

### Chapter 3: Microscopy and Cell Structure

#### Terms:

- **Capsule:** a distinct thick gelatinous material that surrounds some types of microorganisms; sometimes required for an organism to cause disease
- **Chemotaxis:** directed movement of an organism in response to certain chemicals in the environment
- **Cytoplasmic membrane:** a thin lipid bilayer that surrounds the cytoplasm and defines the cell boundary
- **Endospore:** a kind of resting cell, characteristic of species in a limited number of bacterial genera; resistant to heat, radiation, and disinfectants
- **Flagellum:** in bacteria = a long protein appendage composed of subunits of flagellum that provides a mechanism of motility
- **Gram-negative:** bacteria that lose the crystal violet in the gram stain procedure and therefore stain pink; their cell wall is composed of a thin layer of peptidoglycan surrounded by an outer membrane
- **Gram-positive:** bacteria that retain the crystal violet stain in the gram stain procedure and therefore stain purple; their cell wall is composed of a thick layer of peptidoglycan
- **Lipopolysaccharide (LPS):** a part of the outer membrane of gram-negative bacteria
- **Peptidoglycan:** macromolecule found only in bacteria that provides strength to the bacterial cell wall. Made of alternating NAM and NAG units linked by peptide bonds
- **Periplasm:** gel that fills the region between the outer membrane and the cytoplasmic membrane in gram-negative bacteria
- **Pili:** hair like appendages on many gram-negative bacteria that function in conjugation and for attachment
- **Plasmid:** small extrachromosomal circular DNA molecule that replicates independently of the chromosome; often codes for antibiotic resistance
- **Ribosome:** structure that facilitates the joining of amino acids during the process of translation; composed of protein and ribosomal RNA
- **Transport system:** mechanisms cells use to transport molecules across the cytoplasmic membrane

#### To know:

- How different types of microscopy help better magnification, contrast, and resolution
- How gram-staining and acid-fast staining distinguish different types of bacteria
- General morphology of prokaryotic cells
- Membrane transport in bacterial cells; specifically the importance of proton motive force and group translocation
- Structure of bacterial cell wall and basic chemical structure of peptidoglycan

- Difference between the cell walls of gram-negative and gram-positive bacteria
- Bacterial structures that help with motility and surface attachment
- Differences between structures and functions of prokaryotic and eukaryotic cells

## 1. Microscopic techniques

- a. Parameters of microscopy:
  - i. **Magnification:** ratio of an object's image size to its real size
  - ii. **Resolution:** the measure of the clarity of the image, or the minimum distance of two distinguishable points
  - iii. **Contrast:** visible differences in brightness between parts of the sample
- b. **Light microscopy:** visible light passes through a series of lenses to produce a magnified image
  - i. **Ocular lens:** eyepiece with 10x magnification
  - ii. **Specimen stage**
  - iii. **Condenser:** focuses on light
  - iv. **Iris diaphragm:** controls the amount of light that enters objective lens
  - v. **Objective lens:** more magnification
  - vi. **Light source**
  - vii. **Rheostat:** controls brightness of the light
  - viii. Types of light microscopy:
    1. **Bright-field:** illuminates field evenly
    2. **Dark-field:** light is directed at an angle; unstained cells are easy to see because they are bright against a black background
    3. **Phase-contrast:** increases contrast by amplifying the refractive index difference between the medium and that surrounding it
    4. **Differential Interference contrast (DIC):** two light beams pass through and then recombine; looks 3D
    5. **Fluorescence:** cells are stained with a fluorescent dye; UV light is projected and cells emit different wavelengths (colors)
    6. **Scanning laser:** mirrors scan a laser beam across planes of a specimen; computer makes a 3D image
- c. **Electron microscopy:** electron beams are used instead of light (can't use living cells)
  - i. **Transmission (TEM):** transmits beams of electrons through specimen; can observe fine details of cell structure; darker is denser

- ii. **Scanning (SEM):** beam of electrons scan back and forth over the surface; visible 3D surface details
  - iii. **Atomic force:** probe moves in response to slight forces between it and sample. Can see bumps and valleys of atoms on specimen surface
- 2. Staining techniques
  - a. **Differential staining:** distinguishes between two groups of bacteria
    - i. **Gram stain:** due to different chemistries in cell walls
      - 1. Gram positive: remain purple
      - 2. Gram negative: turn pink (counterstained)
    - ii. **Acid-fast staining:**
      - 1. Used for cells that do not easily dye
      - 2. Cells that retain the primary red stain are "acid-fast"
        - a. Have waxy cell wall
      - 3. Methylene blue is used as the counterstain
- 3. Morphology of prokaryotic cells
  - a. Types:
    - i. **Coccus:** spherical
    - ii. **Bacillus:** rod/cylinder
    - iii. **Vibrio:** bean shaped
    - iv. **Spirillum:** corkscrew
    - v. **Spirochete:** corkscrew with flagellum for movement
    - vi. **Pleomorphic:** can have various shapes
  - b. **Typical groupings** of cells: how cells tend to stick together after binary fission (depending on the plane which they divide)
  - c. **Multicellular associations:** signaling process that allows cells to talk to each other until they can take on multicellular behaviours as a population of bacteria
  - d. Parts of prokaryotic cells:
    - i. Ribosomes free in cytoplasm
    - ii. Nucleoid
    - iii. Cell wall (very different from eukaryotic plant cell walls)
    - iv. May have capsule made of polysaccharides
    - v. May have forms of motility: flagella, pilus
  - e. Cytoplasmic membrane
    - i. Structure:
      - 1. Made up of phospholipid bilayer
        - a. Hydrophobic tail
        - b. Hydrophilic head
      - 2. Proteins for many functions
    - ii. Selectively permeable
      - 1. Passive transport: from high to low concentration
    - iii. **Aquaporins:** allow passive transport of water
      - 1. Osmosis
    - iv. Membrane transport
      - 1. Passive transport: move with concentration gradient