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.

**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** contain 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).

| TABLE 3.2: Medium for the Growth and Maintenance of the Green Algal Euglena |
|---------------------------------|----------|
| Component                        | Quantity |
| Sodium chloride                 | 750 mg   |
| Dipotassium hydrogen phosphate  | 5.0 g    |
| Magnesium sulphate              | 1.0 g    |
| Selenium (vi)                   | 10 µg    |
| Molybdenum (vi)                 | 0.5 µg   |
| Zinc sulphate                   | 100 µg   |
| Ethylenediaminetetraacetic acid | 0.1 g    |
| Calcium sulphate                | 700 mg   |
| Glucose                         | 200 g    |
| Yeast extract                   | 10 g     |
| L-arginine                      | 5 mg     |
| L-cystine                       | 100 µg   |
| L-glutamic acid                 | 25 mg    |
| L-histidine                     | 50 µg    |
| L-leucine                       | 5 mg     |
| L-proline                       | 4 mg     |
| L-valine                        | 100 mg   |
| Copper sulphate                 | 3 mg     |
| Sodium selenite                 | 5 µg     |
| Sodium selenite hydrate         | 5 µg     |

Note: These ingredients are dissolved in 1200 ml of water.

Some media are minimal – some require many more ingredients

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

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

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

**Mannitol Salt Agar (MSA) & MacConkey Agar**
- MSA – Selective and Differential

**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 = \( \lambda \).

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 = light wavelength (400 nm) \( \times 2 \times NA \) 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

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**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.

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**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

Confocal

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

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 Scanning Electron Microscopy (SEM)**

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

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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 (silhouette).

**Positive stains**

- **Simple**
  - One dye

- **Differential**
  - Two different colored dyes
    - Ex. Gram stain

- **Special**
  - Emphasize certain cell parts
    - Ex. Capsule stain, flagellum stain

**Table 3.7 Comparison of positive and negative stains**

<table>
<thead>
<tr>
<th>Stains Type</th>
<th>Appearance of Cell</th>
<th>Stained Color</th>
<th>Staining Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive stains</td>
<td></td>
<td></td>
<td>Positive staining</td>
</tr>
<tr>
<td>Negative stains</td>
<td></td>
<td></td>
<td>Negative staining</td>
</tr>
</tbody>
</table>

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

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**Gram Staining**

<table>
<thead>
<tr>
<th>Step</th>
<th>Microscopic Appearance of Cell</th>
<th>Chemical Reaction in Cell Wall</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Crystal violet</td>
<td>Gram (+)</td>
</tr>
<tr>
<td>2</td>
<td>Gancis iodine</td>
<td>Gram (+)</td>
</tr>
<tr>
<td>3</td>
<td>Alcohol</td>
<td>Gram (+)</td>
</tr>
<tr>
<td>4</td>
<td>Saturated (dilute dye)</td>
<td>Red dye has no effect</td>
</tr>
</tbody>
</table>

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**Differential**

- Two different colored dyes

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**Simple Stains**

- Eosin
- Hematoxylin
- Mallory's stain
- Giemsa
- Wrights stain

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**Gram Staining**

- **Gram (+)**
  - Bacteria retain the dye
  - Ex. Bacillus subtilis

- **Gram (-)**
  - Bacteria lose the dye
  - Ex. Streptococcus pneumoniae
Have a great time in lab!!