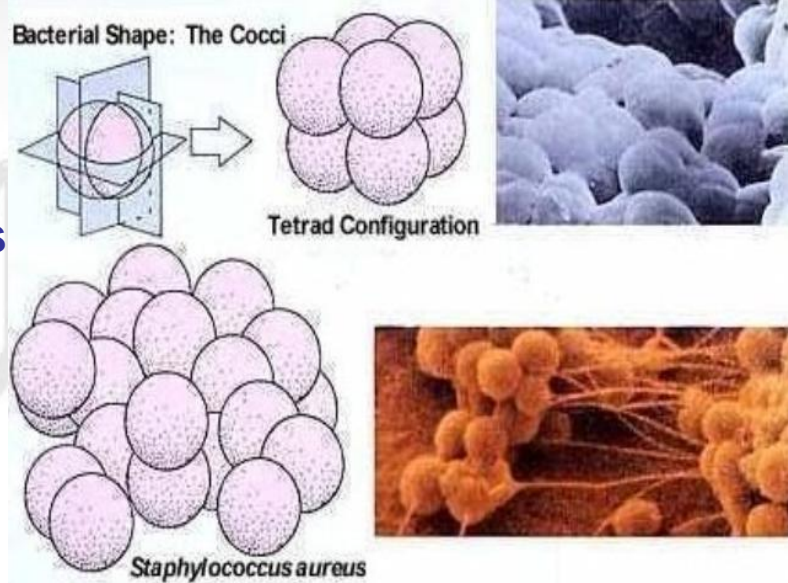


The Rod-Shaped Bacteria



EXAMPLES: *E. coli*, *Yersinia pestis*, *Pseudomonas aeruginosa*

!!!These are only examples of general shapes!!!



With what do we grow bacteria?



Salmonella typhimurium

Gram Negative: growth

Lactose Fermentation: negative
(colorless colonies)



Escherichia coli

Gram Negative: growth

Lactose Fermentation: positive
(pink colonies)



Definitions....😊

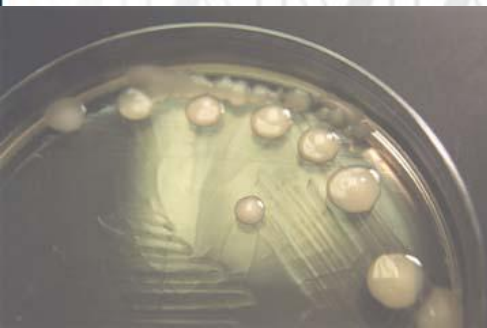


- Chemically defined – exact composition known
- Chemically undefined – some components can't be controlled (beef extract, blood, etc.)
- If solid (versus liquid) growth – 1.5% agar used
- Enrichment media – increase # of specific bacteria in sample by favouring growth of interested species
- Tissue culture media – for cultivating viruses, derived of plant or animal cells

General media requirements



- Bacteria – requirements vary
- Yeasts – high sugar and lower pH
- Anaerobes – must remove oxygen



Selective, differential and S/D media



- Selective media – enhance growth of one bacterial species or suppression of another
- Differential media – differentiate bacteria based on their nutritional requirements and phenotypic characteristics
- Selective / Differential media – very useful in clinical labs (e.g., MacConkey agar)

MacConkey – S/D media

(Bile salts, crystal violet inhibit Gram +ves)



Salmonella typhimurium

Gram Negative: growth

Lactose Fermentation: negative
(colorless colonies)



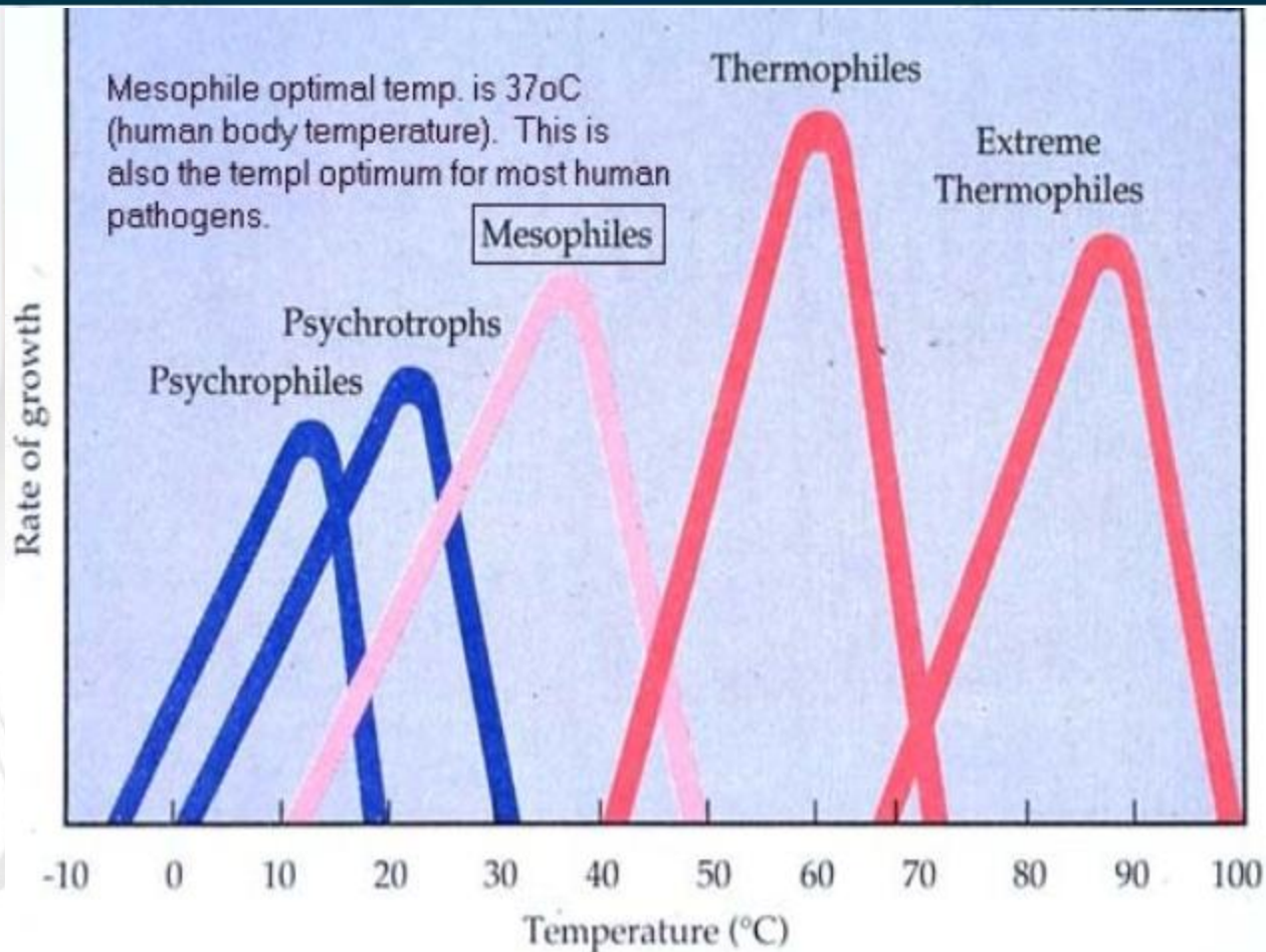
Escherichia coli

Gram Negative: growth

Lactose Fermentation: positive
(pink colonies)



Temperature requirements



Temperature definitions



➤ Psychrophiles

- ✓ grow best at temperatures 15-20°C

➤ Mesophiles

- ✓ grow best at temperatures 25-40°C
- ✓ most bacteria belong here

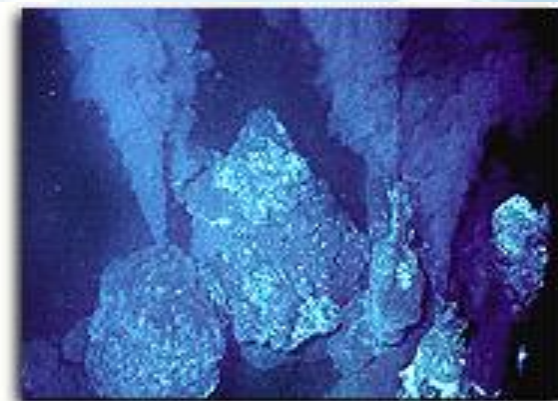
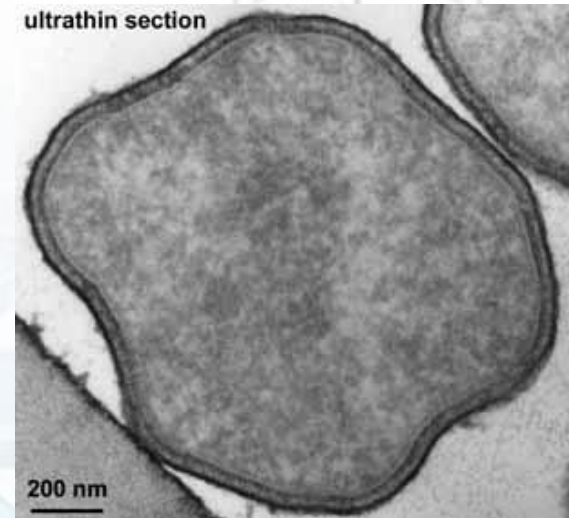
➤ Thermophiles

- ✓ grow best at temperatures 40-85°C

Extreme Thermophiles



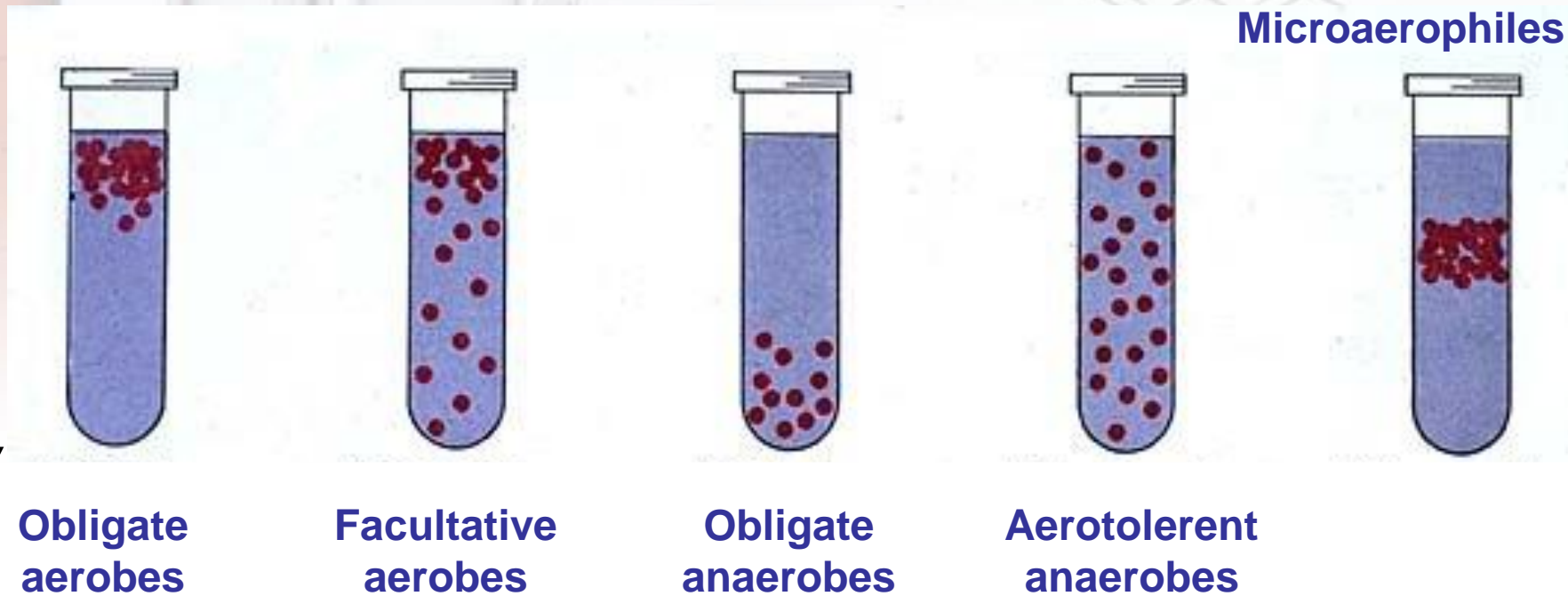
- *Pyrolobus fumarii*
- “fire lobe of the chimney”
- Lobed shape
- Discovered in the walls of a deep sea hydrothermal vent
- Grows between 30 and 113C
 - 106C is optimal



Oxygen requirements



Decreasing oxygen concentration ↓



Growth of anaerobic bacteria



Anaerobic jar



Coy anaerobe chamber



pH and Water requirements



- Optimal pH varies from bacteria to bacteria
- Intracellular pH must be ~7.5
- Growth observed at pH values of 4-9 (optimum 6-8)
- Water (light) can be important for certain microorganisms
- Osmotic pressure (hypertonic, hypotonic, isotonic)