Chapter 08
Lecture
Antibiotic Resistance

- **Staphylococcus aureus**
  - Gram-positive coccus; commonly called Staph
  - Frequent cause of skin and wound infections
  - Since 1970s, treated with penicillin-like antibiotics
    - E.g., methicillin
  - In 2004, over 60% of *S. aureus* strains from hospitalized patients were resistant to methicillin
  - ~2.3 million healthy people in U.S. harbor methicillin-resistant *S. aureus* (MRSA)
  - Healthcare-associated MRSA (HA-MRSA) resistant to other antibiotics, including vancomycin
    - Vancomycin considered drug of last resort
8.1. Genetic Change in Bacteria

- Organisms adapt to changing environments
  - **Natural selection** favors those with greater fitness
  - Bacteria adjust to new circumstances
    - Regulation of gene expression (Chapter 7)
    - Genetic change (Chapter 8)
  - Bacteria excellent system for genetic studies
    - Rapid growth, large numbers
    - More known about *E. coli* genetics than any other
  - Change in organism’s DNA alters **genotype**
    - Sequence of nucleotides in DNA
    - Bacteria are haploid, so only one copy, no backup
  - May change observable characteristics, or **phenotype**
    - Also influenced by environmental conditions
8.1. Genetic Change in Bacteria

- **Mutation and horizontal gene transfer**

![Diagram showing genetic change in bacteria with arrows indicating spontaneous mutation in genome, vertical gene transfer, plasmid transfer, and horizontal gene transfer.](image)
8.2. Spontaneous Mutations

- Transposons (jumping genes)
  - Can move from one location to another
  - Process is transposition
  - Gene insertionally inactivated
    - Function destroyed
  - Most transposons have transcriptional terminators
    - Blocks expression of downstream genes in operon
8.2. Spontaneous Mutations

- Transposons (jumping genes) (continued…)
  - Classic studies carried out by Barbara McClintock
    - Observed color variation in corn kernels resulting from transposons moving into and out of genes controlling pigment synthesis
8.3. Induced Mutations

- Induced mutations result from outside influence
  - Agent that induces change is **mutagen**
  - Geneticists may use mutagens to increase mutation rate
  - Two general types: **chemical, radiation**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Action</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical Agent</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemicals that modify nucleobases</td>
<td>Converts cytosine to uracil</td>
<td>Nucleotide substitution</td>
</tr>
<tr>
<td>Nitrous acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkylating agents</td>
<td>Adds alkyl groups (CH₃ and others) to nucleobases</td>
<td>Nucleotide substitution</td>
</tr>
<tr>
<td>Base analogs</td>
<td>Used in place of normal nucleobases in DNA</td>
<td>Nucleotide substitution</td>
</tr>
<tr>
<td>5-Bromouracil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercalating agents</td>
<td>Inserts between base pairs</td>
<td>Addition or subtraction of nucleotides</td>
</tr>
<tr>
<td>Ethidium bromide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transposons</td>
<td>Randomly insert into DNA</td>
<td>Insertional inactivation</td>
</tr>
<tr>
<td><strong>Radiation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultraviolet (UV) light</td>
<td>Causes intrastrand thymine dimer to form</td>
<td>Errors during repair process</td>
</tr>
<tr>
<td>X rays</td>
<td>Cause single- and double-strand breaks in DNA</td>
<td>Deletions</td>
</tr>
</tbody>
</table>
8.3. Induced Mutations

- **Transposition**
  - Transposons can be used to generate mutations
  - Transposon inserts into cell’s genome
  - Generally inactivates gene into which it inserts
Microorganisms commonly acquire genes from other cells: horizontal gene transfer

- Can demonstrate recombinants with auxotrophs
  - Combine two strains
    - E.g., His\(^-\), Trp\(^-\) with Leu\(^-\), Thr\(^-\)
  - Spontaneous mutants unlikely
  - Colonies that can grow on glucose-salts medium most likely acquired genes from other strain
Horizontal Gene Transfer as a Mechanism of Genetic Change

- Genes naturally transferred by three mechanisms
  - Transformation: naked DNA uptake by bacteria
  - Transduction: bacterial DNA transfer by viruses
  - Conjugation: DNA transfer between bacterial cells

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Main Feature</th>
<th>Size of DNA Transferred</th>
<th>Sensitivity to DNase Addition*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformation</td>
<td>Naked DNA transferred</td>
<td>About 20 genes</td>
<td>Yes</td>
</tr>
<tr>
<td>Transduction</td>
<td>DNA enclosed in a bacteriophage coat</td>
<td>Small fraction of the chromosome</td>
<td>No</td>
</tr>
<tr>
<td>Conjugation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmid transfer</td>
<td>Cell-to-cell contact required</td>
<td>Entire plasmid</td>
<td>No</td>
</tr>
<tr>
<td>Chromosome transfer</td>
<td>Cell-to-cell contact required; only Hfr cells can be donors</td>
<td>Variable fraction of chromosome</td>
<td>No</td>
</tr>
</tbody>
</table>

*DNase is an abbreviation of deoxyribonuclease, an enzyme that degrades DNA.
8.6. DNA-Mediated Transformation

- Naked DNA is not within cell or virus
- Cells release when lysed
- Addition of DNase prevents transformation
- Demonstration of transformation in pneumococci
  - Only encapsulated cells pathogenic

Organisms injected

Living encapsulated cells

Living non-encapsulated cells

Heat-killed encapsulated cells

Heat-killed encapsulated cells +

Living non-encapsulated cells

Results

Mouse dies

No effect

No effect

Mouse dies

Living encapsulated cells isolated
8.6. DNA-Mediated Transformation

- **Transformation**
  - Recipient cell must be **competent**
  - Most take up regardless of origin
    - Some accept only from closely related bacteria (DNA sequence)
  - Process tightly regulated
  - *Bacillus subtilis* has two-component regulatory system
    - Recognizes low nitrogen or carbon
    - High concentration of bacteria (quorum sensing)
    - Only a fraction of population becomes competent
8.7. Transduction

- Transduction: transfer of genes by bacteriophages
  - Specialized transduction: specific genes (Chapter 13)
  - Generalized transduction: any genes of donor cell
    - Rare error during phage assembly
    - Transfer of DNA to new bacterial host

(a) Formation of a transducing particle

1. A bacteriophage attaches to a specific receptor on a host cell.
2. The phage DNA enters the cell. The empty phage coat remains on the outside of the bacterium.
3. Enzymes encoded by the phage genome cut the bacterial DNA into small pieces.
4. Phage nucleic acid is replicated and coat proteins synthesized.
5. During construction of viral particles, bacterial DNA can mistakenly enter a protein coat. This creates a transducing particle that carries bacterial DNA instead of phage DNA.

(b) The process of transduction

1. A transducing particle attaches to a specific receptor on a host cell.
2. The bacterial DNA is injected into a cell.
3. The injected bacterial DNA integrates into the chromosome by homologous recombination.
4. Bacteria multiply with new genetic material. Replaced host DNA is degraded.
Conjugation: DNA transfer between bacterial cells

- Requires contact between donor, recipient cells
- **Conjugative plasmids** direct their own transfer
  - Replicons
- F plasmid (fertility) of *E. coli* most studied
8.8. Conjugation

- Conjugation (continued…)
  - F plasmid of *E. coli*
    - F\(^+\) cells have, F\(^-\) do not
  - Encodes proteins including F pilus
    - Sex pilus
    - Brings cells into contact
    - Enzyme cuts plasmid
    - Single strand transferred
    - Complementary strands synthesized
  - Both cells are now F\(^+\)
8.8. Conjugation

- Chromosomal DNA transfer less common
  - Involves Hfr cells (high frequency of recombination)
    - F plasmid is integrated into chromosome via homologous recombination
  - Process is reversible
  - F’ plasmid results when small piece of chromosome is removed with F plasmid DNA
  - F’ is replicon
8.8. Conjugation

- Chromosomal DNA transfer less common (continued…)
  - Hfr cell produces F pilus
  - Transfer begins with genes on one side of origin of transfer of plasmid (in chromosome)
  - Part of chromosome transferred to recipient cell
  - Chromosome usually breaks before complete transfer (full transfer would take ~100 minutes)
  - Recipient cell remains F⁻ since incomplete F plasmid transferred
Genomics reveals surprising variation in gene pool of even a single species

- Perhaps 75% of *E. coli* genes found in all strains
  - Termed *core genome* of species
- Remaining make up *mobile gene pool*
  - Plasmids, transposons, genomic islands, phage DNA

### TABLE 8.4 The Mobile Gene Pool

<table>
<thead>
<tr>
<th>Composition</th>
<th>Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transposons</td>
<td></td>
</tr>
<tr>
<td>Insertion sequences</td>
<td>Transposase gene flanked by short repeat</td>
</tr>
<tr>
<td>(ISs)</td>
<td>sequences</td>
</tr>
<tr>
<td>Composite transposons</td>
<td>Recognizable gene flanked by insertion</td>
</tr>
<tr>
<td></td>
<td>sequences</td>
</tr>
<tr>
<td>Plasmids</td>
<td>Circular double-stranded DNA replicon; smaller than chromosomes</td>
</tr>
<tr>
<td>Genomic Islands</td>
<td>Large fragment of DNA in a chromosome or plasmid</td>
</tr>
<tr>
<td>Phage DNA</td>
<td>Phage genome</td>
</tr>
</tbody>
</table>
Resistance plasmids (R plasmids)
- Resistance to antimicrobial medications, heavy metals (mercury, arsenic)
  - Compounds found in hospital environments
- Often two parts
  - R genes
  - RTF (resistance transfer factor)
    - Codes for conjugation
- Often broad host range
- Normal microbiota can transfer to pathogens
8.9. The Mobile Gene Pool

- Bacteria can conjugate with plants
  - Natural genetic engineering
  - *Agrobacterium tumefaciens* causes crown gall
    - Different properties, produces opine, plant hormones
    - Piece of tumor-inducing (Ti) plasmid called T-DNA (transferred DNA) is transferred to plant
    - Incorporated into plant chromosome via non-homologous recombination
8.9. The Mobile Gene Pool

- Transposons yielded vancomycin resistant *Staphylococcus aureus* strain
  - Patient infected with *S. aureus*
    - Susceptible to vancomycin
  - Also had vancomycin resistant strain of *Enterococcus faecalis*
    - Transferred transposon-containing plasmid to *S. aureus*
    - Transposon jumped to plasmid in *S. aureus*
Genomic islands: large DNA segments in genome
• Originated in other species
• Nucleobase composition very different from genome
  • G-C base pair ratio characteristic for each species
• May provide different characteristics
  • Utilization of energy sources
  • Acid tolerance
  • Development of symbiosis
  • Ability to cause disease
    – Pathogenicity islands