Agenda

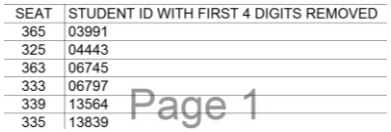
- Final exam information
- Review lectures 5B to 10
- Response to student queries
- Student feedback / evaluations

W2016 ENH222 Final Exam

- Date: Wed Apr 20
- Time: 12:00 – 2:00 pm, 2 hr
- Venue: MTCC-D
- Multiple choice: 60+ Questions
- Weight: 40%
- Cumulative: Covers entire semester's lecture
- Open notes: 2 double-sided letter-sized sheet

Seat Plan

- Tabulated seating list



SEAT	STUDENT ID WITH FIRST 4 DIGITS REMOVED
365	03991
325	04443
363	06745
333	06797
339	13564
335	13839

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- Posted on D2L
- Check seat no. before arriving at Exam Hall
- It will help you to locate your seat more quickly

Final Lecture: Course Review & Wrap-Up

Lecture Learning Objectives

By the end of this lecture, students will be able to:

- Revisit lecture material not covered in mid-term exam
- Identify any outstanding learning issues in these lectures
- Address any questions on the course material

ENH222 Final Lecture
What did we cover?

L1	Introduction & History
L2	Basic Lab Techniques & Microscopy
L3	Prokaryotes & Eukaryotes Agents
L4	Microbial Growth 1 & Nutrition
L5A	Microbial Metabolism
L5B	Microbial Growth 2
L6	Physical Control
L7	Microbiology of Food
L8	Microbiology of Water
L9	Chemical Control
L10	Genetics & Chemotherapeutic Agents

COURSE OBJECTIVES

By the end of this course, students will be able to:

1. Recognize the general characteristics of bacteria, viruses and important eukaryotic micro-organisms
2. Describe important characteristics of bacteria (cellular ultrastructure, growth patterns, growth requirements, factors affecting growth) which play a role in the development and control of foodborne and communicable illness
3. Comprehend terminology of microbial control, the major physical and chemical methods of control and the limitations of each method

COURSE OBJECTIVES

4. Understand the basic concepts of antimicrobial therapy and the development of resistance
5. Describe characteristics of some important food- & waterborne pathogens
6. Recognize fundamental principles underlying HACCP analysis
7. Describe methods used to visualize, isolate, identify and enumerate bacteria and their applications to the evaluation of common, potentially hazardous foods for total microbial load as well as the presence of specific pathogens
8. Understand the principles and applications of bacterial genetics

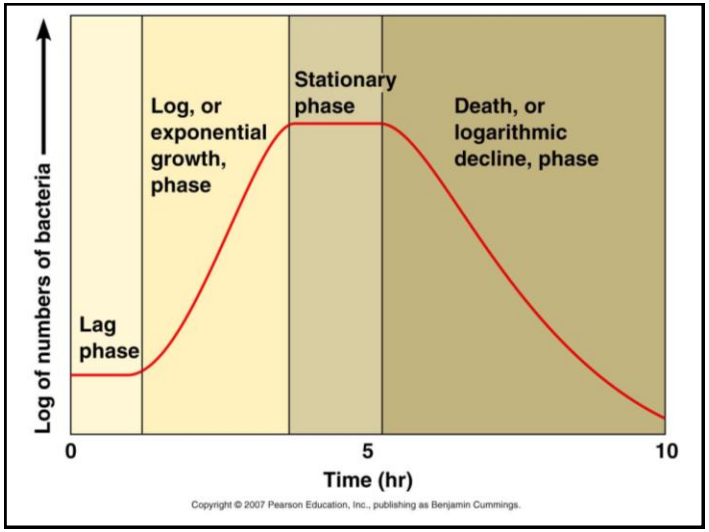
Lecture 5B Learning Objectives
Microbial Growth Enumeration

By the end of this lecture students will be able to:

1. Define bacterial growth by binary fission and generation time
2. Compare the phases of microbial growth
3. Describe direct and indirect methods of measuring cell growth

Generation Time

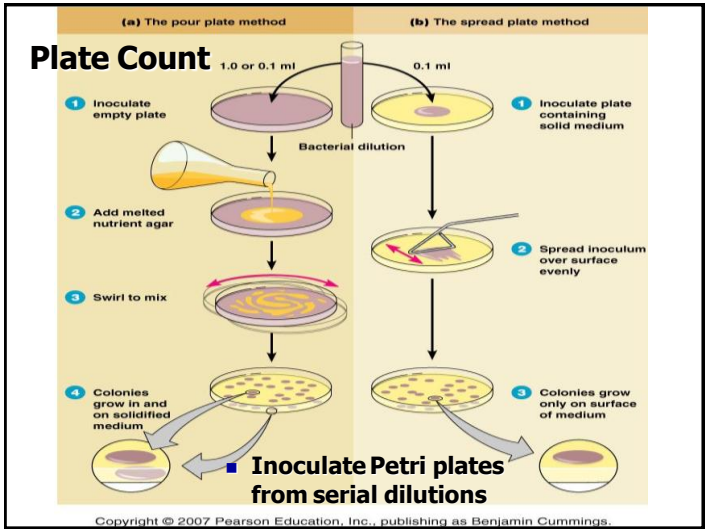
- Time required for a microbial cell to divide and its population to double
- If the generation time is 20 mins then
  - 1 hr will produce ? generations
- If there is 10 bacteria at the start?
  - How many will there be in 1 hr?



Measuring Microbial Growth

Direct methods
- Plate counts
- Filtration
- MPN
- Direct microscopic count

Indirect methods
- Turbidity
- Metabolic activity
- Dry weight



- **Inoculate Petri plates from serial dilutions**

Lecture 6 Learning Objectives Microbial Growth Control

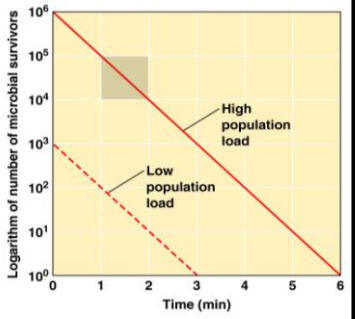
By the end of this lecture students will be able to:

1. Describe patterns of microbial death caused by microbial control agents
2. Described the use of moist and dry heat as well as other physical methods of microbial control
3. Describe the effects of radiation on microbes

Effectiveness of Antimicrobial Treatment

Depends on:

1. Number of microbes
2. Environment (organic matter, temperature, biofilms)
3. Time of exposure
4. Microbial characteristics



(b) The effect of high or low initial load of microbes. If the rate of killing is the same, it will take longer to kill all members of a larger population than a smaller one. This is true for both heat and chemical treatments.

Physical Methods of Microbial Control

- Heat
  - Thermal death point (TDP):
    - Lowest temperature needed to kill all microbes within 10 min.
  - Thermal death time (TDT):
    - Shortest time needed to kill all microbes
  - Decimal reduction time (D value):
    - Time needed to kill 90% of microbes

Physical Methods of Microbial Control: Heat

- Pasteurization – equivalent treatments
  - reduces spoilage organisms and pathogens
  - 63°C for 30 min for milk
  - High temperature, short time (HTST): 72°C for 15 sec
- Commercial Sterilization
  - Ultra high temperature (UHT): 140°C for 4 sec
  - Some thermophilic bacteria may survive

Physical Methods of Microbial Control

- Generally shorter treatment times require higher temperatures…however
  STERILIZATION
- Use **steam under pressure**: autoclave OR
- **Dry heat**: oven (hot-air)
  – Heat in air transfers less readily
  – Needs **longer** time than moist heat

	Hot-air	Autoclave
Equivalent treatments	170°C, 120 min	121°C, 15 min

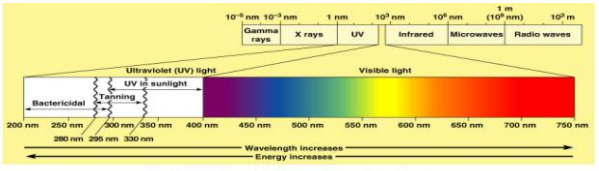
Physical Control of Microbial Growth

- Temperature
  – Dry heat
  – Steam
  – Cold
- Pressure
  – Atmospheric
  – Osmotic
- Radiation

Physical Methods of Microbial Control: Radiation

Radiation damages DNA

- Ionizing radiation
  – X rays, gamma rays, electron beams
  – Nonionizing radiation
    - UV, microwaves (mainly kill by heat)



Lecture 7 Learning Objectives Microbial Contamination of Food

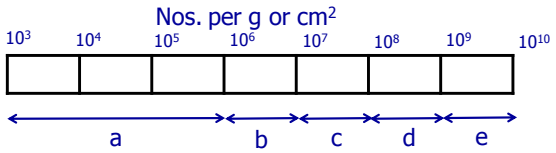
By the end of this lecture students will be able to:

1. Understand how bacterial contamination leads to spoilage in different types of food
2. Understand the effects of physical control of microbial growth for storage and handling of raw and prepared food items to reduce contamination and spoilage
3. Explain Hazards Analysis and Critical Control Points (HACCP)
4. Recognize different foodborne illnesses and their association with certain types of food

Food spoilage and microbial contamination

Relationship between food spoilage and quantitative extent of microbial contamination

a. Raw milk: sour at 10^3 - 10^6
b. Vacuum packed meats: off odour at 10^6 - 10^7
c. Aerobically-stored meats/vegetables: off odour at 10^7 - 10^8
d. Almost all food, aerobically stored meats: slime at 10^8 - 10^9
e. Structural change and decomposition >10^9



Factors Needed for Bacterial Growth

FAT TOM

- Food
- Acidity
- Temperature
- Time
- Oxygen

pH Values of Common Food Items & Acidoduric Microbes

Low pH foods

Product	**pH**
Vegetables	4.3 – 6.5
Fruits	2.0 – 6.7
Yogurt	3.8 – 5.2
Mayonnaise	3.0 – 4.1
Salad Dressing	3.2 – 4.0

Microorganisms	Optimum pH
Acidophiles / Acidoduric	
Yeast (spoilage)	4.0 – 6.5
Molds (spoilage)	4.5 – 6.8

Food Items with Low Water Activity & Tolerant Microbes

Food	A_w
Cured meat	0.87 – 0.95
Jam	0.75 – 0.8
Dried fruit	0.55 – 0.8
Dried vegetables	0.2
Crackers	0.1

Group	Minimum A_w value
Staphylococcus	>0.86
Most yeast	0.88
Molds	0.82

Bacteria survival/growth in abnormal conditions

- Low temp <4°C: *Listeria* & *Yersinia*
- Lower water activity: *Salmonella*
  - Longer D value (decimal reduction time) required to kill 90%
- Higher fat content: Milk
  - Higher thermal death point required to kill all bacteria in 10 mins
- Thermophilic bacterial endospores
  - Longer thermal death time required to kill all bacteria at different temepratures

Hazard Analysis & Critical Control Points (HACCP)

7 Standardized Principles

1. Conduct a hazard analysis
2. Determine the critical control points
3. Establish critical limits
4. Establish monitoring procedures
5. Establish corrective actions
6. Establish verification procedures
7. Establish record-keeping and documentation procedures

http://www.omafra.gov.on.ca/english/food/inspection/haccp/haccp_principles.htm

Hazard Analysis & Critical Control Points (HACCP)

- **Control Point:** Any point at which biological factors can be controlled
- **Critical Control Point:** A point where controls can be applied and a food safety hazard can be prevented, eliminated or reduced to acceptable levels
  - Cooking step (elimination)
  - Chilling step (reduction)
- **Critical Limits:** Defined & measured for each CCP
  - Danger zone upper limit: 60°C
  - Danger zone lower limit: 4°C
  - Max. time in danger zone: 4 hrs

Bacteria-Caused Foodborne Illness

INTOXICATION
(1–12 hr) quicker

- *Staphylococcus* (infected food handler)
- *Bacillus* (cooked rice)
- *Clostridium* (produce, soil)
- *E. coli* (ground beef)

INFECTION
(6-72 hrs) slower

- *Salmonella* (chicken)
- *Shigella* (fecal contamination)
- *Campylobacter* (chicken)

Lecture 8:
Microbial Contamination of Water
Learning Objectives

By the end of this lecture students will be able to:

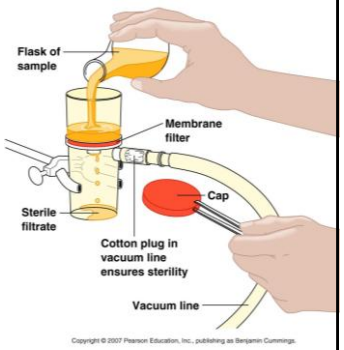
1. Define potable water & recreational water
2. Detect microbiological contamination in water
3. Understand why indicator bacteria such as total coliforms and *Escherichia coli* are used for monitoring water quality
4. List different drinking water sources
5. Discuss treatment for and prevention of water contamination
6. Identify causes and sources of water contamination

Different Types of Water

- Potable water
- Drinking
  - 0 *E. coli* per 100 ml
  - 0 total coliforms per 100 ml
- Recreational water
- fresh or marine
  - 100 E. coli per 100 ml
  - 1000 total coliforms per 100 ml

Membrane Filtration

- Filter water through membrane
- Good for low contamination levels
  - Bacteria trapped & concentrated
- Bacteria removed from filtered water
- Detection & enumeration method



Colilert (ONPG-MUG) Test
Total Coliform and *E. coli*

- Coliform β-galactosidase breaks down ortho-nitrophenyl-β-D-galactopyranoside (ONPG)
- Release yellow ONP indicator
- *E. coli* β-glucuronidase hydrolyzes 4-methyl umbelliferyl-β-D-glucuronide (MUG)
- Release fluorescent umbelliferone derivative
- Very sensitive - 1 CFU/100 ml

Colilert test

Indicator Bacteria
Total Coliforms vs *E. coli*

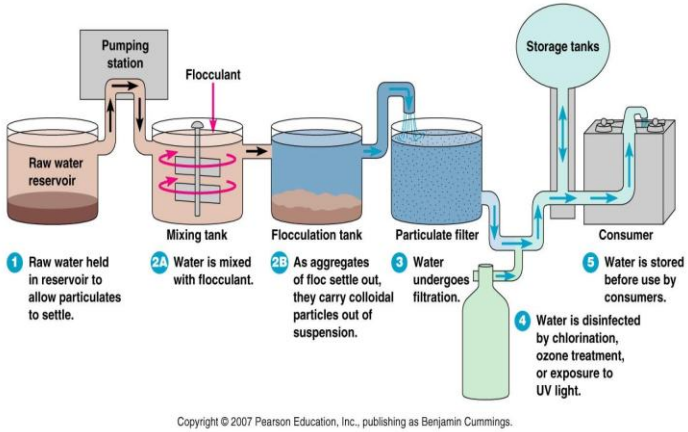
Total coliforms	***E. coli***
• May inhabit mammalian guts	• Mainly inhabits mammalian guts
• Also found occurring naturally in the environment	• Not normally found in environment unless from fecal contamination
• Different types of enterobacteriaceae	• Only 1 type of enterobacteriaceae
• Usually non-pathogenic	• Potentially pathogenic
• Indicator of inadequate treatment of drinking water supplies	• Indicator of fecal pollution in drinking water supplies

Drinking Water Sources

GROUND WATER
- wells
- aquifers
- springs

SURFACE WATER
- lakes
- rivers
- creeks

Municipal Water Treatment



Drinking Water Treatment

MULTIPLE BARRIER APPROACH
- Source water protection
- Effective treatment
  - Coagulation, flocculation and sedimentation
  - Filtration - membrane, sand
  - Disinfection - chlorination, ozonation, UV irradiation
- Well-maintained distribution system
- Routine verification of water quality
- Public education

Drinking Water Contamination

GROUND WATER – wells, aquifers, springs

1. Sewage overflow
2. Shallow well

SURFACE WATER – lakes, river, creeks, (FILTERED)

1. Filter backwash water recycling
2. Water filtration equipment malfunction
3. High turbidity - inadequate flocculation / coagulation

WALKERTON
Summary of Physical Causes

- Heavy rains & flooding
- Shallow well contaminated by surface water
- Water treatment system overwhelmed
- *E. coli* O157:H7 and *Campylobacter* spp. in neighbouring farms
- The primary source of contaminants - manure spread on a farm near Well 5 in late April 2000.
- DNA typing of animals and manure on the farm showed that *E. coli O157:H7* and *Campylobacter* strains on farm matched strains prevalent in human outbreak cases.

Lecture 9:
Microbial Growth Control
Learning Objectives

By the end of this lecture students will be able to:

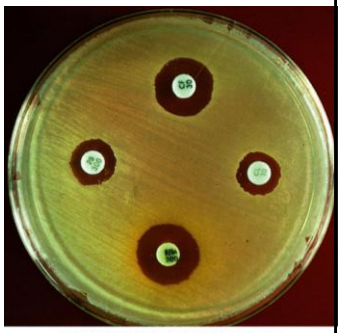
1. List the factors related to effective disinfection.
2. Interpret the results of use-dilution tests and the disk-diffusion method.
3. Identify the methods of action and preferred uses of chemical disinfectants.
4. Explain how the control of microbial growth is affected by the type of microbe.
5. Recognize the chemicals used as preservatives in food.
6. Recognize common antibiotics and their mechanisms of action

Factors related to effective disinfection

- Many types of chemicals
  – Selection important
- Disinfectant concentration
  – Dilute to manufacturer specifications
- Time
  – Different contact times
- Nature of material being disinfected
  – Organic material present may interfere
  – pH of medium affects activity of disinfectant

Disk-Diffusion Test

- Filter disks soaked with known concentrations of disinfectants/antibiotics
- Larger zone of inhibition indicates greater sensitivity
- Bacteria reported as sensitive, intermediate or resistant



Disinfectants

- Methods of action:
  - Plasma membrane disruption
  - Protein denaturation
  - Dissolves lipids

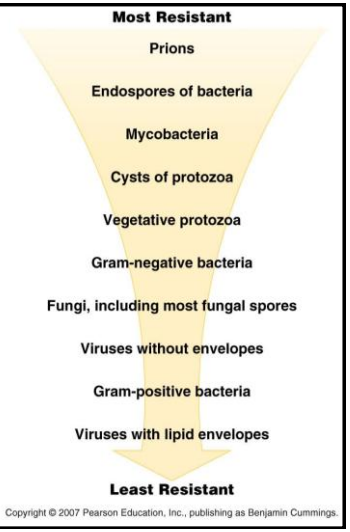
Types of Disinfectants

Type	Use	Gram	Myco-bacteria	Endo-spores
Phenolics	Organic matter	+ve	Good	Poor
Biguanides	Hand scrubs	Both	Poor	Poor
Halogens	Antiseptics Water Treatment	Both	Fair/Good	Poor
Alcohols (70%)	Hand rubs Degerming	Both	Good	Poor
Heavy metals	Ointments	Both	None	None
Quats	Cleaning surfaces	+ve	None	None
Aldehydes	Medical equipment	Both	Good	Fair
Peroxygens	Surfaces Equipment	Both	Good	Good

Microbial Characteristics & Microbial Control

Factors that prevent chemical disinfection

- Lipopolysaccharide layer ar porins of Gram negative bacteria
- Lipid-rich cell wall of *Mycobacteria*
- Bacterial endospores
- Fungal spores
- Protozoan (oo)cysts
- Non-enveloped viruses
- Prions



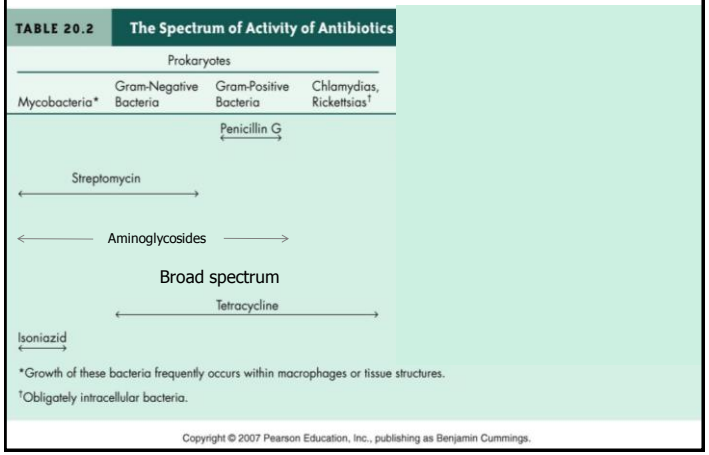
Types of Disinfectants
Chemical food preservatives
Organic acids & salts

Food preservatives

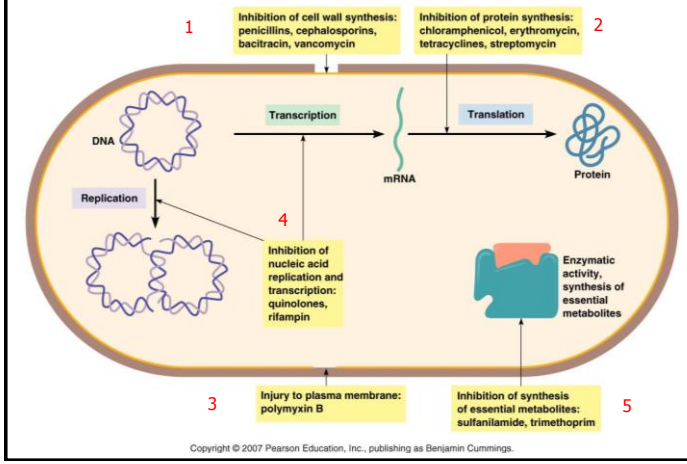
Interfere with metabolism & plasma membranes

- Sorbic acid or sorbate
- Benzoic acid or benzoate
- Propionate
- Nitrate or nitrite
- Nisin and natamycin

Spectrum of Anti-microbial Activity



The Action of Antimicrobial Drugs



Types of Antibiotics

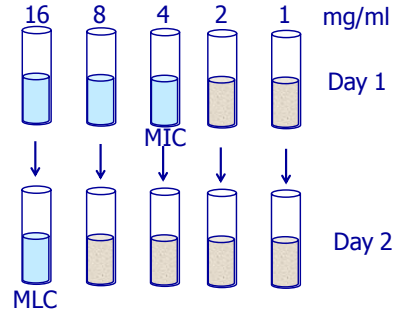
Type	Action	Gram	Mycobacteria
Penicillins	Cell wall	+ve	No
Cephalosporins	Cell wall	+ve	No
Polypeptides	Cell wall	+ve	No
Isoniazid/ Ethambutol	Cell wall	No	Yes
Amino-glycosides	Peptide	Both	Yes
Tetracyclines	Peptide	Both	No
Streptogramins	Peptide	+ve	No
Macrolides	Peptide	+ve	No
Oxazolidinones	Peptide	+ve	No

Types of Antibiotics

Type	Action	Gram	Mycobacteria
Polymyxin B	Plasma membrane	-ve	No
Rifamycin	Nucleic acid		Yes
Quinolones Fluoroquinolones	Nucleic acid	Both	No
Sulphonamides-Trimethoprim	Metabolites	Both	No

Inhibitory Concentrations of Antibiotics

- MIC: lowest antibiotic concentration that prevents visible bacterial growth
- MLC: lowest antibiotic concentration with no further growth in antibiotic free broth



Control of Microbial Growth Summary

Physical
- Temperature
  - Dry heat
  - Steam
  - Cold
- Pressure
  - Atmospheric
  - Osmotic
- Radiation

Chemical
- Disinfectants
- Antibiotics

Lecture 10: Microbial Genetics Learning Objectives

By the end of this lecture students will be able to:

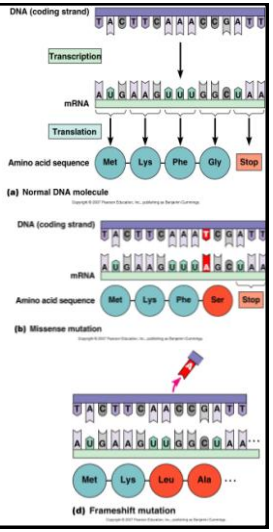
1. Define genetics, genomics, genotype and phenotype
2. Describe different types of mutations
3. Explain genetic transfer and recombination
4. Describe bacterial transformation, plasmids and transposons
5. Understand the mechanisms of antibiotic resistance and the effects of antibiotics used in combination

Terminology Genetics

- Science of heredity
- Gene: A segment of DNA that encodes a functional product, usually a protein.
- Genome: All of the genetic material in a cell
- Genomics: The molecular study of genomes
- Genoype: Genetic or DNA type
- Phenotype: Expression of genotype ie. protein, lipid & polysaccharide type

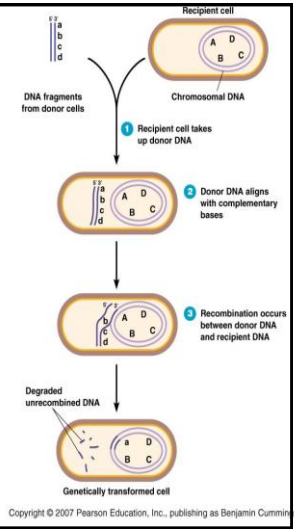
Mutations

- Point mutation
  - Change in one base
    - Synonymous
    - Non-synonymous or missense
    - Nonsense
- Frameshift mutation
  - Insertion or deletion of >=1 base



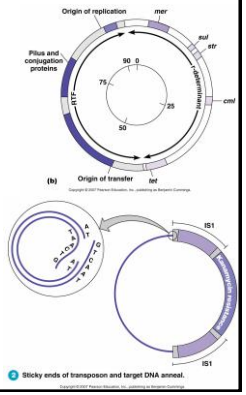
Recombination & Transformation

- Integration of DNA fragments into chromosomes
- Competent recipient cell has altered cell wall making it permeable to large fragments of donor DNA



Plasmids: Resistance Factors

- Serious problem in antibiotic treatment of infectious diseases
- Antibiotic resistance genes
- Transfer between enteric bacteria eg. *Escherichia* & *Salmonella*
- Transposons can insert resistance genes in plasmids & become part of the plasmid



Mechanisms of Antibiotic Resistance

1. Enzymatic destruction of drug
   - Bacterial penicillinases: Beta-lactamases
   - Methicillin & vancomycin resistance in *Staphylococcus aureus and Enterococcus*
2. Prevention of penetration of drug
   - Gram negative mutants alter porin openings
3. Alteration of drug's target site
   - Modification of protein synthesis sites
   - Macrolide & tetracycline resistance
4. Rapid ejection of the drug
   - Rapid efflux pumps out antibiotics
   - Tetracycline resistance

Knowledge of Microorganisms

- Allows us to
  - Prevent food spoilage
  - Prevent disease occurrence
- Led to aseptic techniques to prevent contamination in medicine and in microbiology laboratories.

All the best in your finals!