Chapter 3

Topics

- Methods of Culturing Microorganisms
- Microscope

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Methods of Culturing Microorganisms

- Five basic techniques
- Media
- Microbial growth

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Five basic techniques

1. Inoculate
2. Incubate
3. Isolation
4. Inspection
5. Identification

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Overview of the five major techniques used by microbiologist.

1. Inoculate
2. Incubate
3. Isolation
4. Inspection
5. Identification

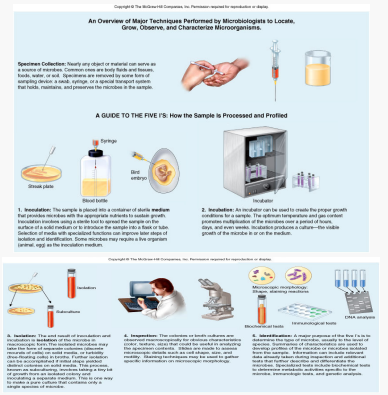

Fig. 3.1 A summary of the general laboratory techniques.

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

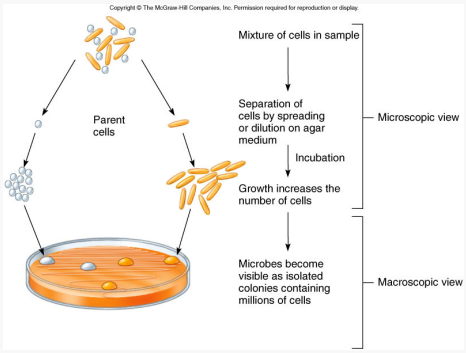

Fig. 3.2 Isolation technique

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Three basic methods of isolating bacteria.

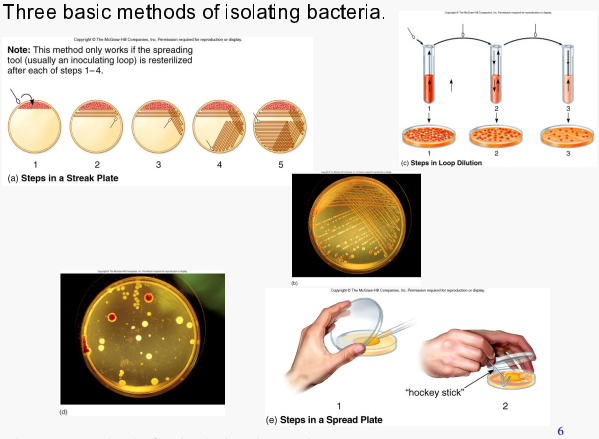

Fig. 3.3 Methods for isolating bacteria.

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Media

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

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Physical State

- Liquid media
- Semi-solid media
- Solid media

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

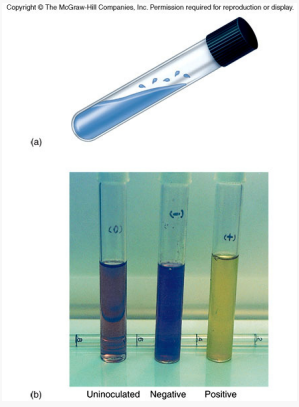

Fig. 3.4 Sample liquid media

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Semi-solid media contain a low percentage (<1%) of agar, which can be used for motility testing.

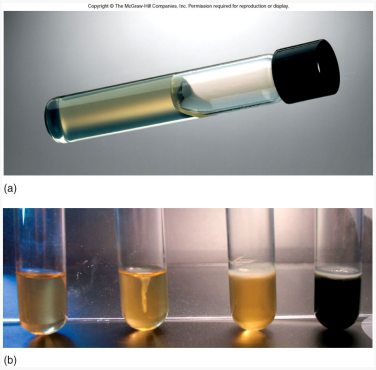

Fig. 3.5 Sample semisolid media

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Solid media contain a high percent (1-5%) of agar, which enables the formation of discrete colonies.

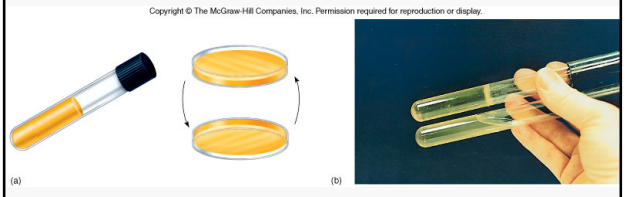

Fig. 3.6 Solid media that are reversible to liquids

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Chemical content

- Synthetic media
- Nonsynthetic or complex media

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

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.

Table 3.2 Medium for the growth and maintenance of the Green Alga Euglena

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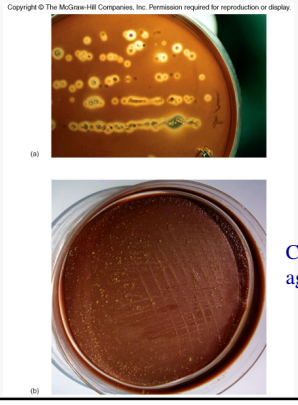
Complex or enriched media contain ingredients that are not chemically defined or pure (i.e. animal extracts).



Blood agar

Chocolate agar

Fig. 3.7 Examples of enriched media

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Functional types of growth media

- Enriched media
- Selective media
- Differential media

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Enriched media are used to grow fastidious bacteria.

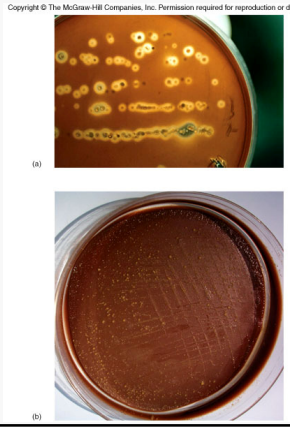

Fig. 3.7 Examples of enriched media

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

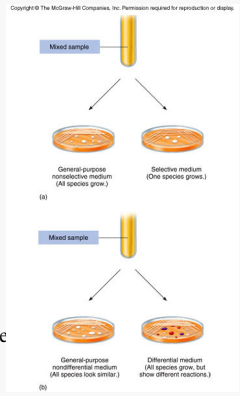

Fig. 3.8 Comparison of selective and different Media with general-purpose media.

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Examples of differential media.

TABLE 3.4 Differential Media

Medium	Substances That Facilitate Differentiation	Differentiates Between
Blood agar	Intact red blood cells	Types of hemolysis
Mannitol salt agar	Mannitol, phenol red, and 7.5% NaCl	Species of *Staphylococcus* NaCl also inhibits the salt-sensitive species
Hektoen enteric (HE) agar	Brom thymol blue, acid fuchsin, sucrose, salicin, thiosulfate, ferric ammonium citrate, and bile	*Salmonella*, *Shigella*, other lactose fermenters from nonfermenters Dyes and bile also inhibit gram-positive bacteria
MacConkey agar	Lactose, neutral red	Bacteria that ferment lactose (lowering the pH) from those that do not
Urea broth	Urea, phenol red	Bacteria that hydrolyze urea to ammonia
Sulfur indole motility (SIM)	Thiosulfate, iron	H_2S gas producers from nonproducers
Triple-sugar iron agar (TSIA)	Triple sugars, iron, and phenol red dye	Fermentation of sugars, H_2S production
XLD agar	Lysine, xylose, iron, thiosulfate, phenol red	*Enterobacter*, *Escherichia*, *Proteus*, *Providencia*, *Salmonella*, and *Shigella*
Birdseed agar	Seeds from thistle plant	*Cryptococcus neoformans* and other fungi

Table 3.4 Differential media

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Mannitol salt agar is a selective media, and MacConkey agar is a differential media.

MSA – Selective (7% NaCl) for Staphylococcus

MacConkey – Selective (Bile, crystal violet) for Gram (-)

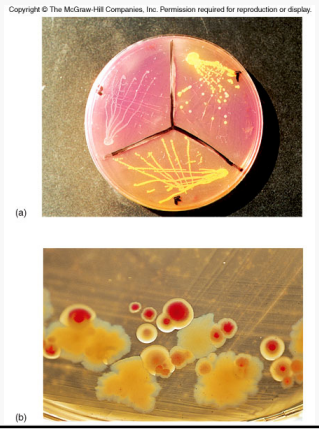

Fig. 3.8 Examples of media that are both selective and differential

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Examples of miscellaneous media are reducing, fermentation and transportation media.

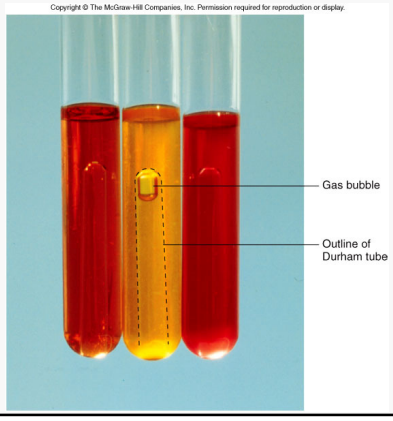

Fig. 3.11 Carbohydrate fermentation broth

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Microbial growth

- Incubation
  - Varied temperatures, atmospheric states
- Inspection
  - Mixed culture
  - Pure culture
- Identification
  - Microscopic appearance
- Maintenance and disposal
  - Stock cultures
  - sterilization

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Microscope

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

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A compound microscope is typically used in teaching and research laboratories.

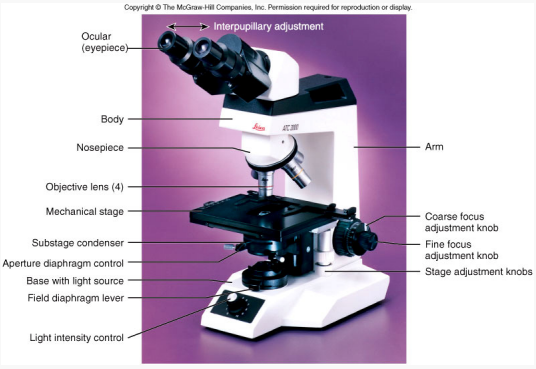

Fig. 3.14 The parts of a student laboratory microscope

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A specimen is magnified as light passes through the objective and ocular lens.

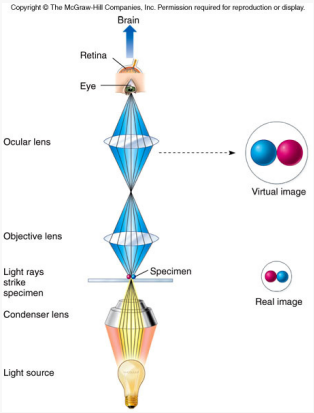

Fig. 3.15 The pathway of light and the two Stages in magnification of a compound microscope.

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Resolution distinguishes magnified objects clearly.

- Capacity to distinguish or separate two adjacent objects from one another.
- -Resolving power (RP) = Wavelength (nm)/2 x NA of objective lens
- RP= 500nm/2 x 1.25

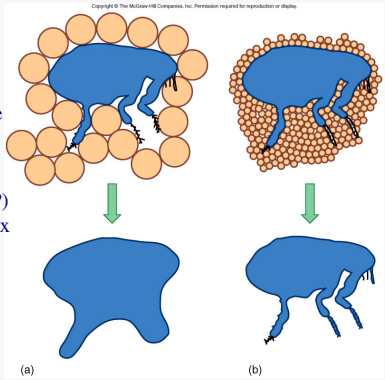

Fig. 3.16 Effect of wavelength on resolution

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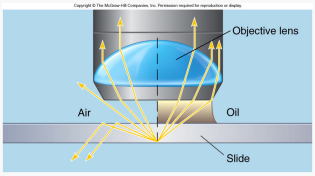
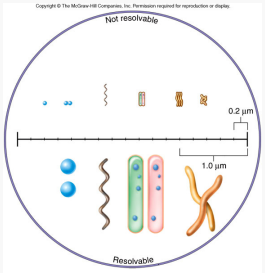
Resolution can be increased by using immersion oil.





Figs. 3.17 and 3.18 Workings of an oil immersion lens, and effect of magnification.

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Optical microscopes

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

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Bright-field

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

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Dark-field

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

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Examples of a bright-field, dark-field, and phase-contrast microscope.

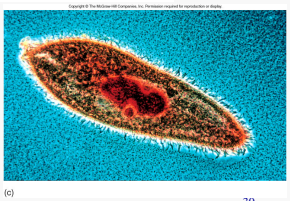

Fig. 3.19 Three views of a basic cell

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Phase-contrast

- Observe live specimens
- View internal cellular detail

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Example of phase-contrast and differential interference.

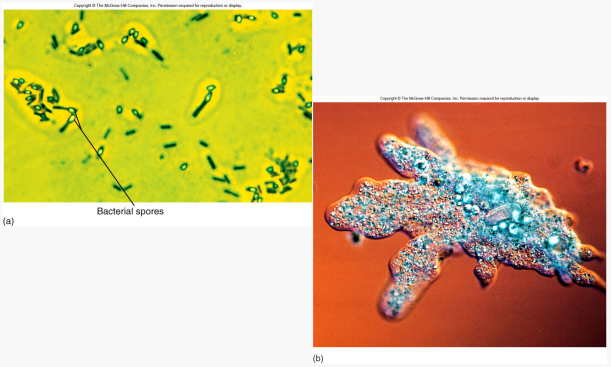

Fig. 3.20 Visualizing internal structures

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Fluorescent

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

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Example of fluorescent microscopy- specimen is stained

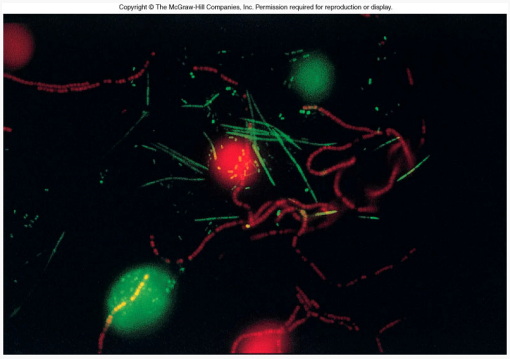

Fig. 3.21 Fluorescent staining on a fresh sample of cheek scrapings from the oral cavity.

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Confocal

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

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Example of a confocal microscope.

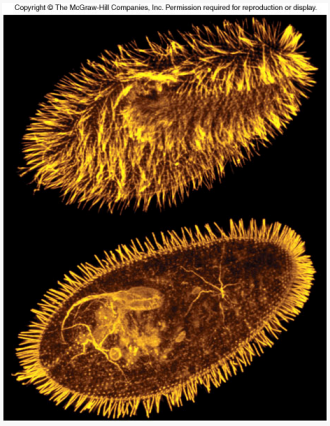

Fig. 3.22 Confocal microscopy of a basic cell

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Electron microscopy

- Very high magnification (100,00X)
- Transmission electron microscope (TEM)
  - View internal structures of cells
- Scanning electron microscope (SEM)
  - Three-dimensional images

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Example of Transmission Electron Microscopy (TEM)

Coronavirus - SARS

100,000X

Toxoplasma

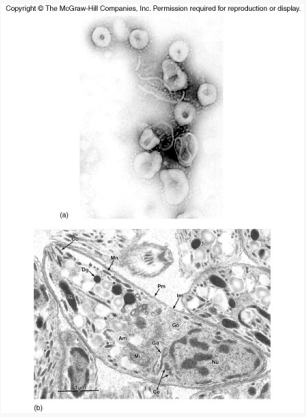

Fig. 3.24 Transmission electron micrograph

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Example of Scanning Electron Microscopy (SEM)

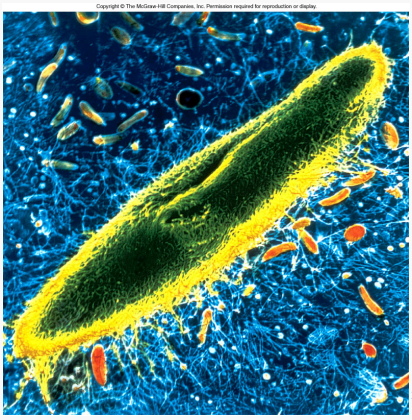

Fig. 3.25 A false-color scanning electron micrograph…

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Summary of optical and electron microscopes.

TABLE 3.5 Comparisons of Types of Microscopy

Microscope	Maximum Practical Magnification	Resolution	Important Features
Visible light as source of illumination			
Bright-field	2,000×	0.2 μm (200 nm)	Common multipurpose microscope for live and preserved stained specimens; specimen is dark, field is white; provides fair cellular detail
Dark-field	2,000×	0.2 μm	Best for observing live, unstained specimens; specimen is bright, field is black; provides outline of specimen with reduced internal cellular detail
Phase-contrast	2,000×	0.2 μm	Used for live specimens; specimen is contrasted against gray background; excellent for internal cellular detail
Differential interference	2,000×	0.2 μm	Provides brightly colored, highly contrasting, three-dimensional images of live specimens
Ultraviolet rays as source of illumination			
Fluorescent	2,000×	0.2 μm	Specimens stained with fluorescent dyes or combined with fluorescent antibodies emit visible light; specificity makes this microscope an excellent diagnostic tool
Confocal	2,000×	0.2 μm	Specimens stained with fluorescent dyes are scanned by laser beam, multiple images (optical sections) are combined into three-dimensional image by a computer; unstained specimens can be viewed using light reflected from specimen
Electron beam forms image of specimen			
Transmission electron microscope (TEM)	100,000×	0.5 nm	Sections of specimen are viewed under very high magnification; finest detailed structure of cells and viruses is shown; used only on preserved material
Scanning electron microscope (SEM)	650,000×	10 nm	Scans and magnifies external surface of specimen; produces striking three-dimensional image

Table 3.5 Comparison of types of microscopy

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Comparison of optical and electron microscopes.

TABLE 3.6 Comparison of Light Microscopes and Electron Microscopes

Characteristic	Light or Optical	Electron (Transmission)
Useful magnification	2,000×	1,000,000× or more
Maximum resolution	200 nm	0.5 nm
Image produced by	Light rays	Electron beam
Image focused by	Glass objective lens	Electromagnetic objective lenses
Image viewed through	Glass ocular lens	Fluorescent screen
Specimen placed on	Glass slide	Copper mesh
Specimen may be alive	Yes	No
Specimen requires special stains or treatment	Not always	Yes
Colored images possible	Yes	No

Table 3.6 Comparison of light microscopes and Electron microscopes

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Stains

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

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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

Sinple vs Differential Stains

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

Examples of simple, differential and special stains.

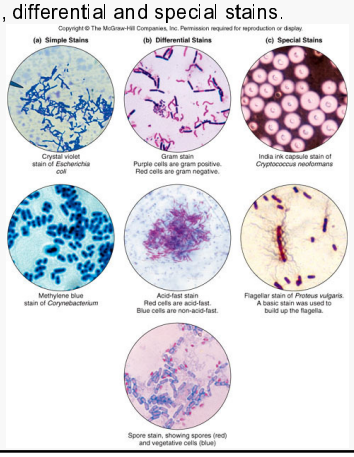
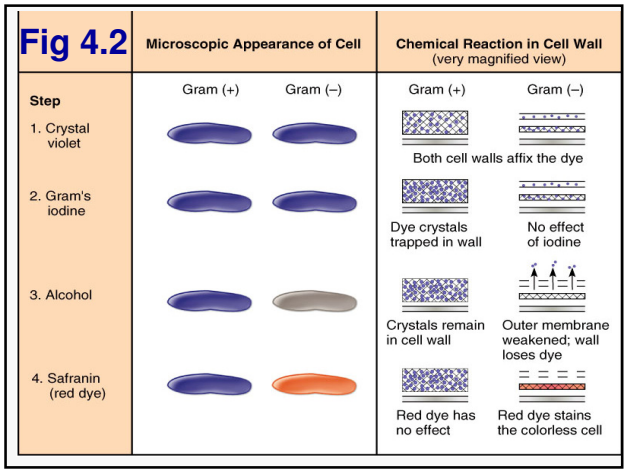


Fig. 3.25 Types of microbial stains



Have a great time in lab!!