Bacterial Structure, Function & Genetics

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Objectives: Bacterial Structure & Function

• Define the cellular organization of bacteria and know the differences between Eukaryotes and Prokaryotes.

• Know major structures of bacteria and its function.

• Know the structure of cell wall of bacteria including the differences between Gram positive and Gram negative bacteria and main functions.
Objectives, cont.,

• Know the external structures of bacteria with and functions.
• Know the cytosol and internal structures of bacteria.
• Describe bacterial spores and its application in the practice of medicine.
Objectives: Bacterial Genetics

• Know basic information about bacterial genetics and replication of bacteria.

• Describe plasmids, its origin, types and its importance in clinical practice.

• Recalls genetics variations, including mutation and mechanisms of gene transfer and its implication on bacterial resistance to antimicrobial agents.
Definition

Bacteria: Is a heterogeneous group of unicellular organisms, about 1-8 μm in diameter.

Bacteria is a Prokaryote (has a primitive nucleus):
- one chromosome
- no nuclear membrane
- no mitochondria
- no sterols

Bacteria contain Plasmids: an extra piece of DNA.
Shapes & Types of Bacteria

• Spherical / Oval .............. Cocci
• Rods .................................. Bacilli
• Very short Bacilli .............. Coccobacilli
• Tapered end ................. Fusiform
• Club-shaped / Curved ...... Vibrio
• Helical / Spiral ............... Spirochaetes
Shapes & Types of Bacteria

- Pneumococci
- Streptococci
- Pseudomonas
- Salmonella typhi
- Staphylococci
- Mycobacterium tuberculosis
- Clostridium tetani
- Treponema
- Leptospira

Types of Bacteria:
- Spheres (Cocci)
- Rods (Bacilli)
- Spirals (Spirochetes)
Arrangements of Bacteria

Arrangements among Cocci:

- Pairs..............Diplococci
- Chains..........Streptococci
- Clusters........Staphylococci
- In four..........Tetrad
- Palisades.....*Corynebacterium*
Major Structures of Bacteria
Cell Wall of Bacteria

- Bacteria are cells with rigid cell wall surround cytoplasmic membrane and internal structures.

Functions of cell wall:
- Rigidity
- Shapes bacteria
- Protection
- Porous / permeable to low molecular weight molecules
- Cell division
- Antigenic determinants
Cell Wall of Bacteria

- Two groups of bacteria depending on reaction to **GRAM STAIN**:
  - **GRAM POSITIVE** BACTERIA: stain blue/purple by Gram stain
  - **GRAM NEGATIVE** BACTERIA: stain red by Gram stain

  **Note**: *Mycoplasma* is naturally have no cell wall.

**Chemical structure of bacterial cell wall:**

- **Peptidoglycan**:
  Rigid part, mucoprotein composed of alternating strands of *
  N- acetyl muramic acid* and *N- acetylb-glucosamine* linked
  with peptide sub units.
Diagram to show chemical structure of cross-linking in peptidoglycan component of cell walls. From Sharon N The Bacterial Cell Wall. Copyright (C) 1969 by Scientific American Inc. All rights reserved.

Three-dimensional representation of Gram-positive bacterial cell wall.
### GRAM STAINING

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong></td>
<td>Wipe bottom of biofilm slide clean</td>
</tr>
<tr>
<td><strong>2</strong></td>
<td>Clean top edges of slide about 2mm</td>
</tr>
<tr>
<td><strong>3</strong></td>
<td>Build up a ridge of petroleum jelly on the top and bottom of a cover slip</td>
</tr>
<tr>
<td><strong>4</strong></td>
<td>Cover slip with petroleum jelly</td>
</tr>
<tr>
<td><strong>5</strong></td>
<td>Biofilm on slide with cover slip held in place by petroleum jelly</td>
</tr>
<tr>
<td><strong>6</strong></td>
<td>Add crystal violet - wait 30 sec.</td>
</tr>
<tr>
<td><strong>7</strong></td>
<td>Wash with water</td>
</tr>
<tr>
<td><strong>8</strong></td>
<td>Add Grams Iodine - wait 1.5 min.</td>
</tr>
<tr>
<td><strong>9</strong></td>
<td>Decolorize with alcohol</td>
</tr>
<tr>
<td><strong>10</strong></td>
<td>Wash with water</td>
</tr>
<tr>
<td><strong>11</strong></td>
<td>Stain with Safranin dye - wait 30 sec.</td>
</tr>
<tr>
<td><strong>12</strong></td>
<td>Wash with water</td>
</tr>
<tr>
<td><strong>13</strong></td>
<td>Examine under oil immersion through the cover slip</td>
</tr>
</tbody>
</table>
Cell Wall of Gram Negative Bacteria

- **Thin** Peptidoglycan
- Outer membrane that contains:
  - specific proteins (porins) important in the transport of hydrophilic molecules
  - lipopolysaccharide & lipid (ENDOTOXIN)
Cell Wall of Gram Positive Bacteria

• Peptidoglycan **thicker** than Gram negative bacteria.

• Closely associated with cytoplasmic membrane.

• **Teichoic acid**: anchors cell wall to cell membrane, epithelial cell adhesion.

• **Antigens**: ~ polysaccharides (Lancefield)
  ~ protein (Griffith)
Diagram showing the structure of Gram-negative and Gram-positive bacterial cell walls.
External Structures of Bacteria

External protrude from the cell into the environment:

• Flagella
• Pili
• Capsule
Flagella

- Helical filaments
- Composed of protein **FLAGELLIN**.
- Found in Gram positive & Gram negative bacteria.

**Distribution:**
- Peritrichous
- Monotrichous
- Lophotrichous
Structure of Flagella

**Basal Body**: a protein arranged as rings on central rod (4 ring in Gram negative, 2 ring in Gram positive).
- outer pair of rings: only in Gram negative, pushed through outer membrane.
- inner pair of rings: inserted into peptidoglycan & cytoplasmic membrane.

**Hook**: a bent structure ~ act as joint

**Long Filament**: a Flagellin protein

**Function of Flagella**: motility & chemotaxis
Structure & Distribution of Flagella
Pili

Fine short filaments extruding from cytoplasmic membrane.

Found on the surface of many Gram negative & Gram positive bacteria.

Composed of protein Pilin.

Two classes:

1. **Common pili** (*fimbriae*): covers the surface—responsible for: adhesion & colonization

2. **Sex pili**: in some bacteria only, responsible for conjugation.
Capsule

- Amorphous material surrounds bacteria.
- Usually polysaccharide
- Occasionally protein

**Function:**
- Inhibits phagocytosis
- Acts as *Virulence factor* in some bacteria by assessing attachment to the surfaces.
Cytoplasmic Membrane

• **Cytoplasmic membrane (cell membrane)**
  – Double layered structure composed of phospholipid & protein
  – Act as semi-permeable membrane (passive diffusion)
  – Site of numerous enzymes involved in active transport of nutrients and various metabolic processes
Internal Structures of Bacteria

Mesosomes: convoluted invaginations of cytoplasmic membrane.

Function:
» Involved in DNA segregation during cell division & respiratory activity
» Contain receptors involved in chemotaxis
» Permeability barrier (active transport of solutes).
Core of Bacteria

• **Composed of**: Cytoplasmic inclusions
  Nucleoid (nuclear body)
  Ribosomes

1. **Cytoplasmic inclusions**: Are nutritional storage granules, examples:
   - Volutin
   - Lipid
   - Starch / or Glycogen
Nucleoid (Nuclear Body)

- Circular single stranded chromosome (bacteria genome or DNA)
- No nuclear membrane
- DNA undergoes semi-conservative replication, bidirectional from a fixed point
Ribosomes of Bacteria

- Distributed throughout the cytoplasm
- Site of protein synthesis
- Composed of RNA and protein
Spores of Bacteria

- Small, dense, metabolically inactive, non-reproductive structures produced by *Bacillus* & *Clostridium*
- Enables the bacteria to survive adverse environmental conditions.
- Contain high concentration of Calcium dipicolonate.
- Resistant to heat, dissecation & disinfectants
- Often remain associated with the cell wall
Spores of Bacteria-cont.

- Spores are described as:
  1. Terminal spores
  2. Sub-terminal spores
  3. Central spores

- Spores germinate when growth conditions become favorable to produce vegetative cells.

- Application in medical practice: spore preparations used for checking the efficacy of Autoclaves, eg. *Bacillus subtilis* & *Bacillus steroethermophilus*. 
Spores of Bacteria
BACTERIAL GENETICS
Bacterial Genetics - definitions

- **Genetics** is the study of inheritance and variation.
- Genetic information encoded in DNA.

**Function of genetic material:**

1. Replication of the genome
2. Expression of DNA to mRNA then to protein.
Definitions-cont.

- **Genotype**: the complete set of genetic determinants of an organism.
- **Phenotype**: expression of specific genetic material.
- **Wild type**: reference (parent) strain
  - **Mutant**: progeny with mutation.

2 types of DNA in bacteria
- ~ Chromosominal
- ~ Extra-chromosominal (Plasmid).
Bacterial Chromosomes

• Haploid, circular molecule of double stranded DNA attached to cell membrane. No nuclear membrane.

• DNA a double helical structure, genetic code in Purine and Pyrimidine bases of nucleotides that makes DNA strand.

• 3 bases comprise one code, each triplet codon codes for one amino acid.

• Replication is by binary fission.
**Procaryote**

- Cell wall
- Single, circular chromosome
- Cytoplasm rich in ribosomes
- Plasmid
- Cell membrane (site of cellular respiration)

**Eucaryote**

- Mitochondrion (site of cellular respiration)
- Cell membrane
- Nuclear membrane
- Lysosome
- Nucleus
- Cytoplasm
- Rough endoplasmic reticulum (ribosomes)
- Smooth endoplasmic reticulum
- Golgi apparatus
Semiconservative Replication

original parent strand

first generation daughter strands

second generation daughter strands

Cytoplasmic membrane

Mesosome

Chromosome of circular double-stranded DNA
Plasmids

- Extra chromosomal DNA composed of double stranded-DNA.
- Found in most species of bacteria.
- Origin?
- Govern their own replication
- **Application**: Genetic exchange, amplify genes
- Transfer by conjugation
Plasmids

- Bacterium
- Plasmid
- Bacterial chromosome

Plasmid maintenance
- Transfer promotion genes
- Self transmissible plasmid typical of coliform bacilli
- Antibiotic resistance or virulence determinants

Maintenance region
- Transducible plasmid (non-self transmissible) typical of staphylococci
- Resistance determinants
Types of Plasmids

1 - **R-plasmids**: genes code for antibiotic resistance particularly Gram negative bacteria.

2 - **Col-plasmids**: in Enterobacteria, codes for extracellular toxins.

3 - **F-plasmids**: (fertility) factor, transfer of chromosome during mating.
Genetic variation in bacteria:
takes place by:
1~ Mutations
2~Gene transfer
Mutation

- Inheritable changes in the structure of genes (DNA).
- Chemical changes in one or more bases of DNA.

Mutation / gene defect leads to alteration in:

- Transcription,
- Amino acid sequences,
- Function eg. Bacteria resistant to antibiotic.
Classification of Mutation

 Depends on biological sequencing:


2 - **Auxotrophic mutation**: affect biosynthetic enzyme resulting in a nutritional requirement of mutant cell.

3 - **Lethal mutation**: leads to death of bacteria.
Mutation Causes Antimicrobial Resistance

Genetic Mutation Causes Drug Resistance

Non-resistant bacteria exist → Bacteria multiply by the billions → Some mutations make the bacterium drug resistant → Drug resistant bacteria multiply and thrive.

- A few of these bacteria will mutate.
- In the presence of drugs, only drug resistant bacteria survive.
Gene Transfer Among Bacteria

Three mechanisms:

1. Transformation
2. Transduction
3. Conjugation.
Transformation

- A fragment of exogenous naked bacterial DNA are taken up and absorbed into recipient cells.

- Common in *Haemophilus influenzae* & *Streptococcus pneumoniae*. Bacteria become resistant to Ampicillin.
Transduction

- Phage mediated transfer of genetic information from donor to recipient cells.

Example:

- **Beta – Lactamase production in**  
  *Staphylococcus aureus*: Bacteria becomes resistant to penicillin.

- **Toxin production by**  
  *Corynebacterium diphtheriae*. 
Transduction

Phage particle containing DNA fragment from host bacterial chromosome

Phage DNA

Phage particles
Conjugation

- Major way bacteria acquire additional genes.
- **Plasmid** mediated
- Cell contact required and genes reside on plasmid resident within donor cells transfer to recipient cell *(mating).*
- Conjugation is the common way of transfer of genes resistance to antibiotics among bacteria in hospitals.
Conjugation

• Mediated by plasmid called F factor (fertility).
• Gene encode changes in surface by producing a sex pilus. This facilitates capture of F^- cells and the formation of a conjugation bridge through which DNA passes from F^+ into F^- cells.
Conjugation in Bacteria
Genetic Recombination

After gene transfer, there are three possible fates:

1. Exogenous DNA degraded by nuclease.
2. Stabilized by circulization and become plasmid.
3. Form a partially hybrid chromosome with segment derived from each source.
Reference Book

*Sherries* Medical Microbiology, an Introduction to Infectious Diseases.