Diagnostic Microbiology

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Diagnostic Microbiology

- Isolation of pure culture from specimen
  - Why? In order to characterize microorganisms, one must isolate the different species from a clinical specimen
- Culture media: nutrient material
  - Type of media used is based on
    - Source of sample tested
    - Species suspected to be in sample
    - Nutritional requirement of the suspected organisms
- Inoculation methods
  - Streak, spread, or pour plate method
    - Must be incubated at appropriate temperatures
    - Colonies are formed when enough cells divide

Preservation of pure cultures

- Short-term: cultures soted in medium refrigeration temperatures
- Long term: cultures need to be maintained by using:
  - Liquid nitrogen
  - Special freezer
  - Lyophilization (freeze drying)

Identification of microorganism after obtaining as pure culture

- Study microorganism by colonial morphology or cellular morphology
- Use magnification
  - Resolving power (resolution) = ability to distinguish two closely located objects as separate, distinct entities
  - Light microscope: lenses that manipulate the path a light beam travels
  - Electron microscope: beam of electrons controlled by magnetic fields used in place of the light source of a light microscope

Pour plates

- Bracteria grow inside agar

- Used for enumeration of bacteria

Identification: Straining Techniques

- Generally, three steps:
  a. Make a smear (thin film of specimen)
  b. Fix dried smear by heat
  c. 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 stain: differentiate acid-fast bacteria vs non-acid-fast bacteria
  - Gram stain: Gram positive and Gram negative; cell wall of bacteria
    - Gram negative cell wall usually thinner than Gram-positive
    - Gram positive cell wall
      - Thick wall of peptidoglycan with teichoic acids
      - Alcohol shrinks pores
    - Gram negative cell wall
      - Outer membrane covering a thin layer of peptidoglycan anchored together by a lipoprotein
      - Selective barrier based on size and charge of molecules
      - Alcohol increased permeability of outer membrane
  - Endospore staining
  - Fluorescence microscopy is used when trying to reveal only objects that are of interest in an otherwise black background
    - Uses a special dye that fluoresces
    - Immunofluorescence
      - Fluorescent dye attaches to a specific antibody and is added to a specimen
      - Any attachment of the antibody to microorganism will fluoresce
  - Electron Microscopy:
    - Transmission electron microscopy: staining with heavy metals of

whole or segment of microorganism
    - Scanning electron microscopy: 3-D image of cell surface coated with fine film of metal

The Gram Stain (Hans Christian Gram)

1. Flood slide with crystal (or gentian) violet.
2. Flood with Gram's iodine
3. Carefully decolorize with 95% ethanol - most critical
4. Flood with safranin (pink color)

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

Other stains

- Endospore
- Capsule
- Flagella

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 um in diameter/width)
- Higher surface area/volume ratio
    - Higher metabolism
    - Faster growth
    - Replication rate (~20 minutes)

Shapes of Bacteria

- Spherical (coccus) - round or ovoid
- Cylindrical or rod (bacillus) - vary in width, length, ends can be square, rounded, tapered or pointed
- Spiral or helical (spirillum) - corkscrew

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 (grape-like clusters)
    - Cubical packet of 8 cells (sarcinae)

Definitions

- Chemically defined media: exact composition known
- Chemically undefined media: some components can't be controlled - natural products added
- If solid (versus liquid) growth: 1.5% agar used
- Enrichment media: increase number 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 (fungi and heterotrophs) - 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; public health microbiology (e.g., MacConkey agar)
    - Bile salts, crystal violet inhibit Gram +ves

Four conditions make up physical conditions for cultivation:

- Temperature
    - Psychrophiles
        - Grow best at temperatures 15-20C
    - Mesophiles

        - Grow best at temperatures 25-40C
        - Most bacteria belong here
    - Thermophiles
        - Grow best at temperatures 40-85C
    - 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
            - 106 is optimal
- Gaseous Atmosphere
    - Gases such as oxygen, carbon dioxide, nitrogen, and methane
        - Aerobic microorganism - 21% oxygen
        - Anaerobic microorganism - no oxygen
        - Facultative microorganism - can grow in air atmosphere or anaerobically; do not need oxygen but can use it for chemical reactions
        - Microaerophilic microorganism - can use oxygen for chemical reaction; 1-15% oxygen level optimal
- pH
    - Varies
    - Intracellular pH must be ~7.5
    - Growth observed at pH values of 4-9 (optimum 6-8)
- Other conditions:
    - Water and light can be important for certain microorganisms
    - Osmotic pressure (hypertonic, hypotonic, isotonic)
        - Hypertonic: higher solute concentration in environment; cell shrivels up
        - Hypotonic: lower solute concentration in environment; cell ruptures from water inflow
        - Isotonic: no net flow