Chapter 3

Topics

- Methods of Culturing Microorganisms
- Microscope

Methods of Culturing Microorganisms

- Different types of media
- Different types of microscopy

A single visible colony represents a pure culture or single type of bacterium isolated from a mixed culture.

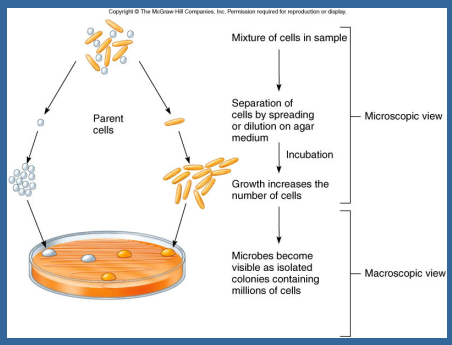
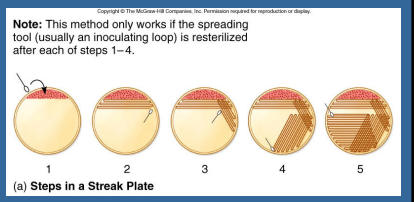
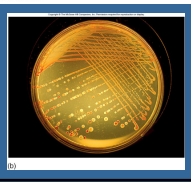


Fig. 3.2 Isolation technique

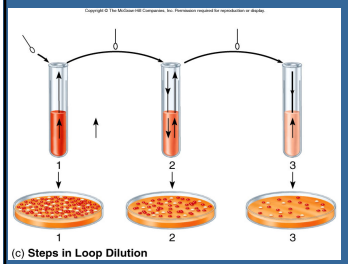
Three basic methods of isolating bacteria.

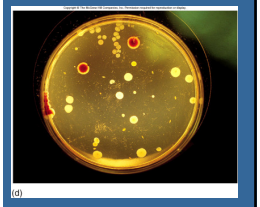


a) STREAK PLATE

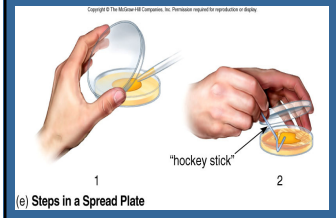


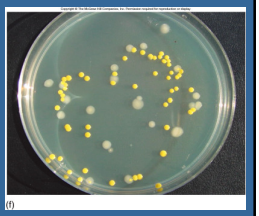
Loop Dilution





Spread Plate





Media

- Classified according to three properties
  - Physical state
  - Chemical composition
  - Functional types

Culture Media:

- **Agar** – a complex polysaccharide from algae is used to provide solid characteristics.

- Frau Angelina Hesse, the American wife of one of Pasteur's colleagues suggested adding agar to liquid media to solidify it

- This enabled Koch to grow bacteria in pure cultures

Bacterial growth media can be divided into 3 main types, depending upon the physical state it is in

- **Liquid media**
- **Semi-solid media**
- **Solid media**

Liquid media are water-based solutions that are generally termed broths, milks and infusions.

Broth culture is one of the most common ways to culture microorganisms – but it does not guarantee a pure culture

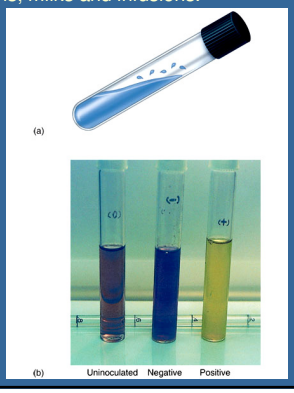
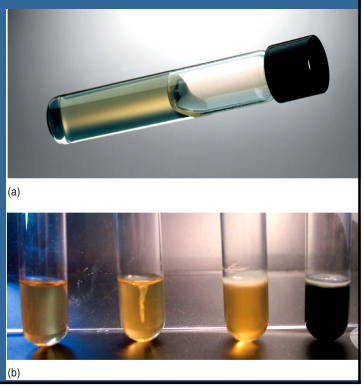


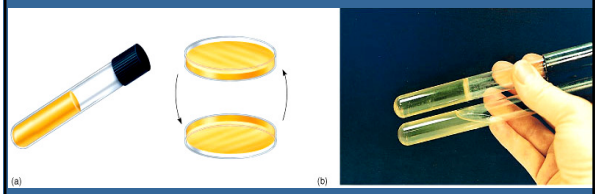
Fig. 3.4

Semi-solid media contain <1% of agar

Semi-solid media is commonly used to test for motility and to ship microorganisms from one place to another – sometimes termed 'slants'



Solid media contains 1-5% of agar



Solid media (agar) is most often used to culture bacteria and fungi as discrete, single colonies – a reliable way to obtain a pure culture – isolation.

Types of Media – based on chemical composition

- Synthetic media (Defined)
- Nonsynthetic or complex media (Undefined)

Synthetic media contain pure organic and inorganic compounds that are chemically defined (i.e. known molecular formula).

Some media are minimal – some require many more ingredients

TABLE 3.2 Medium for the Growth and Maintenance of the Green Alga *Euglena*

Glutamic acid (aa)	6 g
Aspartic acid (aa)	4 g
Glycine (aa)	5 g
Sucrose (c)	30 g
Malic acid (oa)	2 g
Succinic acid (oa)	1.04 g
Boric acid	1.04 mg
Thiamine hydrochloride (v)	12 mg
Monopotassium phosphate	0.6 g
Magnesium sulfate	0.8 g
Calcium carbonate	0.16 g
Ammonium carbonate	0.72 g
Ferric chloride	60 mg
Zinc sulfate	40 mg
Manganese sulfate	6 mg
Copper sulfate	0.62 mg
Cobalt sulfate	5 mg
Ammonium molybdate	1.34 mg

Note: These ingredients are dissolved in 1,000 ml of water.
aa, amino acid; c, carbohydrate; oa, organic acid; v, vitamin; g, gram; mg, milligram.

Green Alga Euglena

For synthetic media – you must know the EXACT growth requirements of a microorganism

Complex or undefined media contain ingredients that are not chemically defined or pure (i.e. animal extracts).

- Not exact chemical formula
- Most are extracts from animals: blood, serum, tissue extracts
- Yeast extract, soybean extract, etc

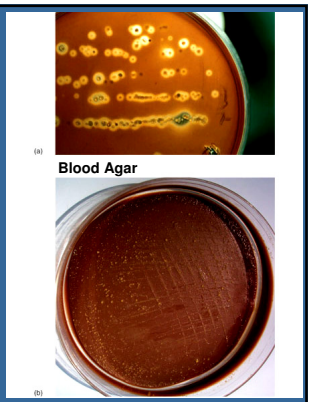
In the clinical (and laboratory) setting there are functional types of growth media

- **Enriched media** – contain complex organic substances that certain species **MUST** have to grow – these organisms are often termed '**fastidious**'
- **Selective media** – contain agents that inhibit growth of certain microbes
- **Differential media** – contain growth agents that promote different phenotype of different organisms on same media

Enriched media are used to grow **fastidious** bacteria.

- Common examples in the clinical laboratory are **blood agar** (hemolytic strains of bacteria – intact RBCs) and **chocolate agar** (*Neisseria gonorrhoeae* – lysed RBCs)

- Plating on enriched media does NOT ensure a single species is present



Blood Agar

Blood Agar

Selective media enables one type of bacteria to grow, while **differential media** allows bacteria to show different reactions (i.e. colony color)

These two types of media can – **often in a single step** – give a preliminary ID for an infectious organism

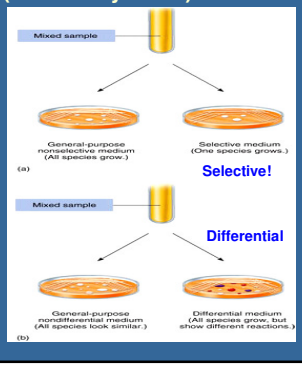


Fig. 3.8 - Selective vs. Differential Media

Examples of selective and differential media

Selective

MacConkey agar – Gram negative enterics

Salmonella/Shigella (SS) agar – specific for these 2 genera

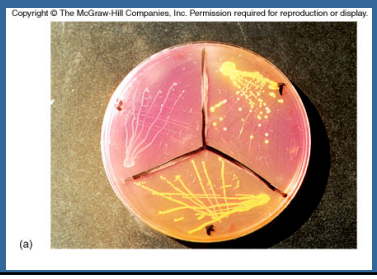
Differential

Blood agar – Distinguish between types of RBC hemolysis

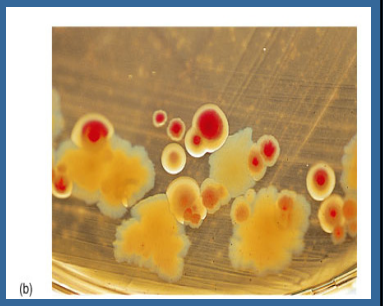
MacConkey agar – Bacteria that ferment lactose – note that this can be used as a selective OR differential media

Mannitol Salt Agar (MSA) & MacConkey Agar

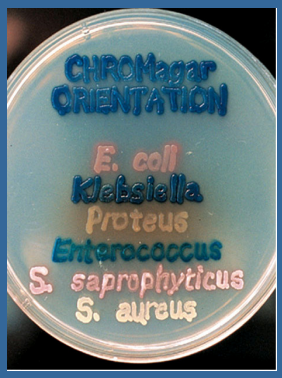
- MSA – Selective and Differential



MacConkey Agar – Selective and differential for Gram (-) enterics



CHROMagar Orientation™ is a single agar that distinguishes between common urinary tract pathogens – by color!

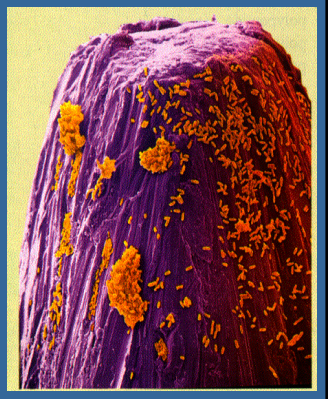


Microscopy

- **Magnification**
- **Resolution**
- **Optical microscopes**
- **Electron microscopes**
- **Stains**

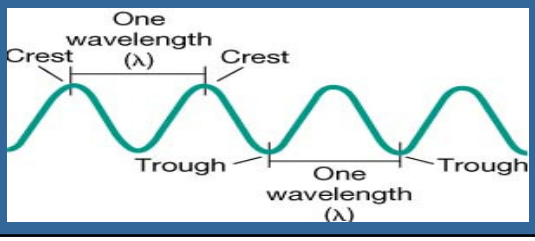
How many bacteria are there on the end of a pin?

How about that pen you are always chewing on????



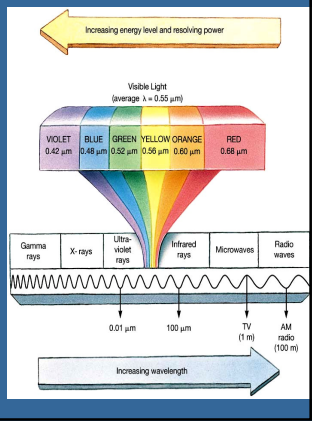
Properties of light:

Wavelength - distance between troughs or crest is wavelength = λ



Wavelength is related to resolution - the ability to see two objects as discrete objects.

Analogy of resolution as a property of wavelength point is: shorter wavelength = better resolution



Magnification

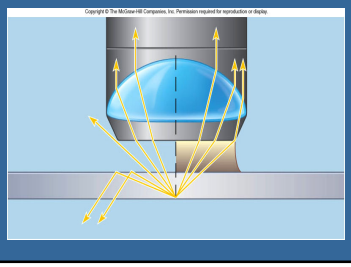
- Ability to enlarge objects
- Given by the OBJECTIVE and OCULAR lens
- For example:
  - 4X objective and 10X ocular lens
  - 100X objective and 10X ocular lens

Resolving Power

- Ability to distinguish or separate two points from one another
- Given by the "quality" of the objective lens
- 4X = 0.45
- 100X = 1.25
- Resolving Power= $\frac{\text{light wavelength (400 nm)}}{\text{2 x NA objective lens}}$

Immersion Oil

- **What is its role?**



Optical microscopes

- All have a maximum magnification of 2000X
  - Bright-field
  - Dark-field
  - Phase-contrast
  - Differential interference
  - Fluorescent
  - Confocal

Bright-field

- Most commonly used in laboratories
- Observe live or preserved stained specimens

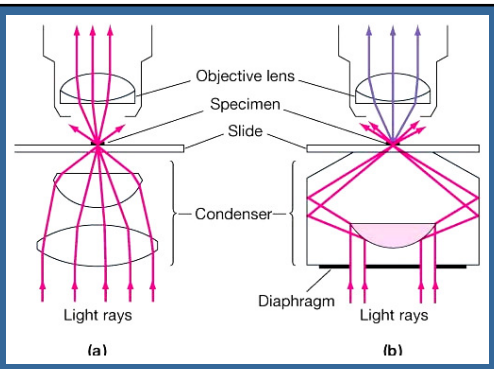
Dark-field

- Observe live unstained specimens
- View an outline of the specimens

Comparison of bright field and dark field microscopy.

The condenser of the bright field scope concentrates light on the specimen and transmits light through the specimen.

In dark field microscopy, the condenser deflects the light rays so that the light is reflected by the specimen. The reflected light is then focused into the image.



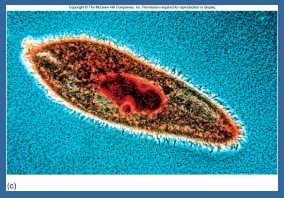
Bright Field **Dark Field**

Example of a bright-field

Example of dark-field

Phase-contrast

- Observe live specimens
- View internal cellular detail
- Denser parts of the cells will affect the passage of light differently and will vary in contrast



Fluorescent Microscopy

- Fluorescence stain or dye
- UV radiation causes emission of visible light from dye
- Diagnostic tool

Example of fluorescent microscopy- specimen is stained

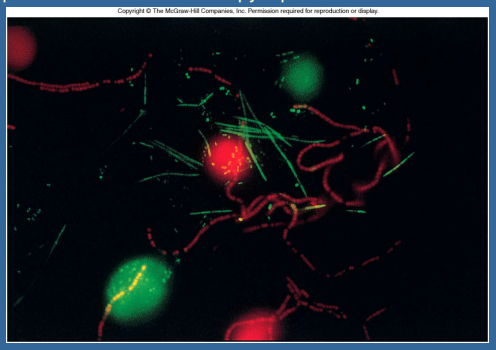


Fig. 3.21 Fluorescent staining on a fresh sample of cheek scrapings from the oral cavity – DH'ers rejoice!

Confocal

- Fluorescence or unstained specimen images are combined to form a three-dimensional image.

Example of a confocal microscope.

Fig. 3.22 Confocal microscopy of a basic cell

Electron microscopy

- Very high magnification (100,000X)
- Transmission electron microscope (TEM)
  - View internal structures of cells
- Scanning electron microscope (SEM)
  - Three-dimensional images

Example of Transmission Electron Microscopy (TEM)

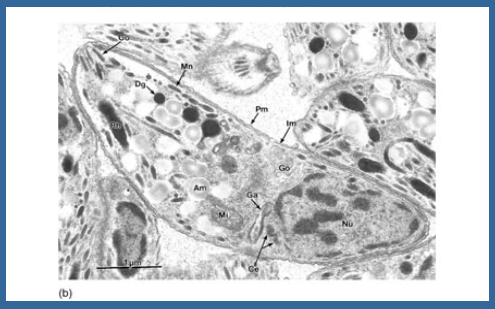


Fig. 3.24 Transmission electron micrograph

Example of Scanning Electron Microscopy (SEM)

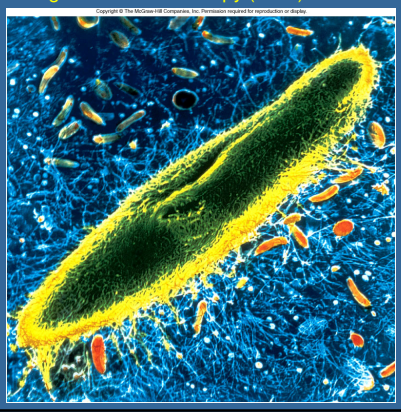


Fig. 3.25 A **false-color** scanning electron micrograph…

Stains

- **Positive stains**
  - Dye binds to the specimen
- **Negative stains**
  - Dye does not bind to the specimen, but rather around the specimen (silhouette).

Positive stains are basic dyes (positive charge) that bind negative charge cells, and **negative stains** are acidic dyes (negative charge) that bind the background.

TABLE 3.7 Comparison of Positive and Negative Stains

	Positive Staining	Negative Staining
Appearance of cell	Colored by dye	Clear and colorless
		
Background	Not stained (generally white)	Stained (dark gray or black)
Dyes employed	Basic dyes: Crystal violet Methylene blue Safranin Malachite green	Acidic dyes: Nigrosin India ink
Subtypes of stains	Several types: Simple stain Differential stains Gram stain Acid-fast stain Spore stain Special stains Capsule Flagella Spore Granules Nucleic acid	Few types: Capsule Spore

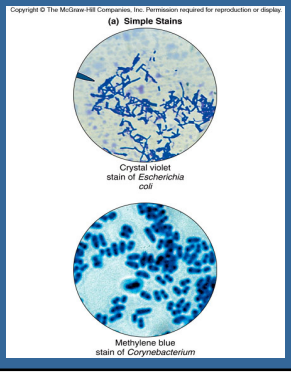
Table 3.7 Comparison of positive and negative stains

Positive stains

- **Simple**
  - One dye
- **Differential**
  - Two-different colored dyes
    - Ex. Gram stain
- **Special**
  - Emphasize certain cell parts
    - Ex. Capsule stain, flagellum stain

Positive stains

Simple Stains

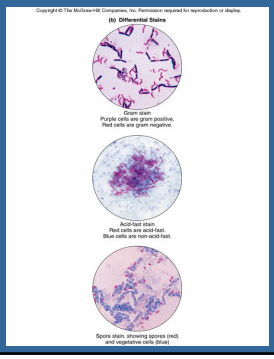


Differential - Two-different colored dyes

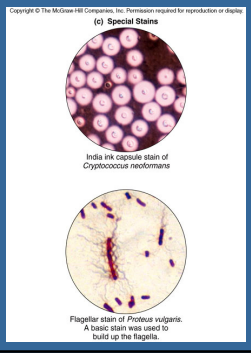
Gram Staining

Step	Microscopic Appearance of Cell: Gram (+)	Microscopic Appearance of Cell: Gram (–)	Chemical Reaction in Cell Wall (very magnified view): Gram (+)	Chemical Reaction in Cell Wall (very magnified view): Gram (–)
1. Crystal violet			 Both cell walls affix the dye	
2. Gram's iodine			 Dye crystals trapped in wall	 No effect of iodine
3. Alcohol			 Crystals remain in cell wall	 Outer membrane weakened; wall loses dye
4. Safranin (red dye)			 Red dye has no effect	 Red dye stains the colorless cell

Differential



Special Staining



Have a great time in lab!!