Game plan

Lecture

Binary fission
Growth curves
Physical requirements for growth
Chemical requirements for growth

Bring books and APO-3 for next class

Lab

Lab Exam

Prelabs

Growth Curve

Microbial growth and clinical implications

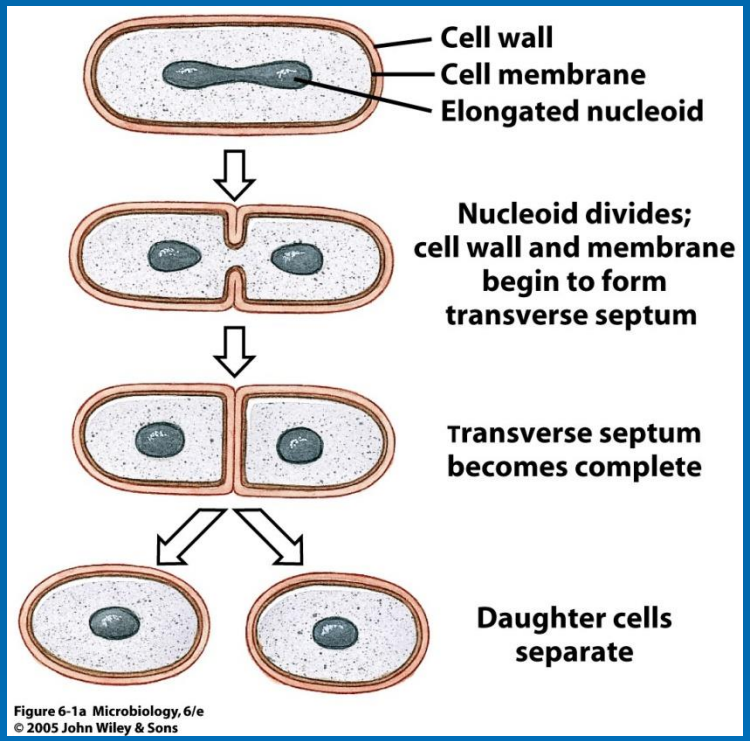

Figure 6-1a Microbiology, 6/e
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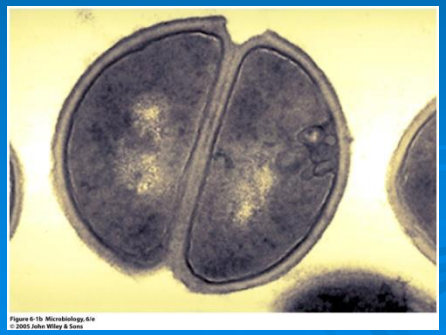


Eukaryotes

Mitosis

Budding (yeast)

Spores (fungi)

Filament fragmentation (fungi)

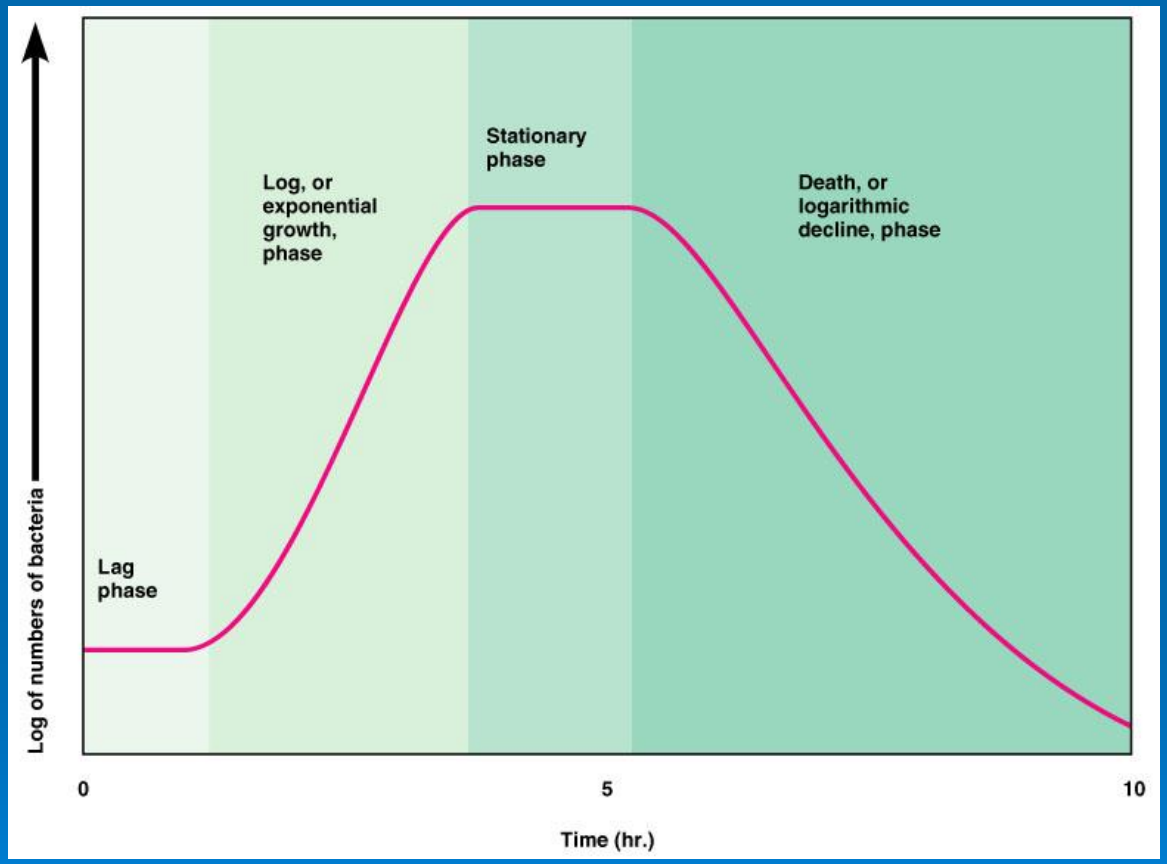
Prokaryotes

Binary fission

Filament fragmentation

Generation time: time it takes for population of cells to double in number

Standard growth curve



1. Lag phase
   - Metabolically active
   - No change in cell #

2. Log phase- exponential growth

Numbers of Cells	Numbers Expressed as a Power of 2	Visual Representation of Numbers
1	2^0	●
2	2^1	●●
4	2^2	●●●●
8	2^3	●●●●●●●●
16	2^4	●●●●●●●●●●●●●●●●
32	2^5	●●●●●●●●●●●●●●●●●●●●●●●●●●●●●●

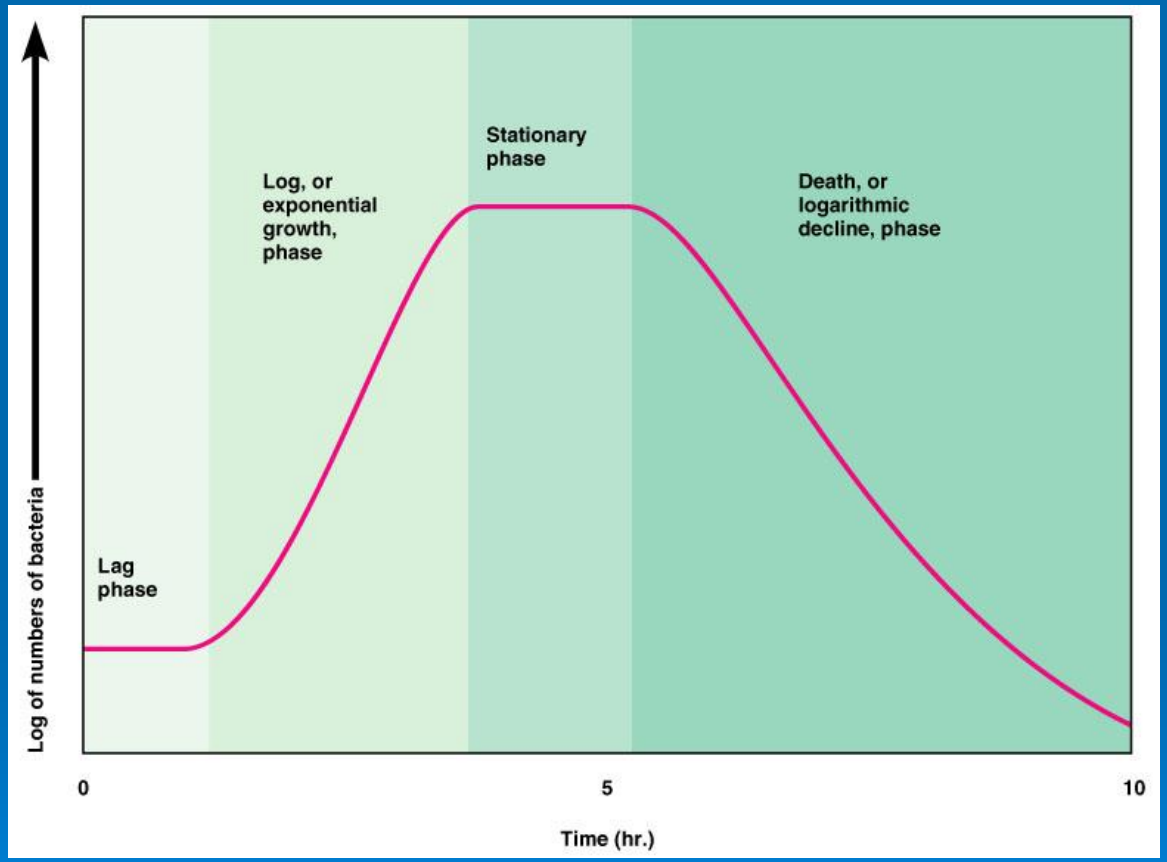
(a) Visual representation of increase in bacterial number over five generations. The number of bacteria doubles in each generation. The superscript indicates the generation, that is, $2^5 = 5$ generations.

Generation Number	Number of Cells	Log_{10} of Number of Cells
0	$2^0 = 1$	0
5	$2^5 = 32$	1.51
10	$2^{10} = 1{,}024$	3.01
15	$2^{15} = 32{,}768$	4.52
16	$2^{16} = 65{,}536$	4.82
17	$2^{17} = 131{,}072$	5.12
18	$2^{18} = 262{,}144$	5.42
19	$2^{19} = 524{,}288$	5.72
20	$2^{20} = 1{,}048{,}576$	6.02

(b) Conversion of the number of cells in a population into the logarithmic expression of this number. To arrive at the numbers in the center column, use the y^x key on your calculator. Enter 2 on the calculator; press y^x; enter 5; then press the = sign. The calculator will show the number 32. Thus, the fifth-generation population of bacteria will total 32 cells. To arrive at the numbers in the right-hand column, use the log key on your calculator. Enter the number 32; then press the log key. The calculator will show, rounded off, that the $\log_{10}$ of 32 is 1.51.

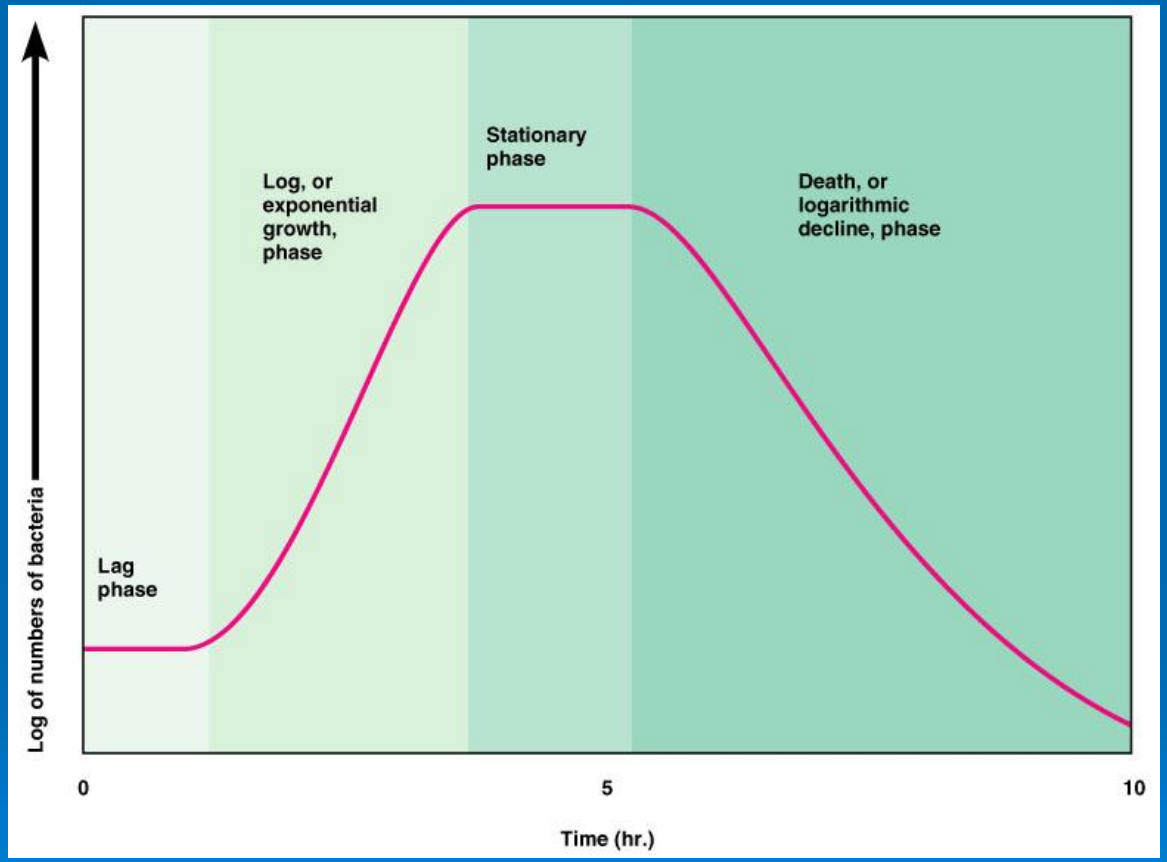
Figure 6.12- Overview

Standard growth curve



1. Lag phase
   - Metabolically active
   - No change in cell #
2. Log phase
   - Exponential growth
3. Stationary phase
   - Death rate= growth rate

Standard growth curve



1. Lag phase
   - Metabolically active
   - No change in cell #
2. Log phase
   - Exponential growth
3. Stationary phase
   - Death rate= growth rate
4. Death/decline phase
   - Death rate > growth rate

Sporulation

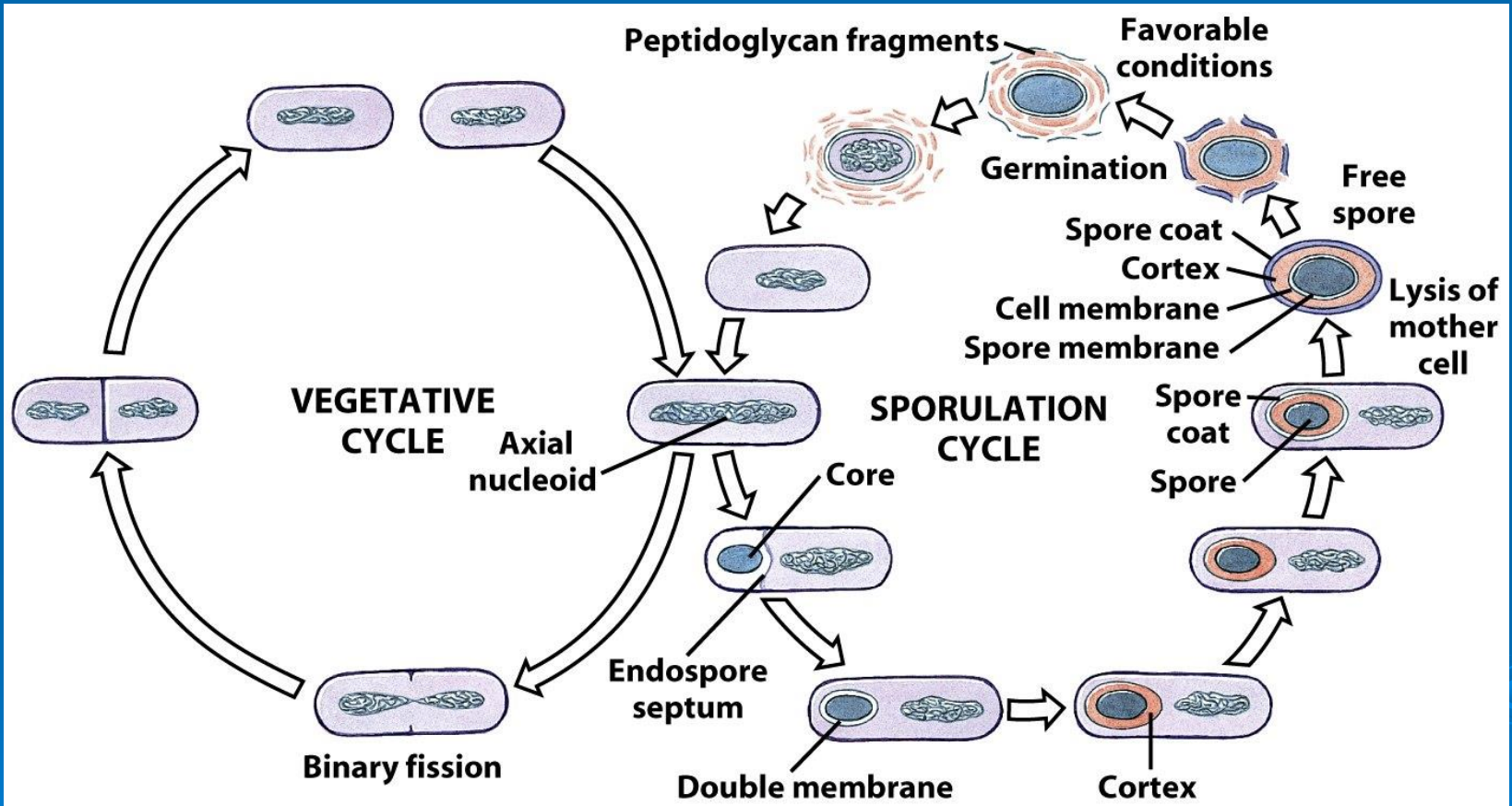


Figure 6-17 Microbiology, 6/e

Physical requirements for growth: temperature

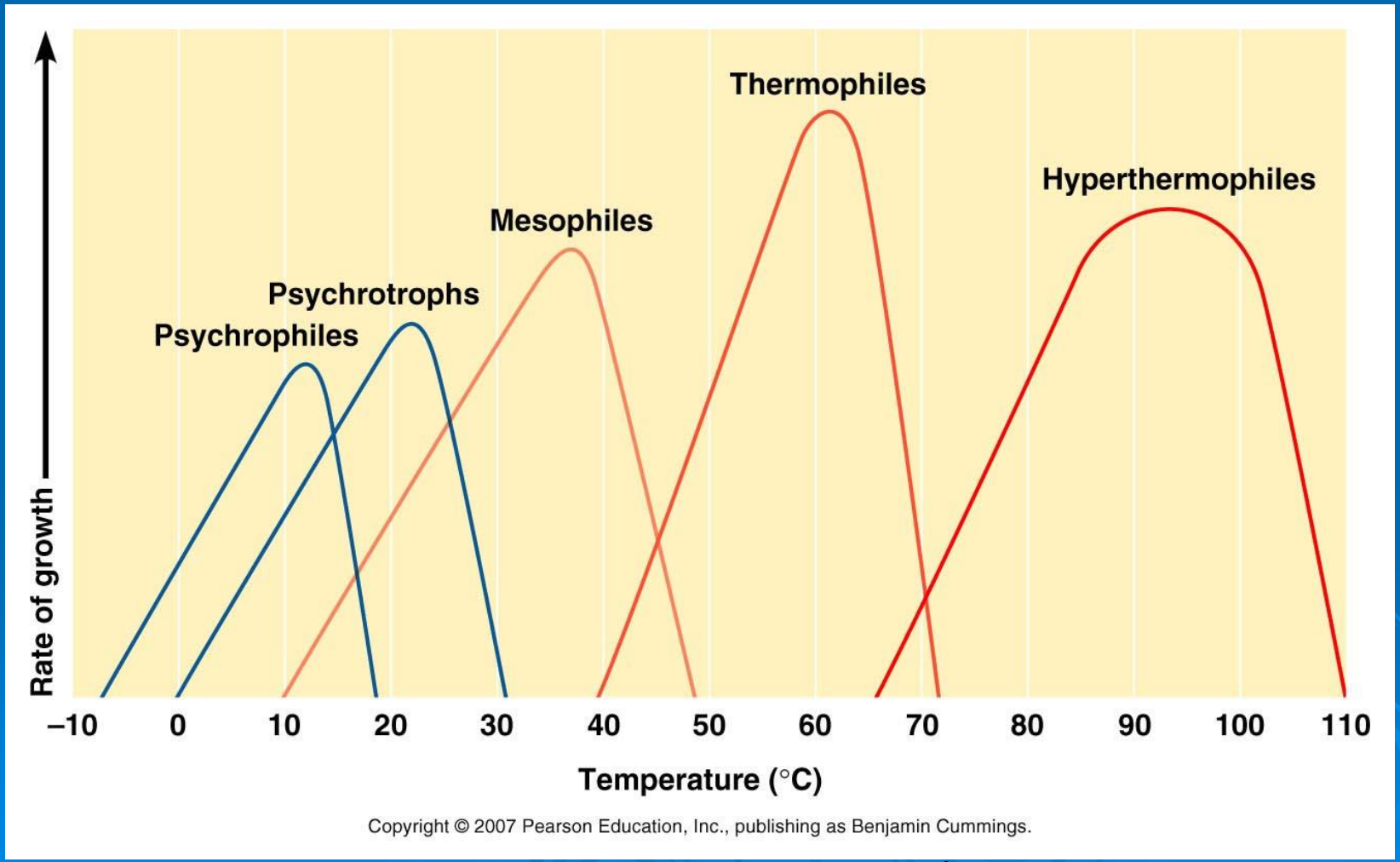


Figure 6.1

Clinical implications: Refrigeration prevents food poisoning

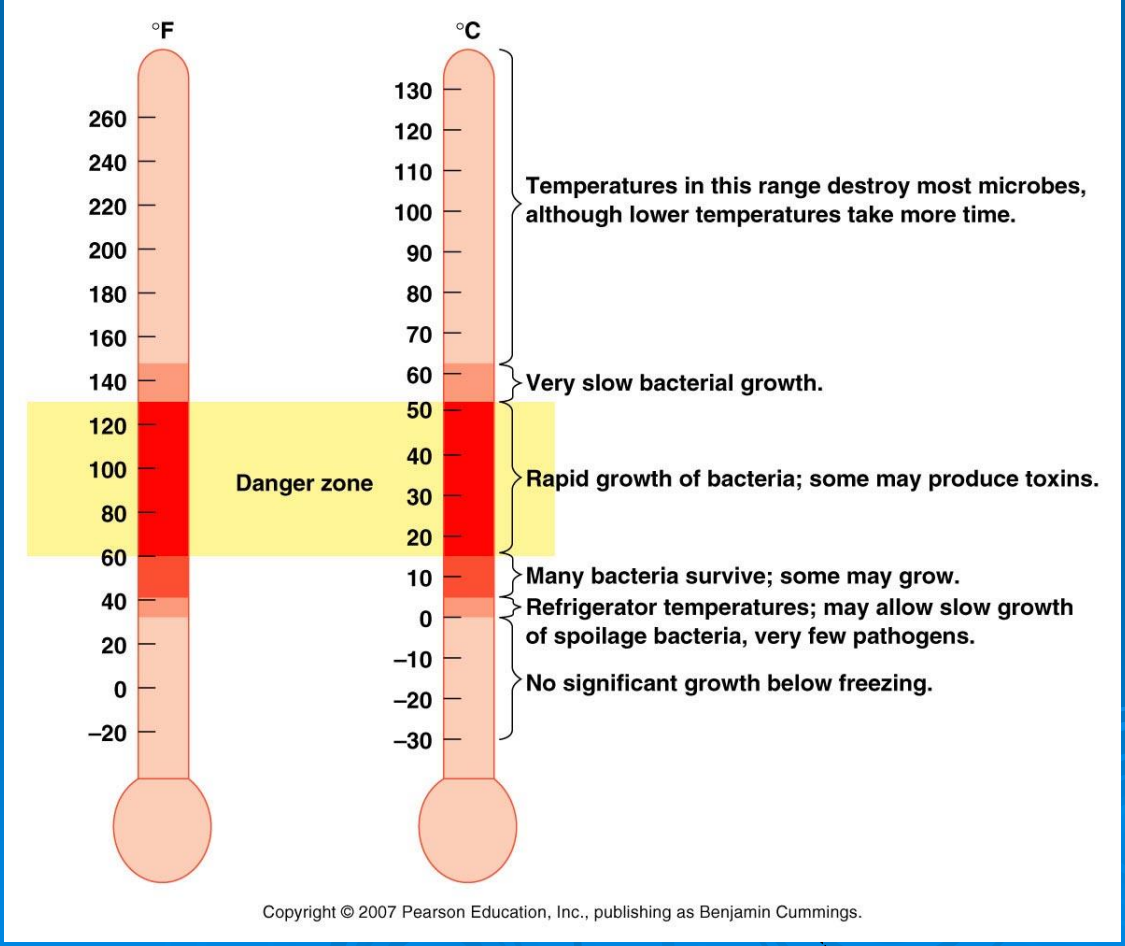
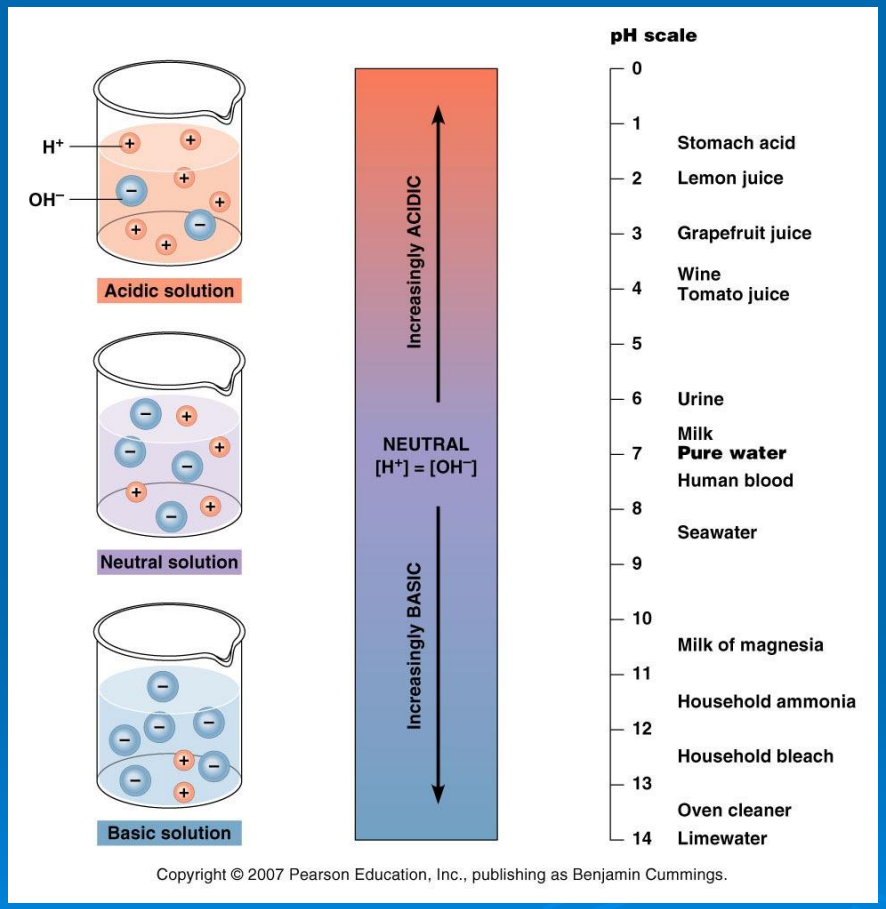


Figure 6.2

Physical requirements for growth: pH



-Lactobacillus
-Propionibacterium acnes
-Ferroplasma

-Cyanobacteria
-Vibrio cholerae

Physical requirements for growth: osmotic pressure

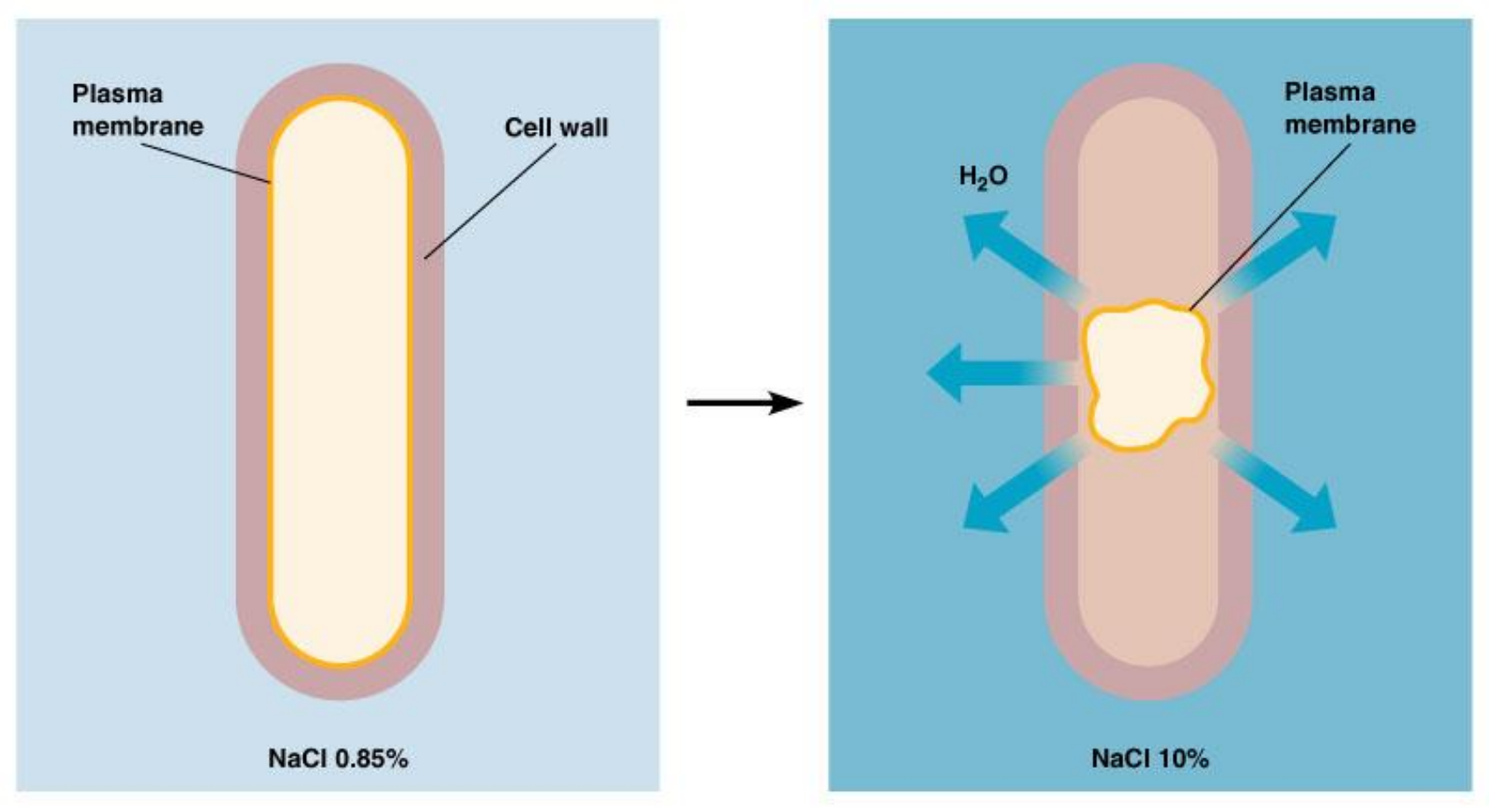


Figure 6.4 - Overview

Physical requirements for growth: osmotic pressure

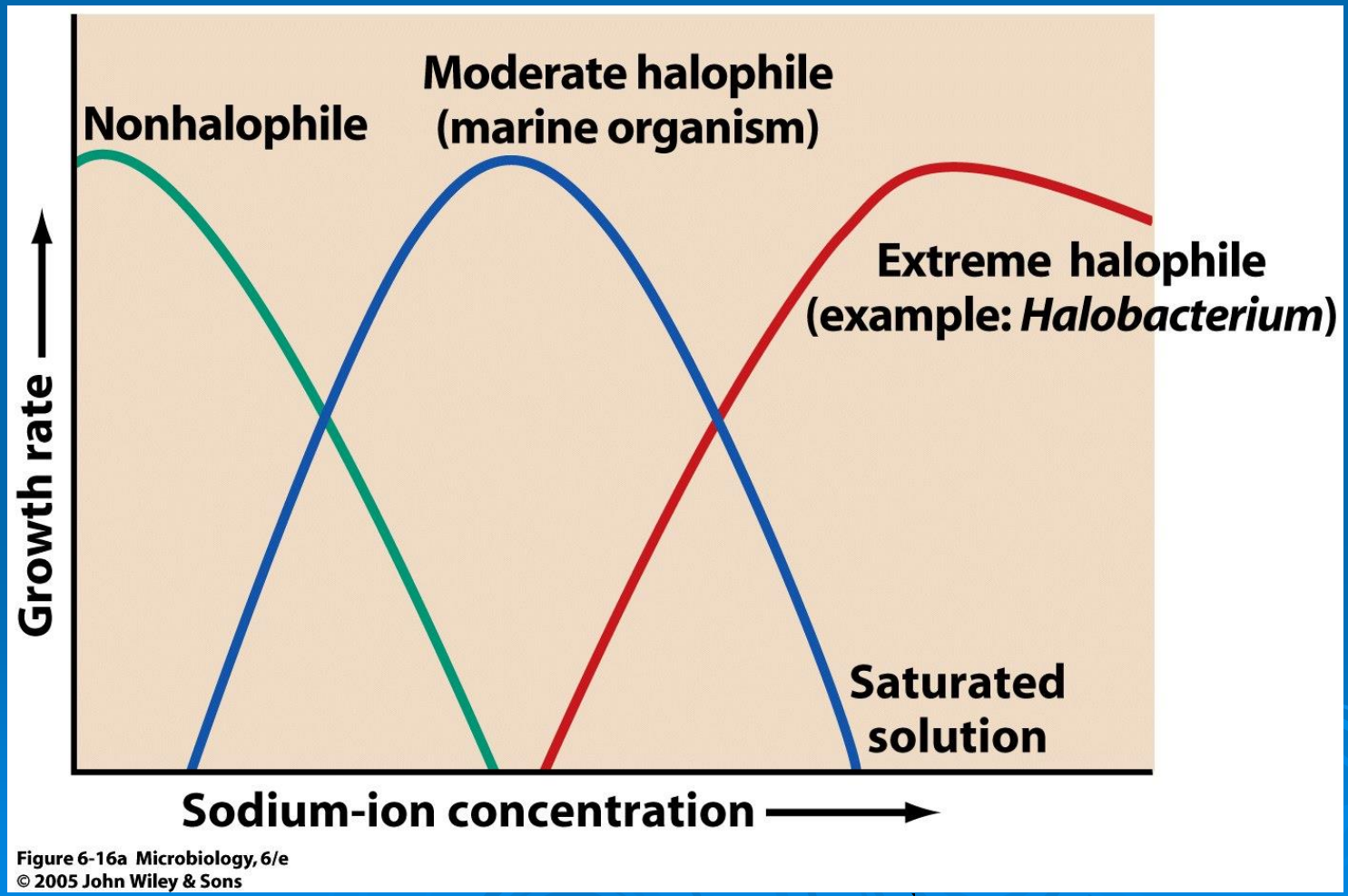
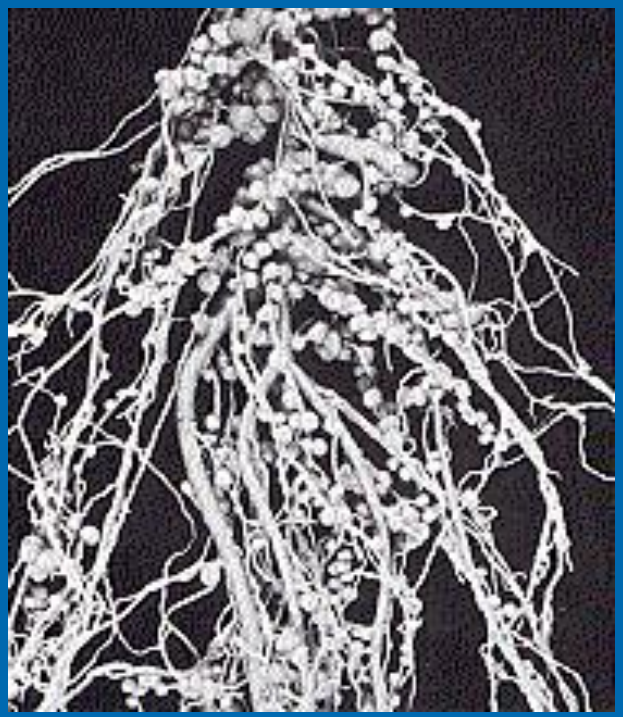


Figure 6-16a Microbiology, 6/e

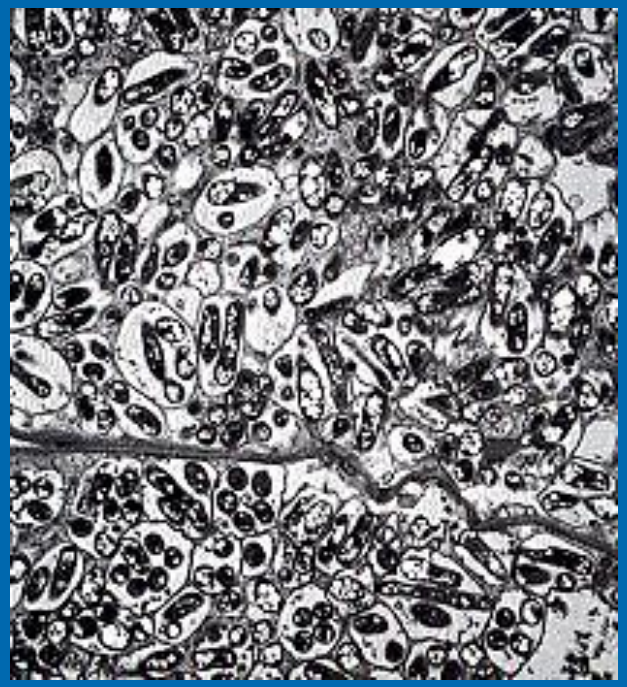
Chemical requirements for growth

Chemical	Used for…	Primary source	Alternative sources
Carbon			
Nitrogen			
Sulfur			
Phosphorus			
Trace elements			
Organic growth factors			

Nitrogen fixation



Leguminous root nodules



TEM of rhizobia in root nodule cell

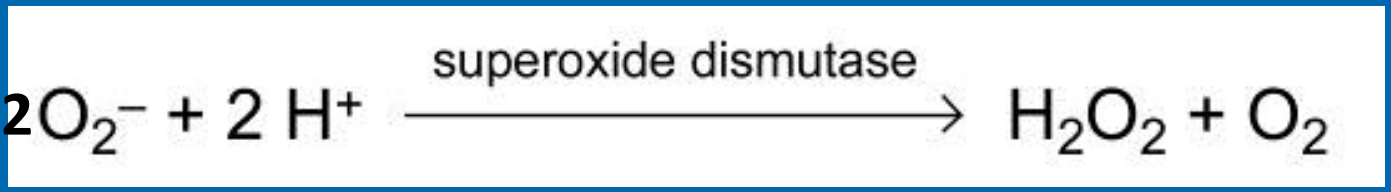
Rhizobium in symbiosis

Azotobacter
Klebsiella
some *Clostridium*

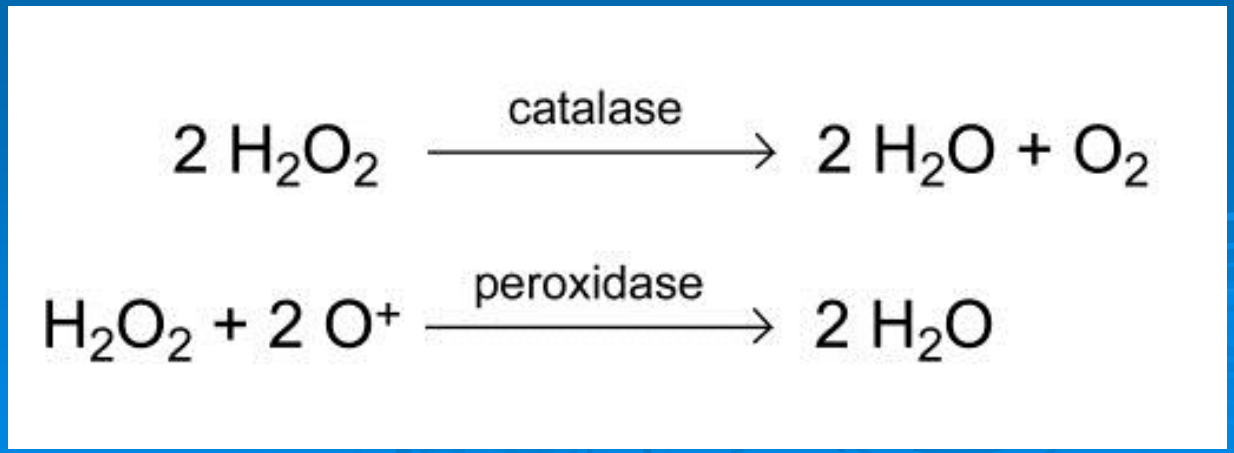
Oxygen

With a little oxygen toxicity…

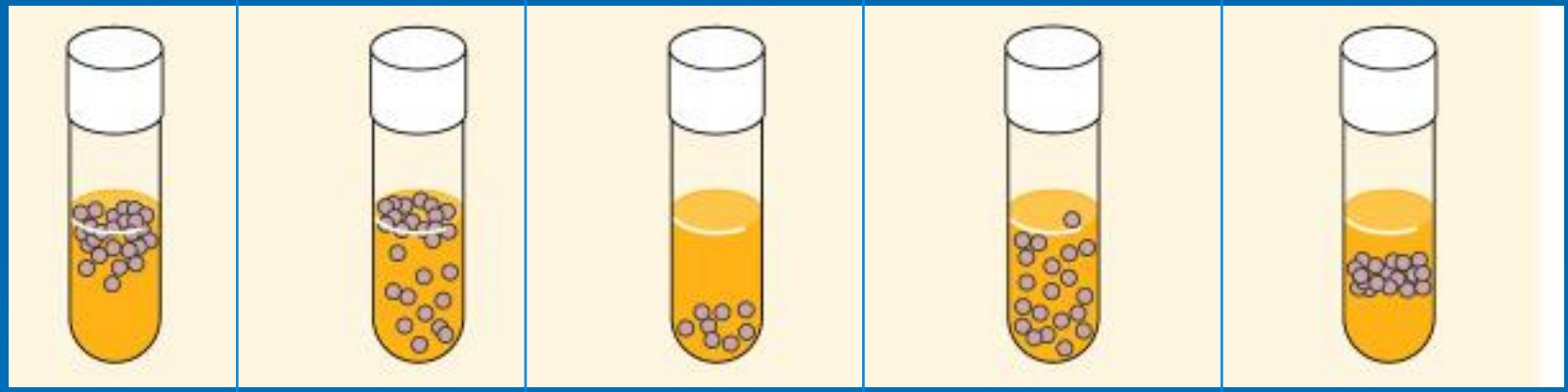
Superoxide free radical (O_2^-)



Hydrogen peroxide contains peroxide anion (O_2^{-2})



Oxygen requirements of bacteria

Obligate aerobes	Facultative anaerobes	Obligate anaerobes	Aerotolerant anaerobes	Microaerophiles
				

What type of metabolism?
What oxygen-detoxification enzymes are present?

Independent Study

1. Determine enzyme content and metabolism of obligate aerobes, obligate anaerobes, facultative anaerobes, and aerotolerant anaerobes.
Using this information, propose a hypothetical evolutionary sequence for these organisms, assuming that early earth had no oxygen in it's atmosphere. Be able to explain your choice using enzyme content/metabolism.

2. Look at preferred uses and mechanism of action for physical, chemical and antibiotic methods of microbial growth control (Tables 7.5, 7.7, and 7.8).
You will use this information next time for APO-3.

Game plan

Lecture

Clinical applications: media
Microbial isolation and measurement

APO-3: Growth control

Lab

Calculate direct generation times
Effects on growth: O2, pH, temp

Pre-labs

Growth control: temp and UV

Clinical implications of growth: media

TABLE 6.2 A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

Defined medium

TABLE 6.3 A Chemically Defined Medium for Growing a Fastidious Chemoheterotrophic Bacterium, Such as *Neisseria gonorrhoeae*

Constituent	Amount	Constituent	Amount
Carbon and energy sources		**Amino acids**	
Glucose	9.1 g	Cysteine	1.5 g
Starch	9.1 g	Arginine, proline (each)	0.3 g
Sodium acetate	1.8 g	Glutamic acid, methionine (each)	0.2 g
Sodium citrate	1.4 g	Asparagine, isoleucine, serine (each)	0.2 g
Oxaloacetate	0.3 g	Cystine	0.06 g
Salts		**Organic growth factors**	
Potassium phosphate, dibasic (K_2HPO_4)	12.7 g	Calcium pantothenate	0.02 g
Sodium chloride (NaCl)	6.4 g	Thiamine	0.02 g
Potassium phosphate, monobasic (KH_2PO_4)	5.5 g	Nicotinamide adenine dinucleotide	0.01 g
Sodium bicarbonate ($NaHCO_3$)	1.2 g	Uracil	0.006 g
Potassium sulfate (K_2SO_4)	1.1 g	Biotin	0.005 g
Sodium sulfate (Na_2SO_4)	0.9 g	Hypoxanthine	0.003 g
Magnesium chloride ($MgCl_2$)	0.5 g	**Reducing agent**	
Ammonium chloride (NH_4Cl)	0.4 g	Sodium thioglycolate	0.00003 g
Potassium chloride (KCl)	0.4 g	**Water**	1 liter
Calcium chloride ($CaCl_2$)	0.006 g		
Ferric nitrate [$Fe(NO_3)_3$]	0.006 g		

SOURCE: R. M. Atlas, *Handbook of Microbiological Media,* Ann Arbor, MI: CRC Press, 1993.

Defined medium for a fastidious organism

Clinical implications of growth: media

TABLE 6.2 A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *Escherichia coli*

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ($NH_4H_2PO_4$)	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Potassium phosphate, dibasic (K_2HPO_4)	1.0 g
Water	1 liter

Defined

TABLE 6.4 Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria

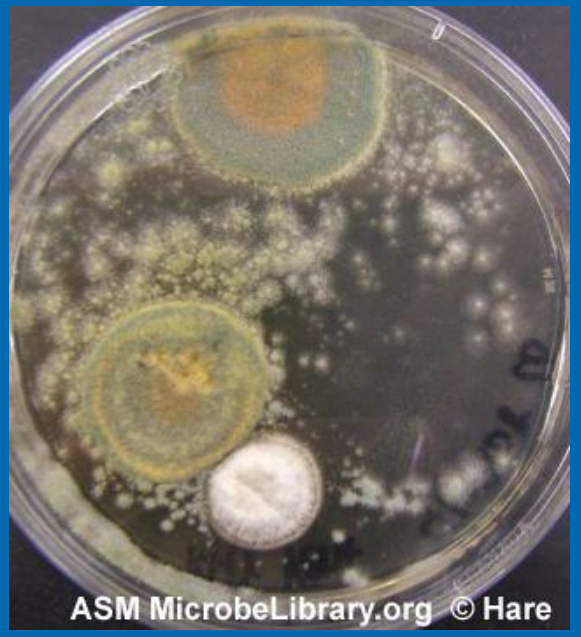
Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

Complex

Clinical implications of growth: culture media

Selective

Sabouraud's dextrose agar

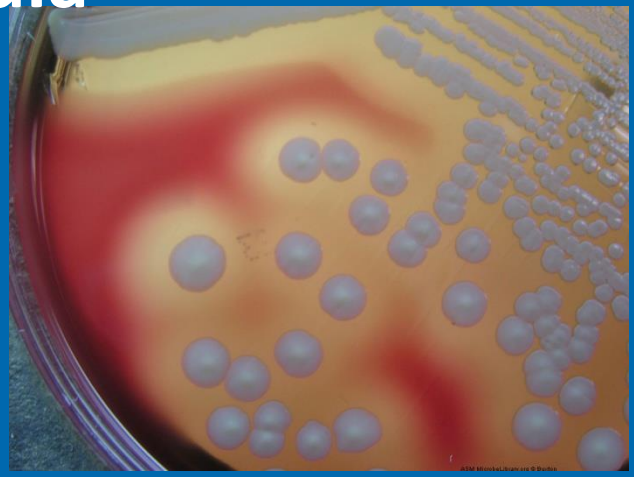


Fungal infections from AIDS

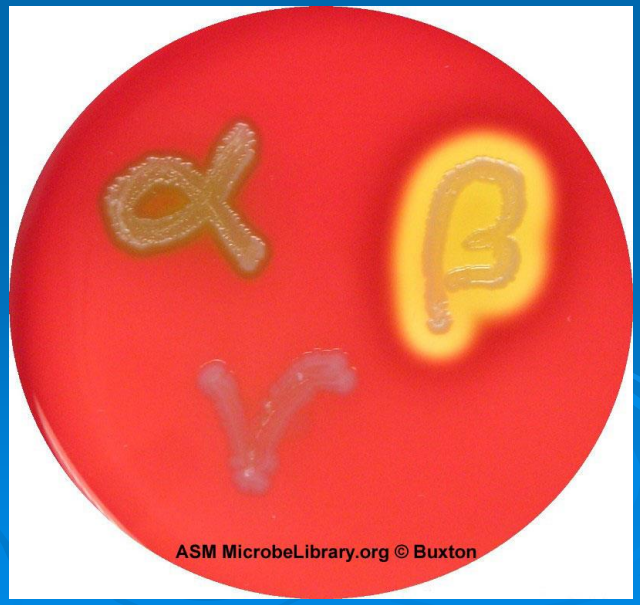
Clinical implications of growth: culture media

Selective
Sabouraud's dextrose agar

Differential
Blood agar



β- hemolytic *S. aureus*

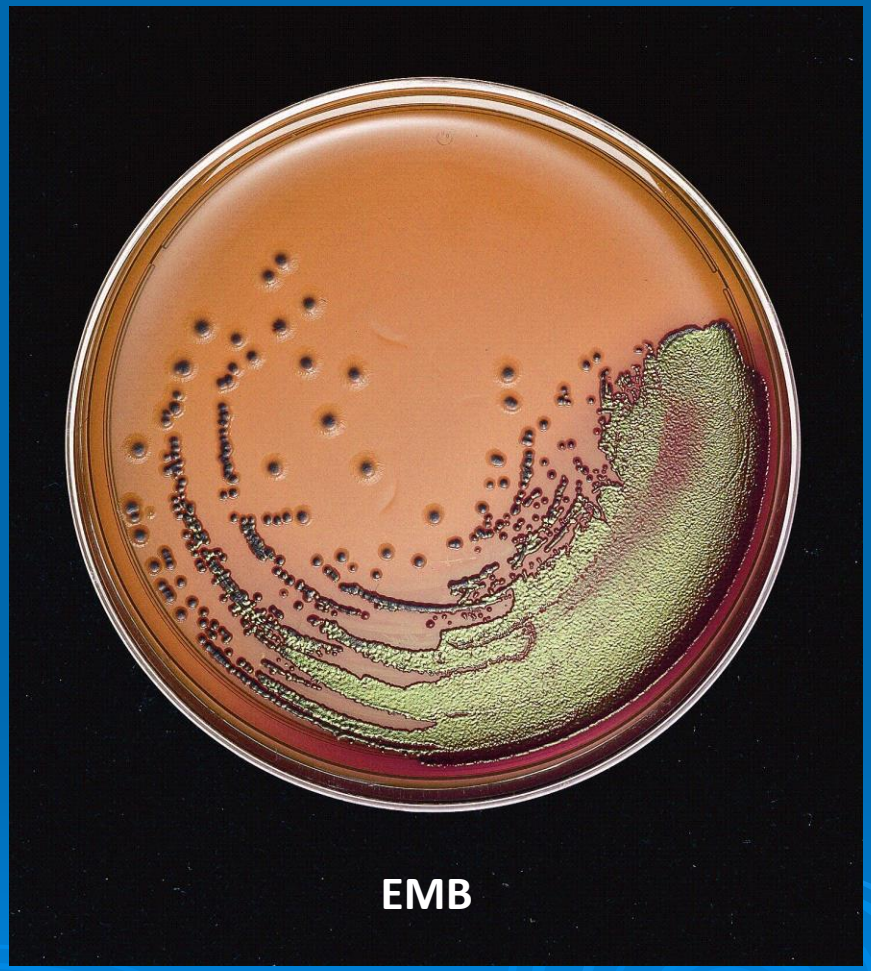


Clinical implications of growth: culture media

Selective
Sabouraud's dextrose

Differential
Blood agar

Selective/ differential
Eosin methylene blue (EMB)



E. coli

Image courtesy of BD Medical

Clinical implications of growth: culture media

Selective
Sabouraud's dextrose

Differential
Blood agar

Selective/ differential
Eosin methylene blue (EMB)
Mannitol salt agar (MSA)

MSA with multiple *Staphylococcus sp*.

Clinical implications of growth: culture media

Selective
Sabouraud's dextrose

Differential
Blood agar

Selective/ differential
Eosin methylene blue (EMB)
Mannitol salt agar (MSA)
MacConkey's agar

MacConkey agar with *E. coli* and *S. marcesens*

Clinical implications of growth: culture media

Selective
Sabouraud's dextrose

Differential
Blood agar (BA)

Selective/ differential
Eosin methylene blue (EMB)
Mannitol salt agar (MSA)
MacConkey's agar

Enrichment media
Gram-negative broth
Heat shock media
Chocolate agar…

Clinical implications of growth: anaerobic conditions

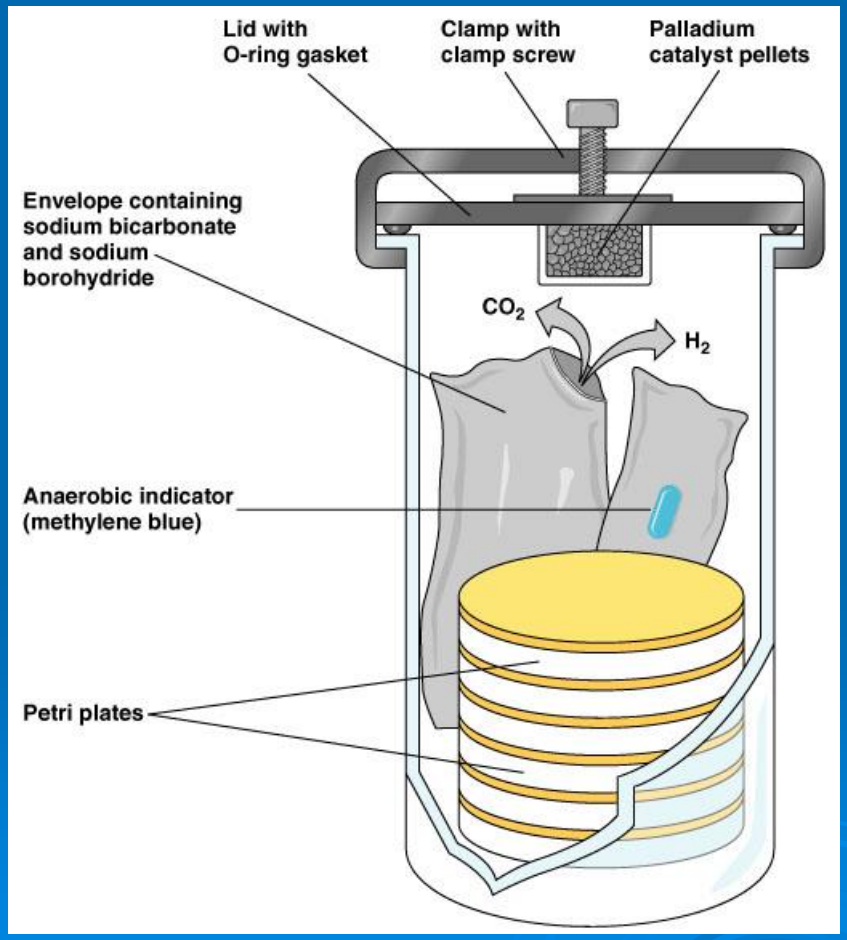


Figure 6.5

Anaerobic jar

Figure 6-23 Microbiology, 6/e
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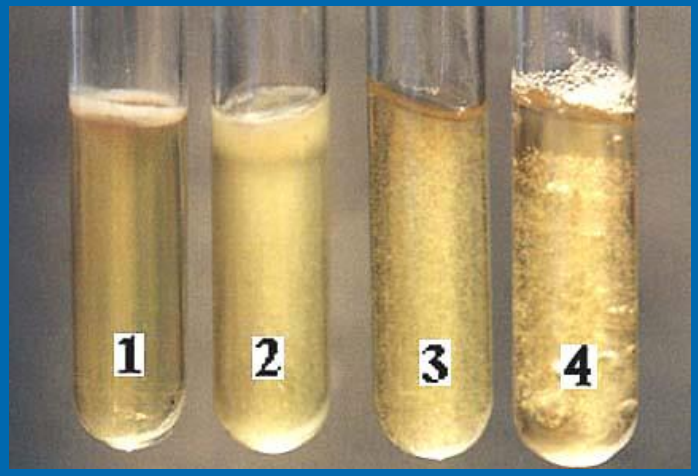
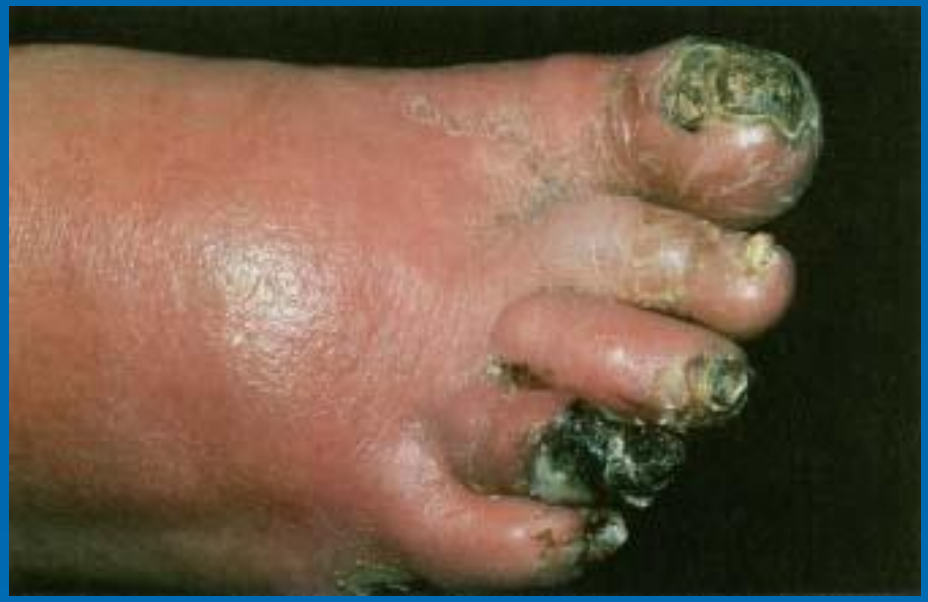
Anaerobic chamber

Clinical implications of growth: anaerobic thioglycollate medium



Corresponding tube no. above	1	2	3	4
Oxygen relationship designation	OBLIGATE AEROBE	FACULTATIVE ANAEROBE	AEROTOLERANT ANAEROBE	OBLIGATE ANAEROBE
Aerobic respiration*	+	+	–	–
Fermentation*	–	+	+	+
Ability to grow aerobically (oxygen tolerance)	+	+	+	–
Ability to grow anaerobically	–	+	+	+
Catalase reaction	+	+	–	–

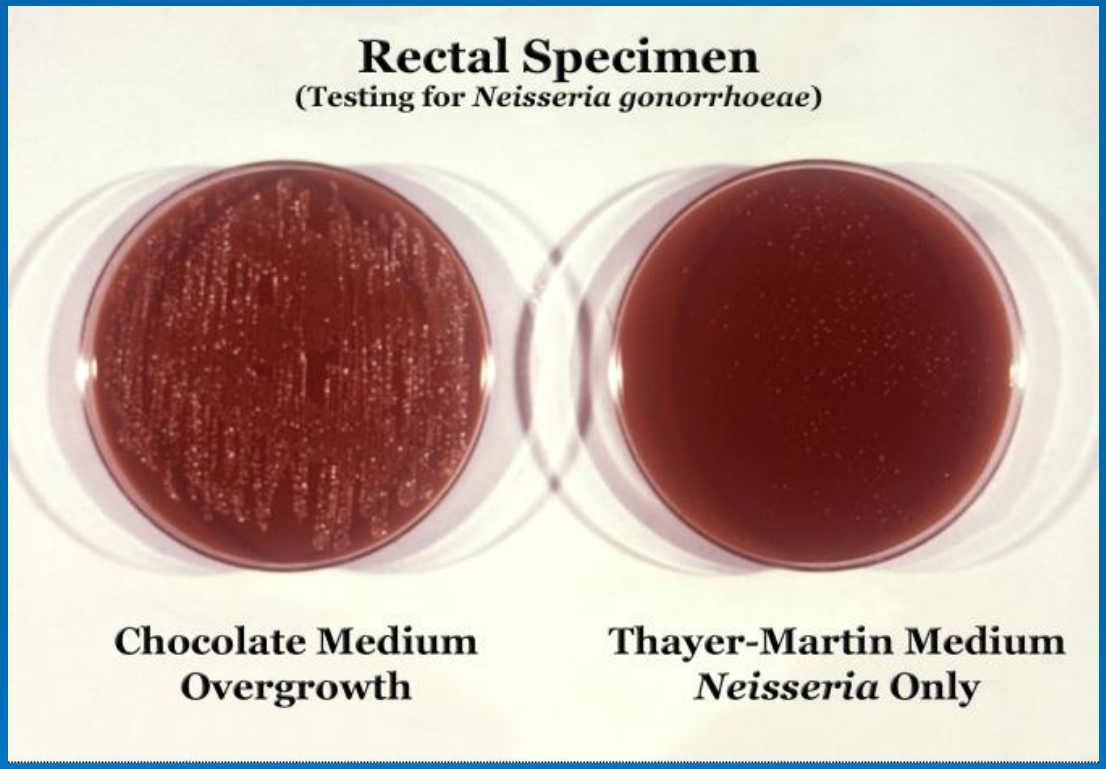
Clinical implications of growth: anaerobes



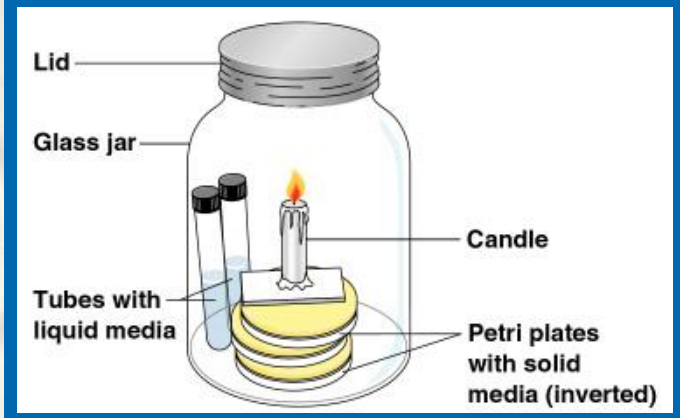
Clostridium perfringens- obligate anaerobe that causes gas gangrene

http://www.humanillnesses.com/images/hdc_0001_0001_0_img0044.jpg

Clinical implications of growth: CO_2-loving capnophiles



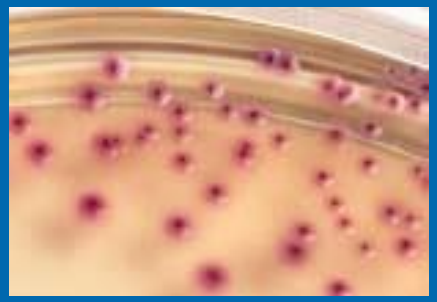
Neisseria gonorrhoeae



Candle extinction jar

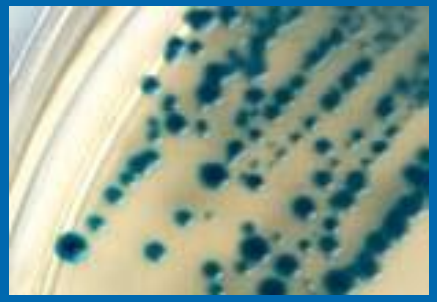
Figure 6.7 - Overview

Clinical implications of growth: HardyChrom UTI differential medium

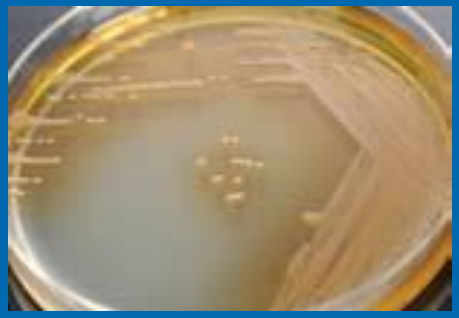


E. coli

Enterococcus faecalis



Klebsiella pneuomoniae

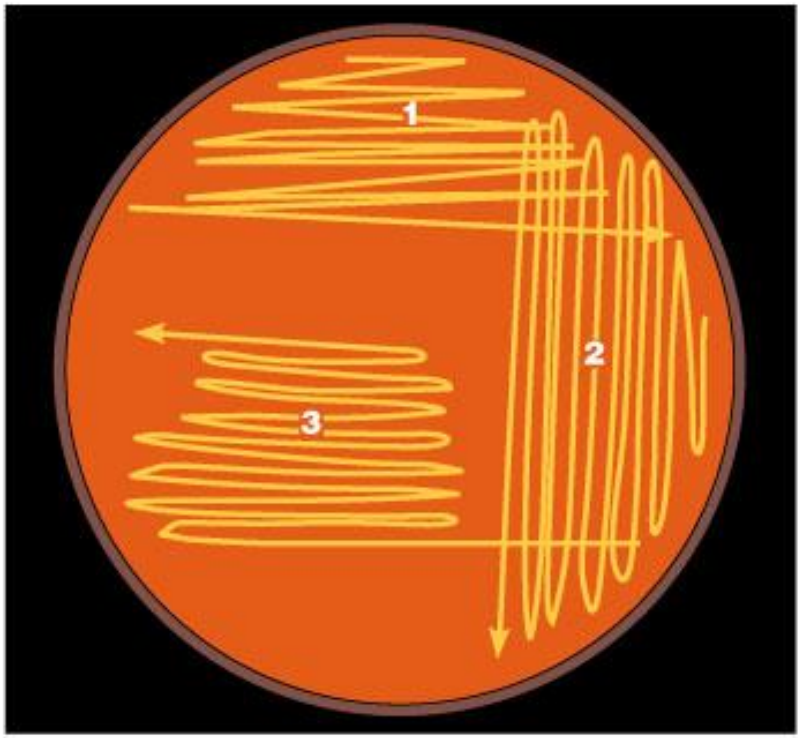


Proteus mirabilis

Staphylococcus aureus

Pseudomonas aeruginosa

Microbe isolation and measurement: the streak plate



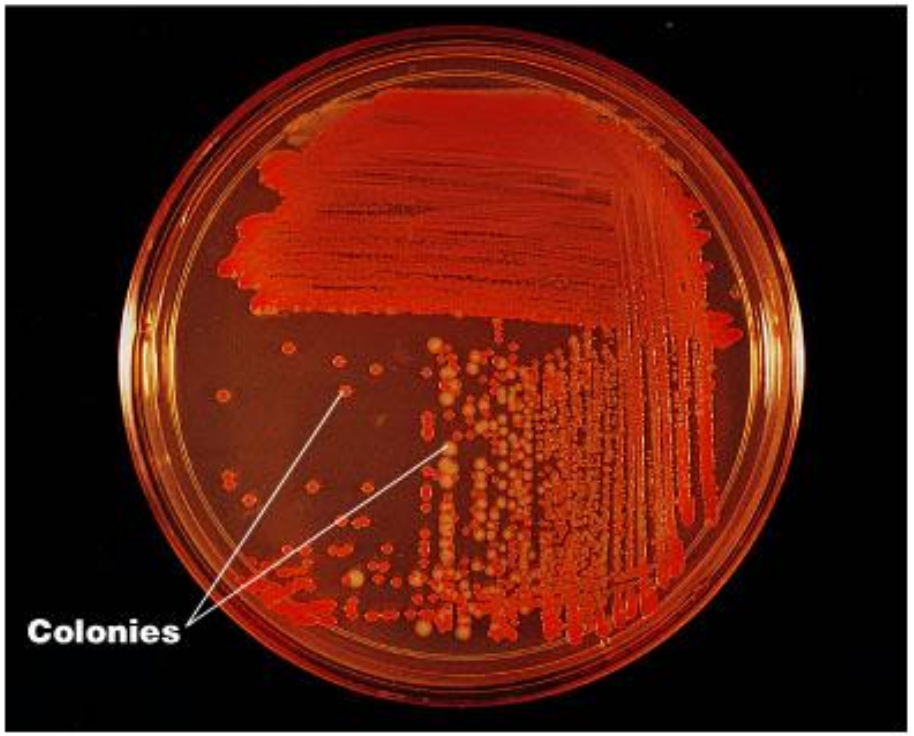
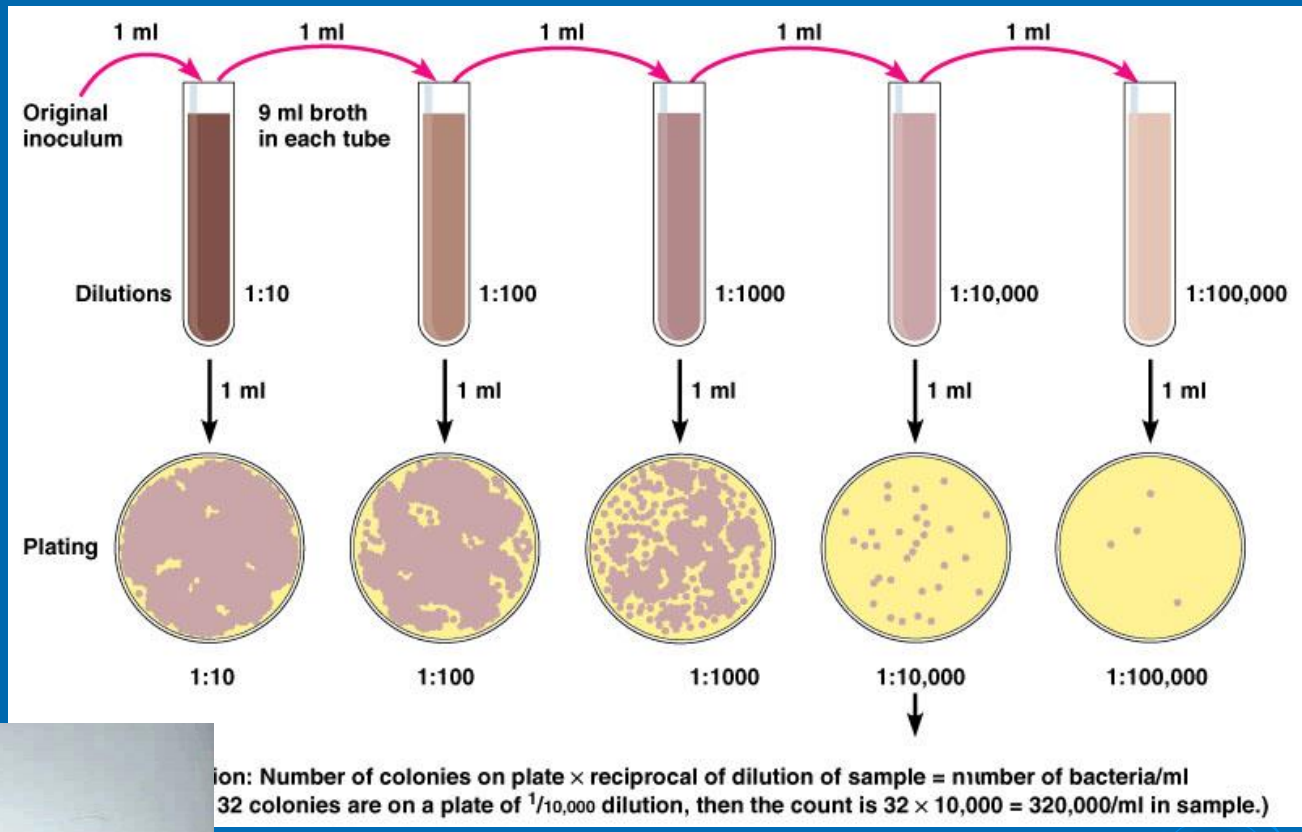


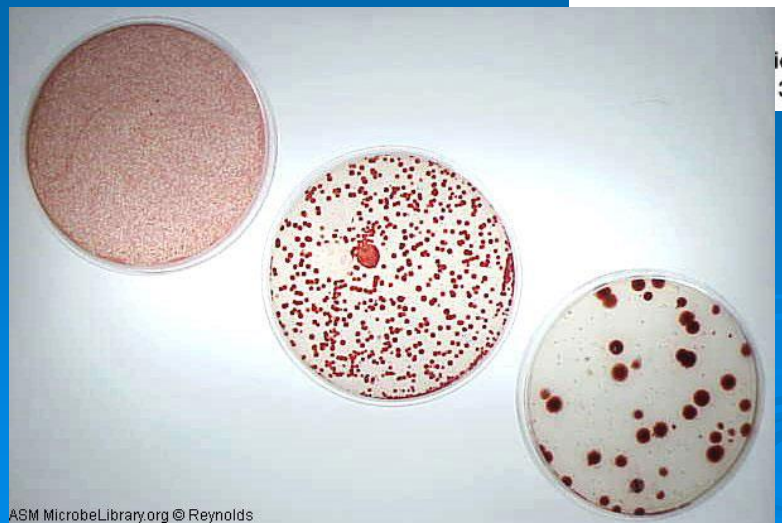
Figure 6.10 - Overview

Microbe isolation and measurement

Plate counts



ion: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000/ml in sample.)



Microbe isolation and measurement

Plate counts

Filtration

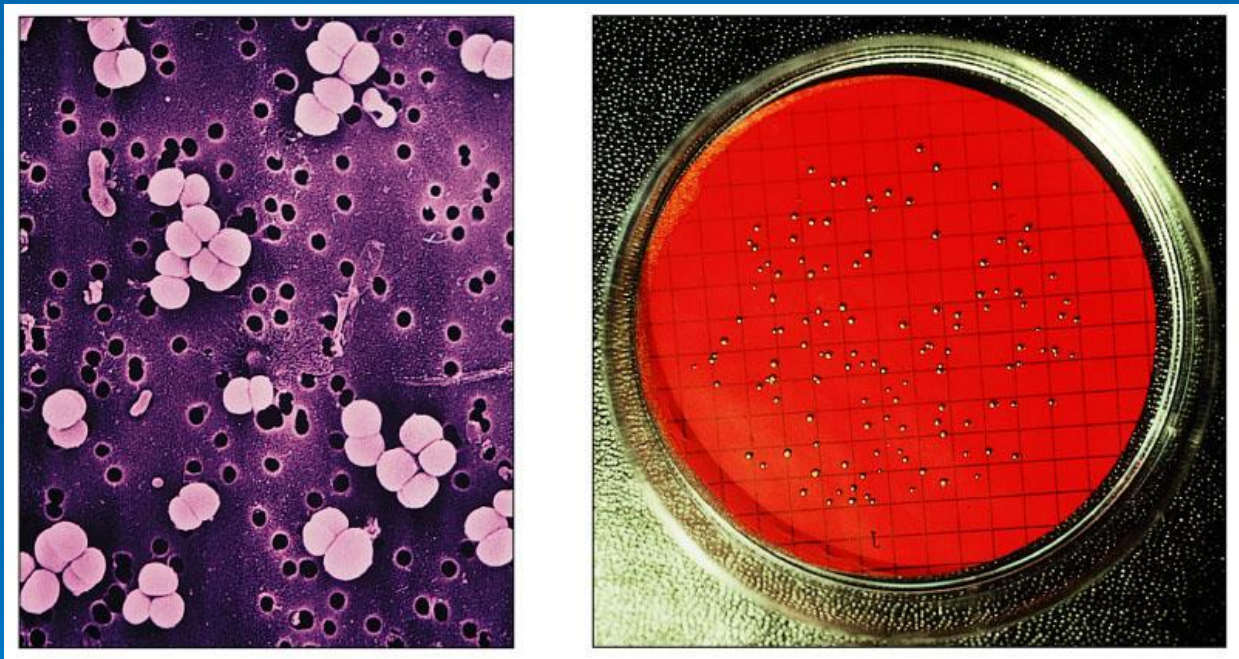


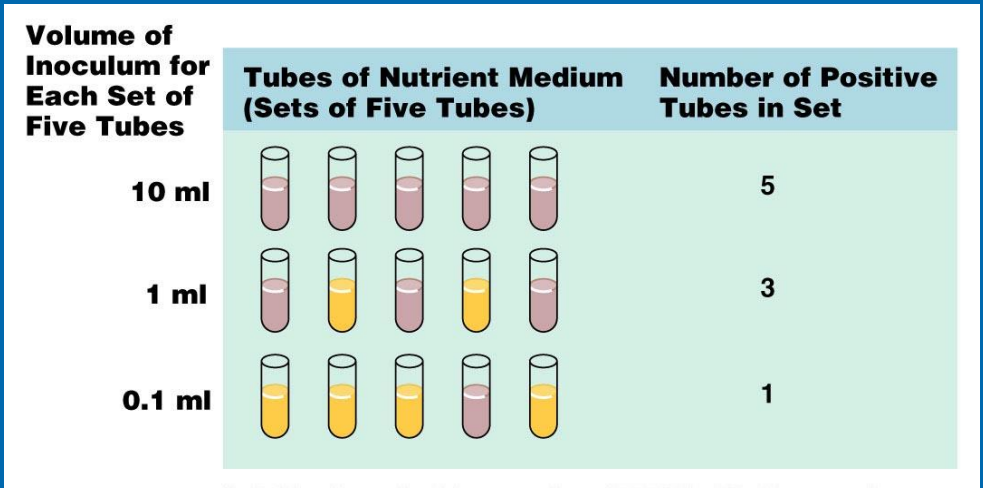
Figure 6.17 - Overview

Microbe isolation and measurement

Plate counts

Filtration

Most probable number (MPN)



Combination of Positives	MPN Index/ 100 ml	95% Confidence Limits	
		Lower	Upper
4-2-0	22	9	56
4-2-1	26	12	65
4-3-0	27	12	67
4-3-1	33	15	77
4-4-0	34	16	80
5-0-0	23	9	86
5-0-1	30	10	110
5-0-2	40	20	140
5-1-0	30	10	120
5-1-1	50	20	150
5-1-2	60	30	180
5-2-0	50	20	170
5-2-1	70	30	210
5-2-2	90	40	250
5-3-0	80	30	250
5-3-1	110	40	300
5-3-2	140	60	360

Microbe isolation and measurement

Plate counts

Filtration

Most probable number (MPN)

Direct count

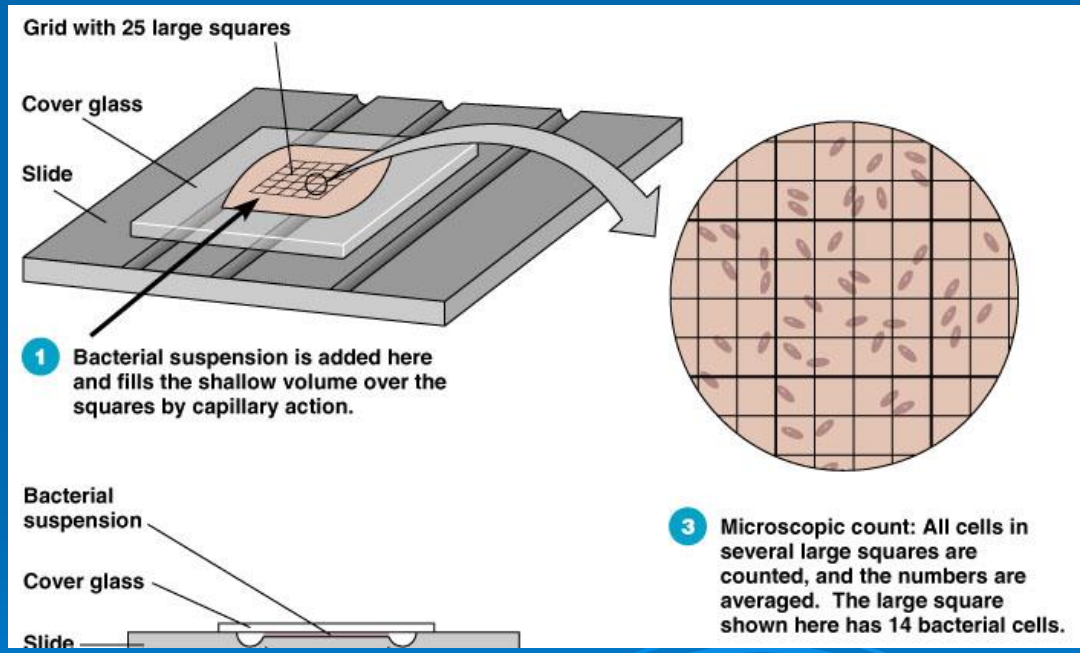


Figure 6.19 - Overview

Microbe isolation and measurement

Plate counts

Filtration

Most probable number (MPN)

Direct count

Turbidity

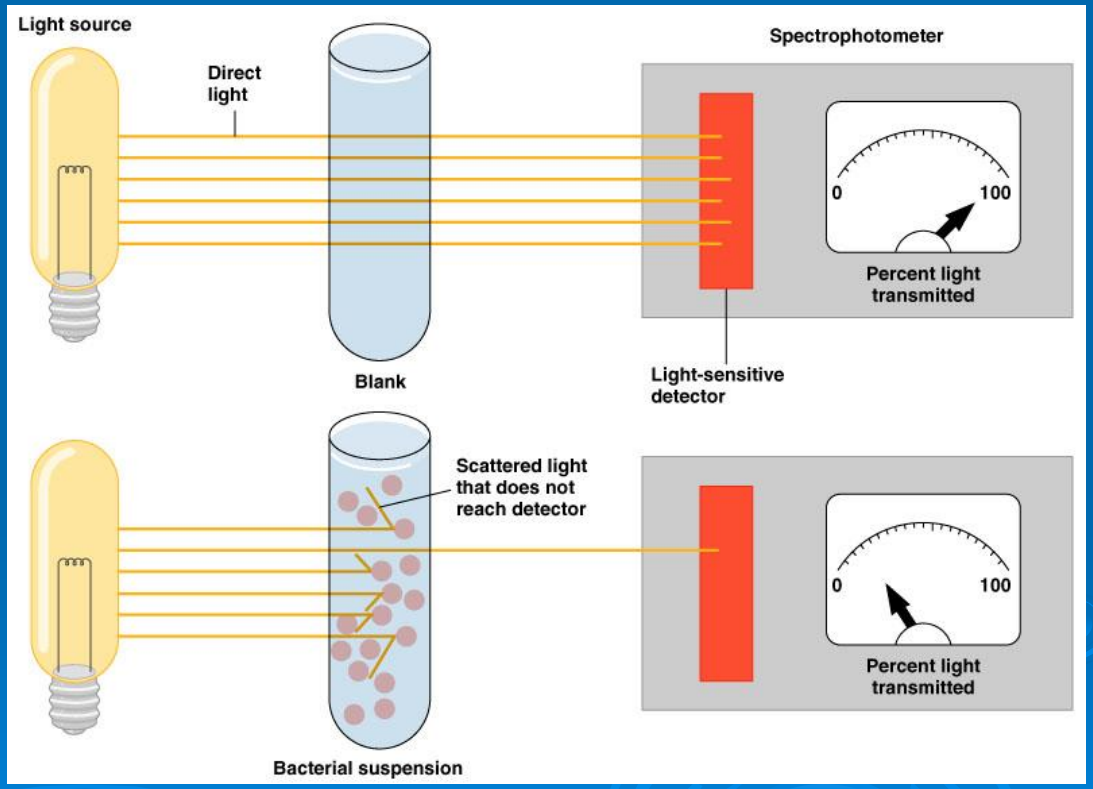


Figure 6.20 - Overview (1 of 3)

Microbe growth control terminology

Sterilization: destruction or removal of ALL forms of life (including endospores)

Commercial sterilization: heat treatment to kill endospores of *Clostridium botulinum* in canned food

Disinfection: destruction of vegetative pathogens

Antisepsis: destruction of vegetative pathogens on living tissue

Degerming: removal of microbes from a limited area

Sanitization: treatment intended to lower microbial counts on eating and drinking utensils to safe public health levels

Table 7.1

Factors influencing antimicrobial effectiveness

-Number of microbes

-Environmental influences
- -Organic matter
- -Biofilms
- -Medium conditions

-Time of exposure

-Microbial characteristics

Physical antimicrobials

Table 7.5 Physical Methods Used to Control Microbial Growth

Methods	Mechanism of Action	Comment	Preferred Use
Heat			
1. Moist heat			
a. Boiling or flowing steam	Protein denaturation	Kills vegetative bacterial and fungal pathogens and almost all viruses within 10 min; less effective on endospores	Dishes, basins, pitchers, various equipment
b. Autoclaving	Protein denaturation	Very effective method of sterilization; at about 15 psi of pressure (121°C), all vegetative cells and their endospores are killed in about 15 min	Microbiological media, solutions, linens, utensils, dressings, equipment, and other items that can withstand temperature and pressure
2. Pasteurization	Protein denaturation	Heat treatment for milk (72°C for about 15 sec) that kills all pathogens and most nonpathogens	Milk, cream, and certain alcoholic beverages (beer and wine)
3. Dry heat			
a. Direct flaming	Burning contaminants to ashes	Very effective method of sterilization	Inoculating loops
b. Incineration	Burning to ashes	Very effective method of sterilization	Paper cups, contaminated dressings, animal carcasses, bags, and wipes
c. Hot-air sterilization	Oxidation	Very effective method of sterilization but requires temperature of 170°C for about 2 hr	Empty glassware, instruments, needles, and glass syringes
Filtration	Separation of bacteria from suspending liquid	Removes microbes by passage of a liquid or gas through a screenlike material; most filters in use consist of cellulose acetate or nitrocellulose	Useful for sterilizing liquids (enzymes, vaccines) that are destroyed by heat

Physical antimicrobials

Table 7.5 Physical Methods Used to Control Microbial Growth

Methods	Mechanism of Action	Comment	Preferred Use
Cold			
1. Refrigeration	Decreased chemical reactions and possible changes in proteins	Has a bacteriostatic effect	Food, drug, and culture preservation
2. Deep-freezing (see Chapter 6, page 170)	Decreased chemical reactions and possible changes in proteins	An effective method for preserving microbial cultures, in which cultures are quick-frozen between −50° and −95°C	Food, drug, and culture preservation
3. Lyophilization (see Chapter 6, page 170)	Decreased chemical reactions and possible changes in proteins	Most effective method for long-term preservation of microbial cultures; water removed by high vacuum at low temperature	Food, drug, and culture preservation
High Pressure	Alteration of molecular structure of proteins and carbohydrates	Preservation of colors, flavors, nutrient values	Fruit juices
Desiccation	Disruption of metabolism	Involves removing water from microbes; primarily bacteriostatic	Food preservation
Osmotic Pressure	Plasmolysis	Results in loss of water from microbial cells	Food preservation
Radiation			
1. Ionizing	Destruction of DNA	Not widespread in routine sterilization	Sterilizing pharmaceuticals and medical and dental supplies
2. Nonionizing	Damage to DNA	Radiation not very penetrating	Control of closed environment with UV (germicidal) lamp

Chemical antimicrobials

Table 7.8 Chemical Agents Used to Control Microbial Growth

Chemical Agent	Mechanism of Action	Preferred Use	Comment
Phenol and Phenolics			
1. Phenol	Disruption of plasma membrane, denaturation of enzymes.	Rarely used, except as a standard of comparison.	Seldom used as a disinfectant or antiseptic because of its irritating qualities and disagreeable odor.
2. Phenolics	Disruption of plasma membrane, denaturation of enzymes.	Environmental surfaces, instruments, skin surfaces, and mucous membranes.	Derivatives of phenol that are reactive even in the presence of organic material; O-phenylphenol is an example.
3. Bisphenols	Probably disruption of plasma membrane.	Disinfectant hand soaps and skin lotions.	Triclosan is an especially common example of a bisphenol. Broad spectrum, but most effective against gram-positives.
Biguanides (Chlorhexidine)	Disruption of plasma membrane.	Skin disinfection, especially for surgical scrubs.	Bactericidal to gram-positives and gram-negatives; nontoxic, persistent.
Halogens	Iodine inhibits protein function and is a strong oxidizing agent; chlorine forms the strong oxidizing agent hypochlorous acid, which alters cellular components.	Iodine is an effective antiseptic available as a tincture and an iodophor; chlorine gas is used to disinfect water; chlorine compounds are used to disinfect dairy equipment, eating utensils, household items, and glassware.	Iodine and chlorine may act alone or as components of inorganic and organic compounds.
Alcohols	Protein denaturation and lipid dissolution.	Thermometers and other instruments; in swabbing the skin with alcohol before an injection, most of the disinfecting action probably comes from a simple wiping away (degerming) of dirt and some microbes.	Bactericidal and fungicidal, but not effective against endospores or nonenveloped viruses; commonly used alcohols are ethanol and isopropanol.

Chemical antimicrobials

Table 7.8 Chemical Agents Used to Control Microbial Growth

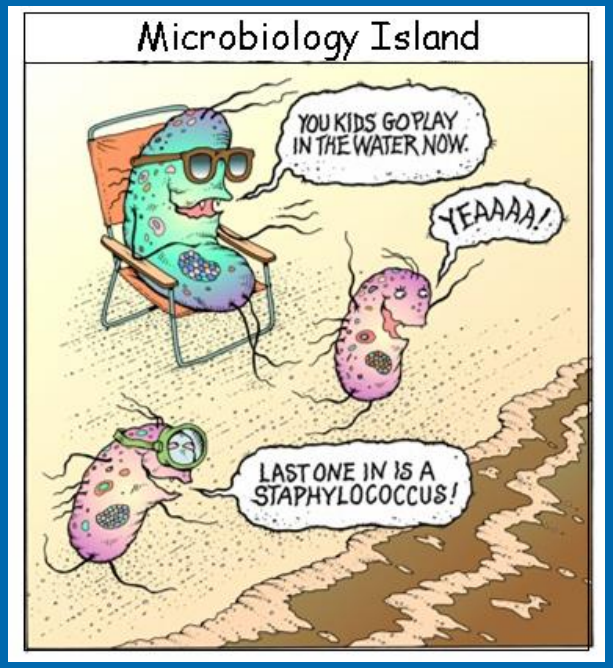
Chemical Agent	Mechanism of Action	Preferred Use	Comment
Heavy Metals and Their Compounds	Denaturation of enzymes and other essential proteins.	Silver nitrate may be used to prevent gonorrheal neonatal ophthalmia; silver-sulfadiazine used as a topical cream on burns; copper sulfate is an algicide.	Heavy metals such as silver and mercury are biocidal.
Surface-Active Agents			
Soaps and detergents	Mechanical removal of microbes through scrubbing.	Skin degerming and removal of debris.	Many antibacterial soaps contain antimicrobials.
Acid-anionic sanitizers	Not certain; may involve enzyme inactivation or disruption.	Sanitizers in dairy and food-processing industries.	Wide spectrum of activity; nontoxic, noncorrosive, fast-acting.
Quaternary ammonium compounds (cationic detergents)	Enzyme inhibition, protein denaturation, and disruption of plasma membranes.	Antiseptic for skin, instruments, utensils, rubber goods.	Bactericidal, bacteriostatic, fungicidal, and virucidal against enveloped viruses; examples of quats are Zephiran and Cepacol.
Chemical Food Preservatives			
Organic acids	Metabolic inhibition, mostly affecting molds; action not related to their acidity.	Sorbic acid and benzoic acid effective at low pH; parabens much used in cosmetics, shampoos; calcium propionate used in bread.	Widely used to control mold and some bacteria in foods and cosmetics.
Nitrates/nitrites	Active ingredient is nitrite, which is produced by bacterial action on nitrate. Nitrite inhibits certain iron-containing enzymes of anaerobes.	Meat products such as ham, bacon, hot dogs, sausage.	Prevents growth of *Clostridium botulinum* in food; also imparts a red color.

Chemical antimicrobials

Table 7.8 Chemical Agents Used to Control Microbial Growth

Chemical Agent	Mechanism of Action	Preferred Use	Comment
Aldehydes	Protein denaturation.	Glutaraldehyde (Cidex) is less irritating than formaldehyde and is used for disinfecting medical equipment.	Very effective antimicrobials.
Chemical Sterilization			
Ethylene oxide and other gaseous sterilants	Inhibits vital cellular functions.	Mainly for sterilization of materials that would be damaged by heat.	Ethylene oxide is the most commonly used. Heated hydrogen peroxide and chlorine dioxide have special uses.
Plasma sterilization	Inhibits vital cellular functions.	Especially useful for tubular medical instruments.	Usually hydrogen peroxide excited in a vacuum by an electromagnetic field.
Supercritical fluids	Inhibits vital cellular functions.	Especially useful for sterilizing organic medical implants.	Carbon dioxide compressed to a supercritical state.
Peroxygens and Other Forms of Oxygen	Oxidation.	Contaminated surfaces; some deep wounds, in which they are very effective against oxygen-sensitive anaerobes.	Ozone is widely used as a supplement for chlorination; hydrogen peroxide is a poor antiseptic but a good disinfectant. Peracetic acid is especially effective.

Survivor: Microbiology Island



You are stranded on an island with your fellow classmates. Luckily, you have a vast array of anti-microbial tools at your disposal. Working in groups, use the growth control tools provided to complete the team challenges and answer any questions.

Independent Study

1. Be familiar with the physical and chemical antimicrobial methods, including mechanism of action, for those listed on the study guide.

2. Review DNA structure and the basics of replication, transcription and translation. (Figures 8.5, 8.7, and 8.9 are good references)