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Unit 1: Introduction to Microbial World and You

Microbiology:

The study of microorganisms.

Microorganisms: Small living organisms that generally can not be seen with the naked eye.

Include:

- ☐ Bacteria
- ☐ Fungi (yeasts and molds)
- ☐ Protozoa
- ☐ Algae
- ☐ Multicellular parasites

Also include nonliving infectious agents:

- ◆ Viruses
- ◆ Prions

History of Microbiology

Early Studies

Before 17th century, study of microbiology was hampered by the lack of appropriate tools to observe microbes.

- ❑ **Robert Hooke:** In 1665 built a compound light microscope and used it to observe thin slices of cork. Coined the word cell.
- ❑ **Anton van Leeuwenhoeck:** In 1673 was the first person to observe live microorganisms which he called “*animalcules*” (bacteria, protozoa), using single-lens microscopes that he designed.

History of Microbiology

Spontaneous Generation vs Biogenesis

- Before 1860s many scientists believed in **Spontaneous generation**, i.e.: That living organisms could arise spontaneously from nonliving matter:
 - Mice come from rags in a basket.
 - Maggots come from rotting meat.
 - Ants come from honey.
 - Microbes come from spoiled broth.

History of Microbiology

Spontaneous Generation vs Biogenesis

- **Theory of Biogenesis:** Belief that living cells can only arise from other living cells.
- **Francesco Redi:** In 1668 proved that maggots do not arise spontaneously from decaying meat.
- **Lazaro Spallanzani:** In 1765 found that nutrient broth that had been heated in a sealed flask would not become contaminated with microbes.
 - Some proponents of spontaneous generation argued that boiling had destroyed the “life force” of air in flask.
 - Others argued that microbes were different from other life forms.

History of Microbiology

Spontaneous Generation vs Biogenesis

Debate was finally settled by Pasteur.

- **Louis Pasteur:** In 1861 finally disproved spontaneous generation when he demonstrated that microorganisms in the environment were responsible for microbial growth in nutrient broth.
 - Designed **swan neck flasks** that allowed air in, but trapped microbes in neck.
 - Developed **aseptic technique**: Practices that prevent contamination by unwanted microorganisms.

History of Microbiology

Golden Age: 1857-1914

Rapid advances led to the development of microbiology as a science.

Pasteur's Contributions to Microbiology:

- ❑ **Fermentation:** Pasteur found that yeasts were responsible for converting sugar into alcohol in the absence of air.
- ❑ Souring and spoilage were caused by bacterial contamination of beverages.

History of Microbiology

Golden Age: 1857-1914

Pasteur's Contributions:

- ❑ **Pasteurization:** Developed a process in which liquids are heated (at 65°C) to kill most bacteria responsible for spoilage.
- ❑ **Disease Causes:** Identified three different microbes that caused silkworm diseases.
- ❑ **Vaccine:** Developed a vaccine for rabies from dried spinal cords of infected rabbits.
- ❑ Directed Pasteur Institute until his death in 1895.

History of Microbiology

Golden Age: 1857-1914

Germ Theory of Disease: Belief that microbes cause diseases. Before, most people believed diseases were caused by divine punishment, poisonous vapors, curses, witchcraft, etc.

- **Agostino Bassi (1835):** Found that a fungus was responsible for a silkworm disease.
- **Ignaz Semmelweis (1840s):** Demonstrated that childbirth fever was transmitted from one patient to another, by physicians who didn't disinfect their hands. He was ostracized by colleagues.

History of Microbiology

Golden Age: 1857-1914

Germ Theory of Disease:

- **Joseph Lister (1860):** Used disinfectant to treat surgical wounds, greatly reducing infection rates. Considered the father of antiseptic surgery.
- **Robert Koch (1876):** First person to conclusively prove that a specific bacterium caused a disease.
 - **Germ Theory:** One microbe causes one specific disease.
 - Proved that *Bacillus anthracis* causes anthrax in cattle.
 - Later identified bacterium that causes tuberculosis.

History of Microbiology

Modern Microbiology: After 1914

Chemotherapy: Treatment of a disease by using a chemical substance. Chemical must be more poisonous to microbe than host.

- **Quinine:** First known chemical to treat a disease (malaria). Used by Spanish conquistadors.
- **Synthetic Drugs:** Made in the laboratory.
- **Antibiotics:** Produced naturally by fungi and bacteria.

History of Microbiology

Modern Microbiology: After 1914

- ❑ **Paul Ehrlich (1910):** Search for “magic bullet”.
 - ❑ Discovered salvarsan, an arsenic derivative, was effective against syphilis.
- ❑ **Alexander Fleming (1928):** Discovered that penicillin produced by the mold *Penicillium notatum* was able to prevent microbial growth.
 - ❑ Penicillin was not mass produced until the 1940s.
- ❑ **Rene Dubos (1939):** Discovered two antibiotics (gramidin and tyrocidine) produced by bacterium (*Bacillus brevis*).

History of Microbiology

Modern Microbiology: After 1914

Problems with Chemotherapy:

- Toxicity
- Drug resistant microbes

Microbial nutrition, growth, and metabolism

Overview

- Growth requirements and classification
- Physical parameters that effect growth and classification based on growth patterns
- Chemical parameters that effect growth and classification based on growth patterns
- Population growth -- growth curve
- Population growth -- Methods

Temperature and Microbial Growth

□ Cardinal temperatures

- minimum
- optimum
- maximum

□ Temperature is a major environmental factor controlling microbial growth.

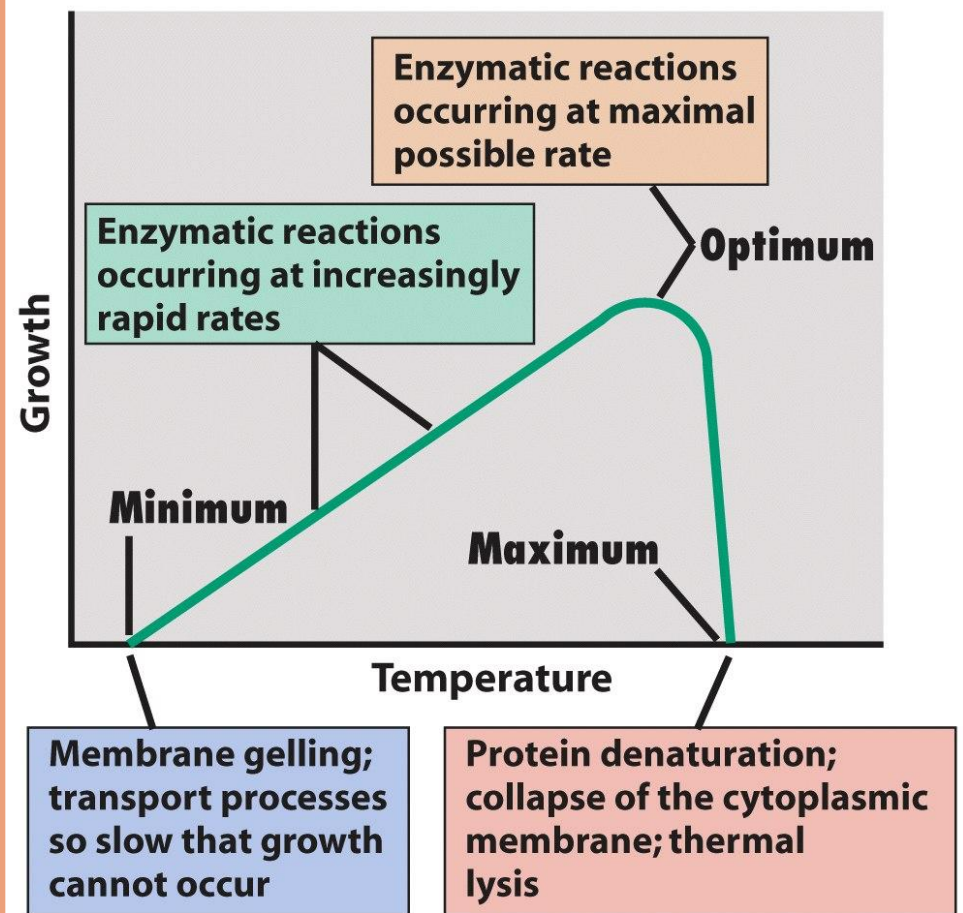


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Temperature

- **Minimum Temperature:** Temperature below which growth ceases, or lowest temperature at which microbes will grow.
- **Optimum Temperature:** Temperature at which its growth rate is the fastest.
- **Maximum Temperature:** Temperature above which growth ceases, or highest temperature at which microbes will grow.

Classification of Microorganisms by Temperature Requirements

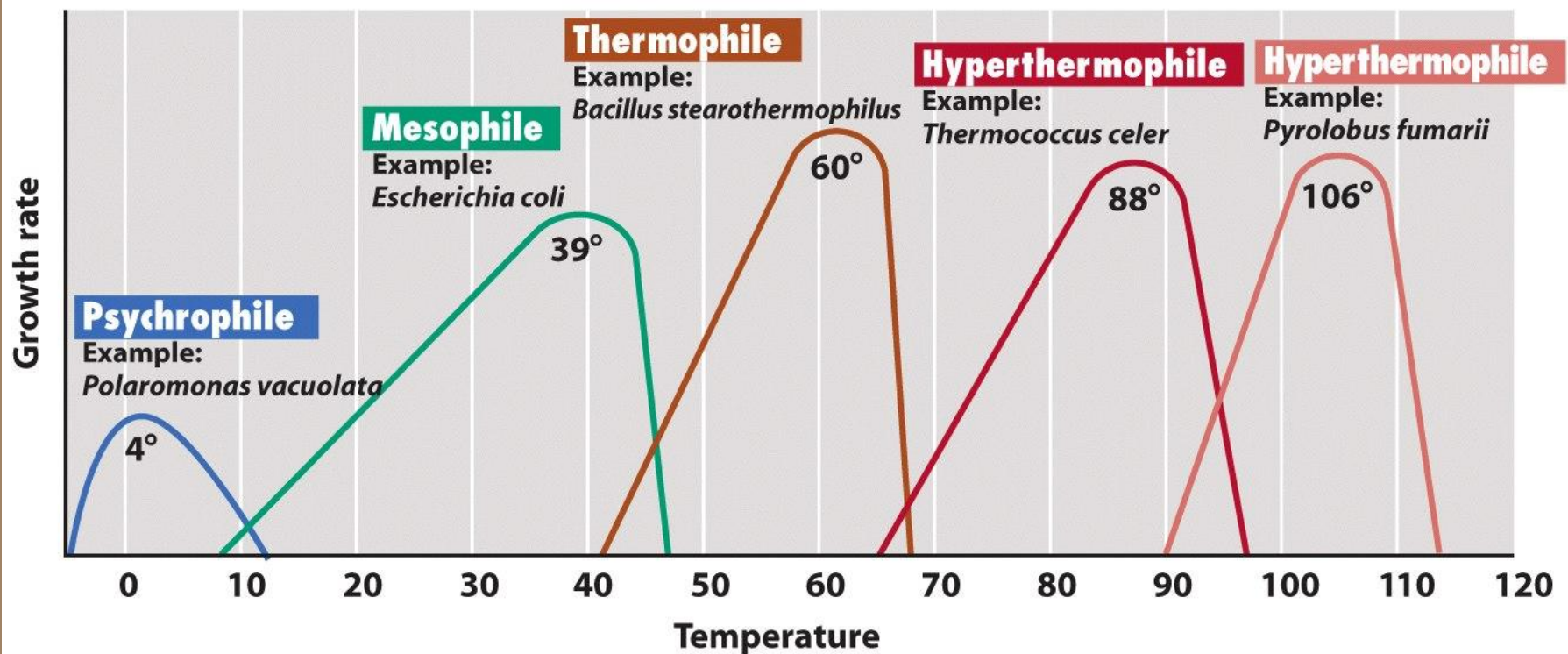


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Temperature Classes of Organisms

□ **Mesophiles (20 – 45C)**

- Midrange temperature optima
- Found in warm-blooded animals and in terrestrial and aquatic environments in temperate and tropical latitudes

□ **Psychrophiles (0-20C)**

- Cold temperature optima
- Most extreme representatives inhabit permanently cold environments

□ **Thermophiles (50- 80C)**

- Growth temperature optima between 45°C and 80°C

□ **Hyperthermophiles**

- Optima greater than 80°C
- These organisms inhabit hot environments including boiling hot springs, as well as undersea hydrothermal vents that can have temperatures in excess of 100°C

Table 6.5**Temperature Ranges for Microbial Growth**

Microorganism	<i>Cardinal Temperatures (°C)</i>		
	Minimum	Optimum	Maximum
Nonphotosynthetic Procaryotes			
<i>Bacillus psychrophilus</i>	-10	23-24	28-30
<i>Micrococcus cryophilus</i>	-4	10	24
<i>Pseudomonas fluorescens</i>	4	25-30	40
<i>Staphylococcus aureus</i>	6.5	30-37	46
<i>Enterococcus faecalis</i>	0	37	44
<i>Escherichia coli</i>	10	37	45
<i>Neisseria gonorrhoeae</i>	30	35-36	38
<i>Thermoplasma acidophilum</i>	45	59	62
<i>Bacillus stearothermophilus</i>	30	60-65	75
<i>Thermus aquaticus</i>	40	70-72	79
<i>Sulfolobus acidocaldarius</i>	60	80	85
<i>Pyrococcus abyssi</i>	67	96	102
<i>Pyrodictium occultum</i>	82	105	110
<i>Pyrolobus fumarii</i>	90	106	113
Photosynthetic Bacteria			
<i>Rhodospirillum rubrum</i>	ND ^a	30-35	ND
<i>Anabaena variabilis</i>	ND	35	ND
<i>Oscillatoria tenuis</i>	ND	ND	45-47
<i>Synechococcus eximius</i>	70	79	84

Table 6.1**Presently known upper temperature limits
for growth of living organisms**

Group	Upper temperature limits (°C)
Animals	
Fish and other aquatic vertebrates	38
Insects	45–50
Ostracods (crustaceans)	49–50
Plants	
Vascular plants	45
Mosses	50
Eukaryotic microorganisms	
Protozoa	56
Algae	55–60
Fungi	60–62
Prokaryotes	
<i>Bacteria</i>	
Cyanobacteria	70–74
Anoxygenic phototrophs	70–73
Chemoorganotrophic/chemolithotrophic	95
<i>Bacteria</i>	
<i>Archaea</i>	
Chemoorganotrophic/chemolithotrophic	113 ^a
<i>Archaea</i>	

^a The upper temperature limit for growth of the organism *Pyrolobus fumarii*.
Related species of *Pyrodicticum* may be able to grow up to as high as 121°C.

pH and Microbial Growth

pH – measure of $[H^+]$

each organism has a pH range and a pH optimum

acidophiles – optimum in pH range 1-4

alkalophiles – optimum in pH range 8.5-11

lactic acid bacteria – 4-7

Thiobacillus thiooxidans – 2.2-2.8

fungi – 4-6

internal pH regulated by **BUFFERS** and near neutral
adjusted with ion pumps

Human blood and tissues has pH 7.2 ± 0.2

pH and Microbial Growth

- The acidity or alkalinity of an environment can greatly affect microbial growth.
- Most organisms grow best between pH 6 and 8, but some organisms have evolved to grow best at low or high pH. The internal pH of a cell must stay relatively close to neutral even though the external pH is highly acidic or basic.
 - **Acidophiles** : organisms that grow best at low pH (*Helicobacter pylori*, *Thiobacillus thiooxidans*)
 - **Alkaliphiles** : organisms that grow best at high pH (*Vibrio cholera*)
 - Most of pathogenic bacteria are neutrophiles

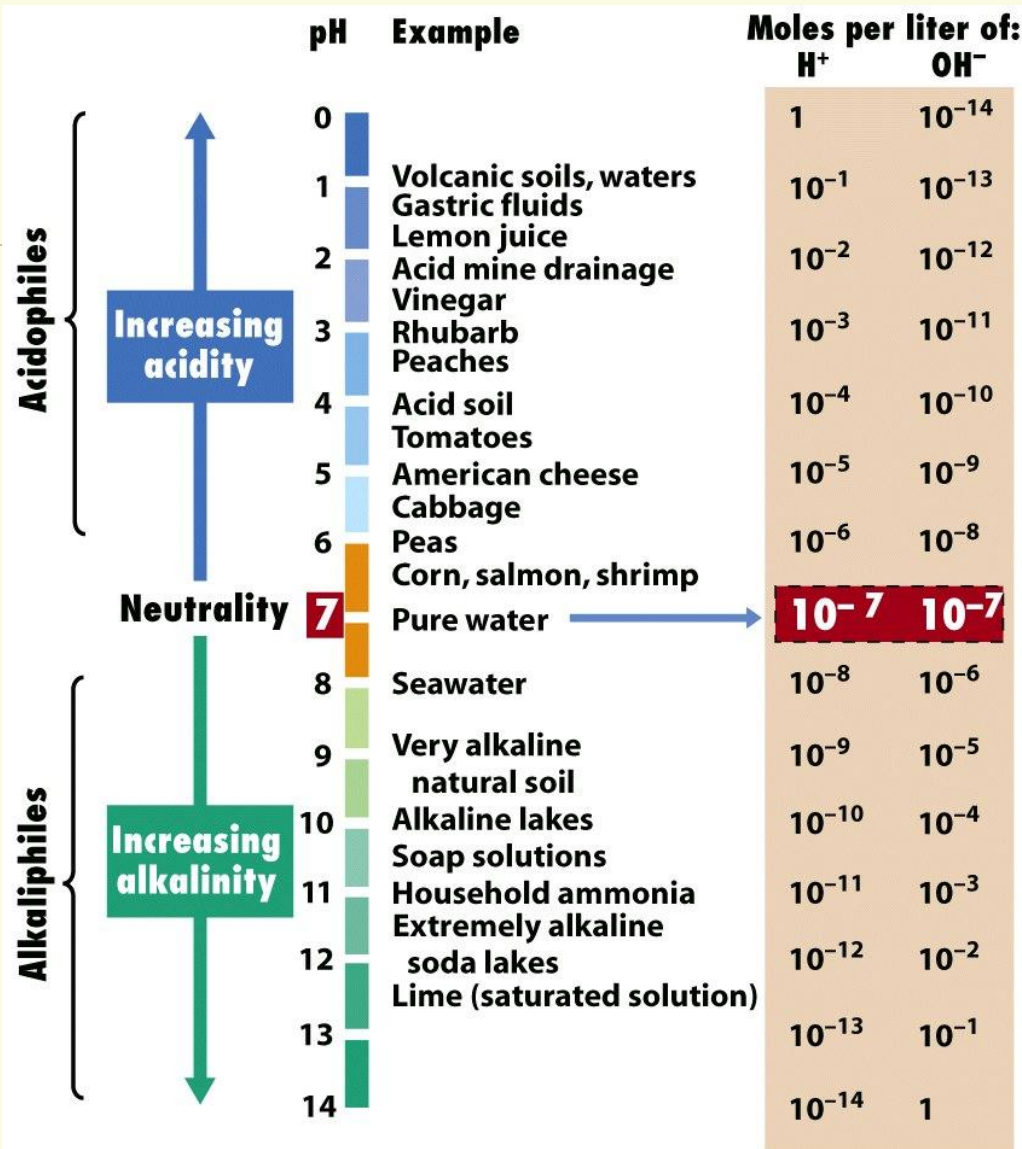


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Osmotic Effects on Microbial Growth

- Osmotic pressure depends on the surrounding solute concentration and water availability
- Water availability is generally expressed in physical terms such as water activity (a_w)
- Water activity is the ratio of the vapor pressure of the air in equilibrium with a substance or solution to the vapor pressure of pure water (a_w 1.00).

$$a_w = \frac{P_{\text{solu}}}{P_{\text{water}}}$$

Environmental factors and growth

1. Osmotic Effect and water activity

organisms which thrive in high solute – osmophiles

organisms which tolerate high solute – osmotolerant

organisms which thrive in high salt – halophiles

organisms which tolerate high salt – halotolerant

organisms which thrive in high pressure – barophiles

organisms which tolerate high pressure – barotolerant

Table 6.2 Water activity of several substances

Water activity (a_w)	Material	Example organisms^a
1.000	Pure water	<i>Caulobacter</i> , <i>Spirillum</i>
0.995	Human blood	<i>Streptococcus</i> , <i>Escherichia</i>
0.980	Seawater	<i>Pseudomonas</i> , <i>Vibrio</i>
0.950	Bread	Most gram-positive rods
0.900	Maple syrup, ham	Gram-positive cocci such as <i>Staphylococcus</i>
0.850	Salami	<i>Saccharomyces rouxii</i> (yeast)
0.800	Fruit cake, jams	<i>Saccharomyces bailii</i> , <i>Penicillium</i> (fungus)
0.750	Salt lakes, salted fish	<i>Halobacterium</i> , <i>Halococcus</i>
0.700	Cereals, candy, dried fruit	<i>Xeromyces bisporus</i> and other xerophilic fungi

^a Selected examples of prokaryotes or fungi capable of growth in culture media adjusted to the stated water activity.

Halophiles and Related Organisms

- In nature, osmotic effects are of interest mainly in habitats with high salt environments that have reduced water availability
- **Halophiles** : have evolved to grow best at reduced water potential, and some (**extreme halophiles** e.g. *Halobacterium*, *Dunaliella*) even require high levels of salts for growth.
- **Halotolerant** : can tolerate some reduction in the water activity of their environment but generally **grow best in the absence of the added solute**
- **Xerophiles** : are able to grow in **very dry environments**

Nonhalophile

Example:
Escherichia coli

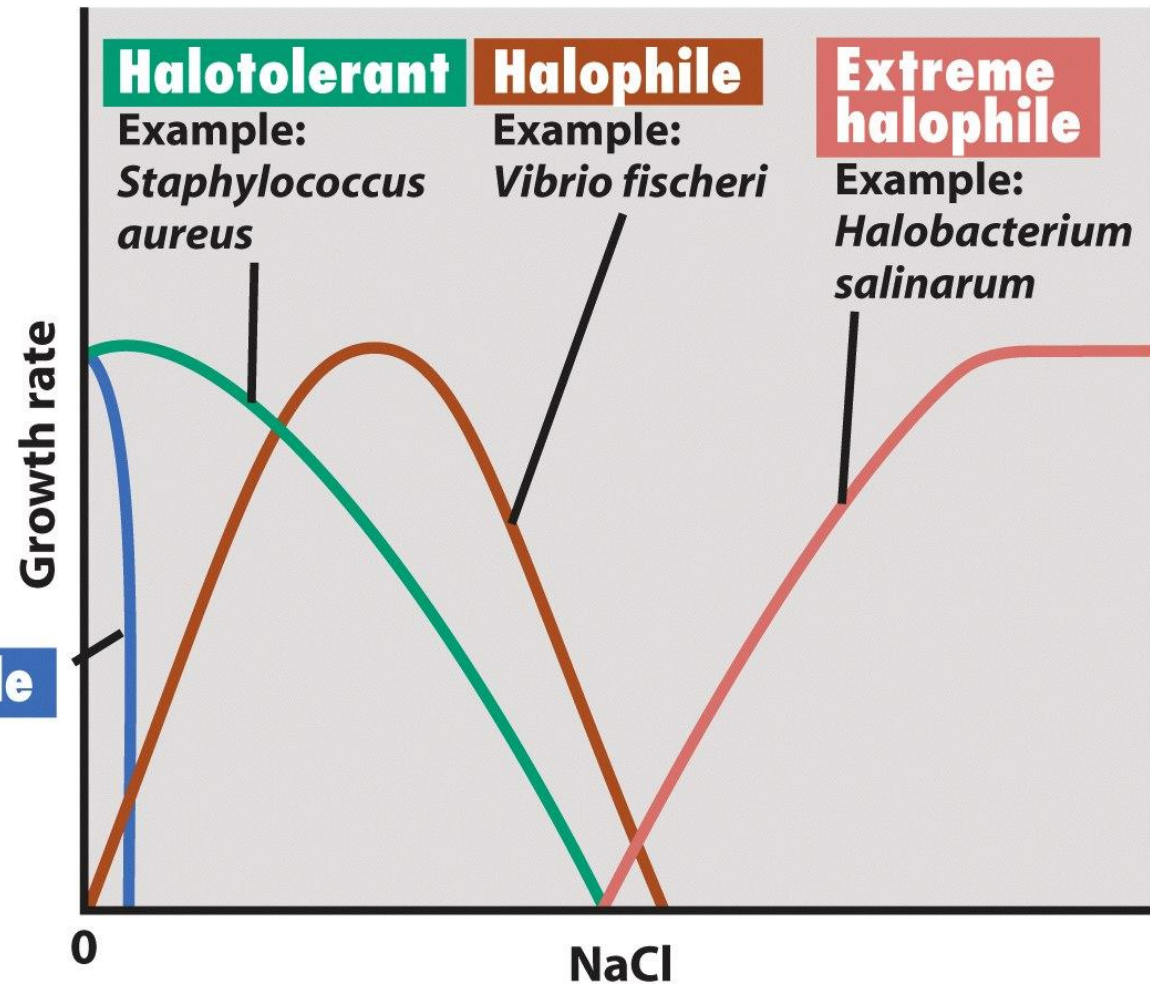


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Microbial Nutrition

□ Why is nutrition important?

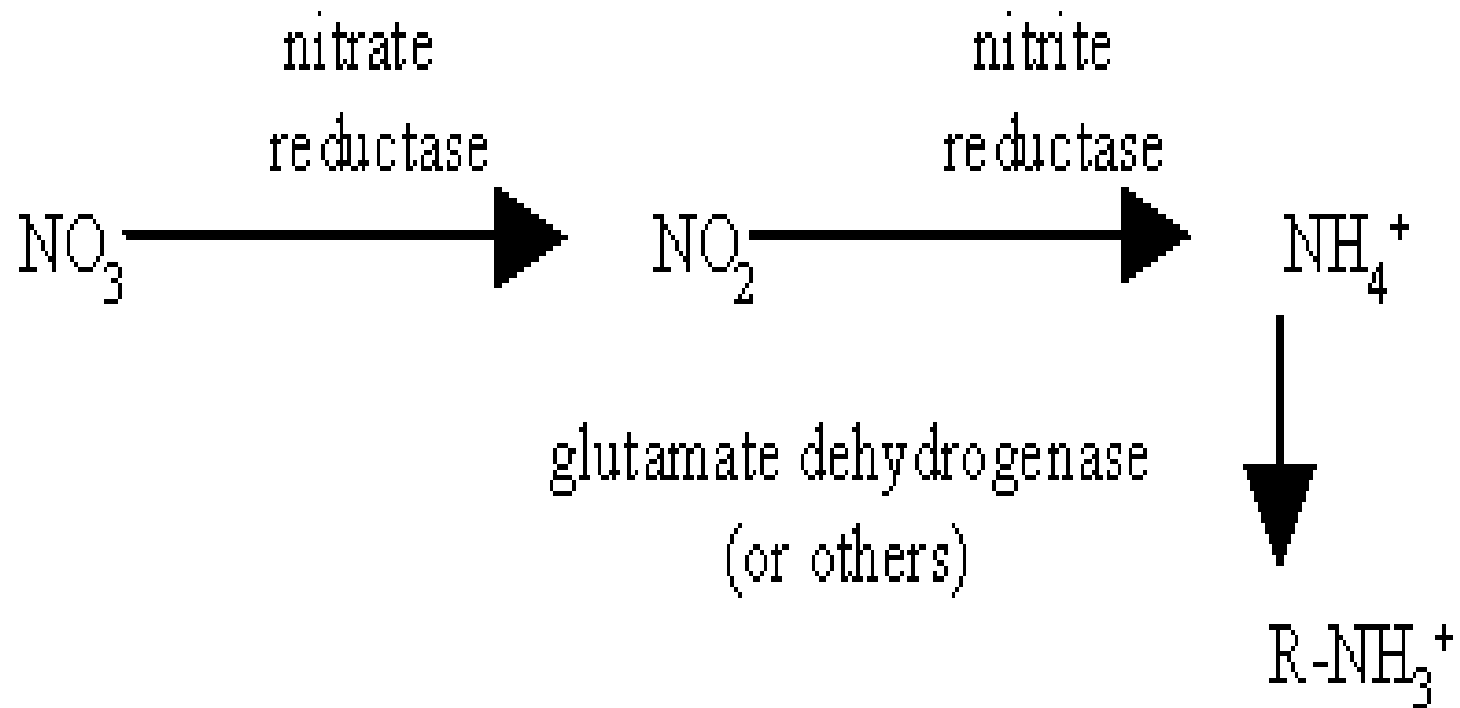
- The hundreds of chemical compounds present inside a living cell are formed from nutrients.

□ **Macronutrients** : elements required in fairly large amounts

□ **Micronutrients** : metals and organic compounds needed in very small amounts

Main Macronutrients

- Carbon (C, 50% of dry weight) and nitrogen (N, 12% of dry weight)
- **Autotrophs** are able to build all of their cellular **organic molecules from carbon dioxide**
- Nitrogen mainly incorporated in proteins, nucleic acids
- Most Bacteria can use Ammonia - NH_3 and many can also use NO_3^-
- Nitrogen fixers can utilize atmospheric nitrogen (N_2)



Microbial growth requirements

- Source of carbon for basic structures
- Source of cellular energy (ATP or related compounds) to drive metabolic reactions
- Source of high energy electrons/H, reducing power, typically in form of NADH/NADPH

Classification of organisms based on sources of C and energy used

		Energy Source	
		Light (<i>photo</i> -)	Chemical compounds (<i>chemo</i> -)
Carbon Source	Carbon dioxide (<i>auto</i> -)	<i>Photoautotrophs</i> - Plants, algae, and cyanobacteria use H₂O to reduce CO₂, producing O₂ as a byproduct - Photosynthetic green sulfur and purple sulfur bacteria do not use H₂O nor produce O₂	<i>Chemoautotrophs</i> Hydrogen, sulfur, and nitrifying bacteria
	Organic compounds (<i>hetero</i> -)	<i>Photoheterotrophs</i> Green nonsulfur and purple nonsulfur bacteria	<i>Chemoheterotrophs</i> - Aerobic respiration: most animals, fungi, and protozoa, and many bacteria - Anaerobic respiration: some animals, protozoa, and bacteria - Fermentation: some bacteria and yeasts

Nitrogen requirements

- Although many biological components within living organisms contain N, and N_2 is the most abundant component of air, very few organisms can “fix” or utilize N_2 by converting it to NH_3
- N is often growth limiting as organisms must find source as NH_4^+ for biosynthesis
- Photosynthetic organisms and many microbes can reduce NO_3^- to NH_4^+

Other Macronutrients

- Phosphate (P), sulfur (S), potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe)
- Iron plays a major role in cellular respiration, being a key component of cytochromes and iron-sulfur proteins involved in electron transport.
- **Siderophores** : Iron-binding agents that cells produce to obtain iron from various insoluble minerals.

Representative Siderophore

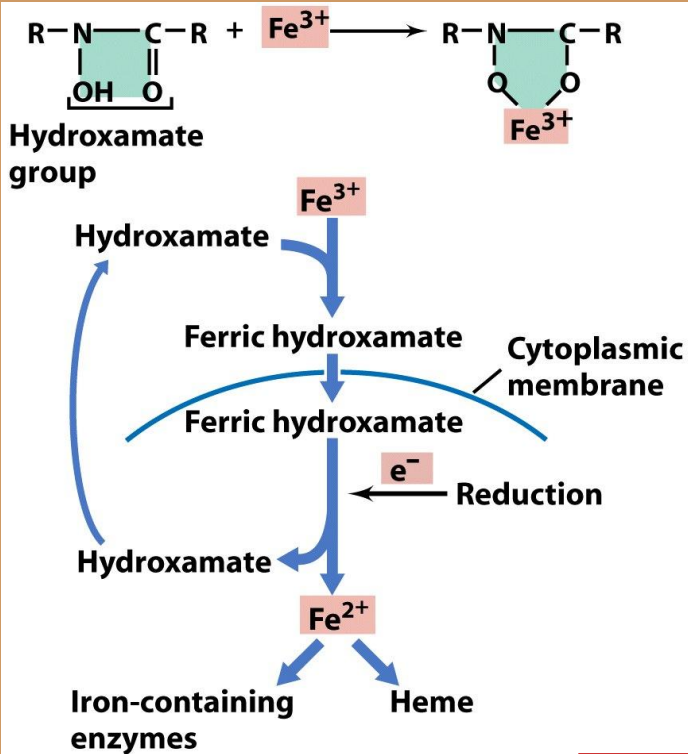
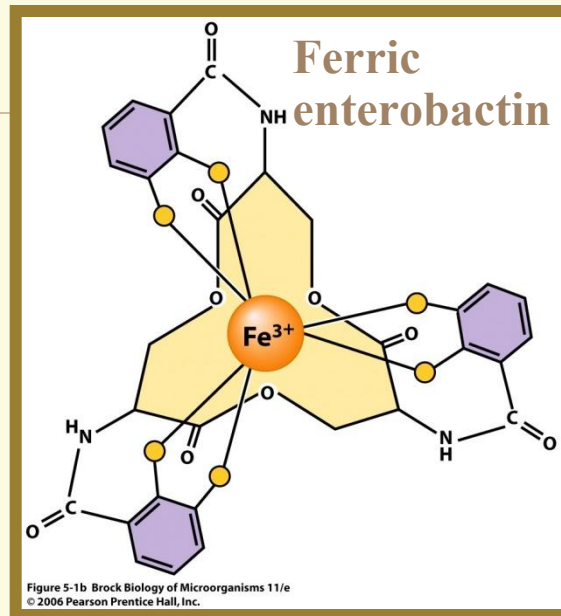


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Aquachelin

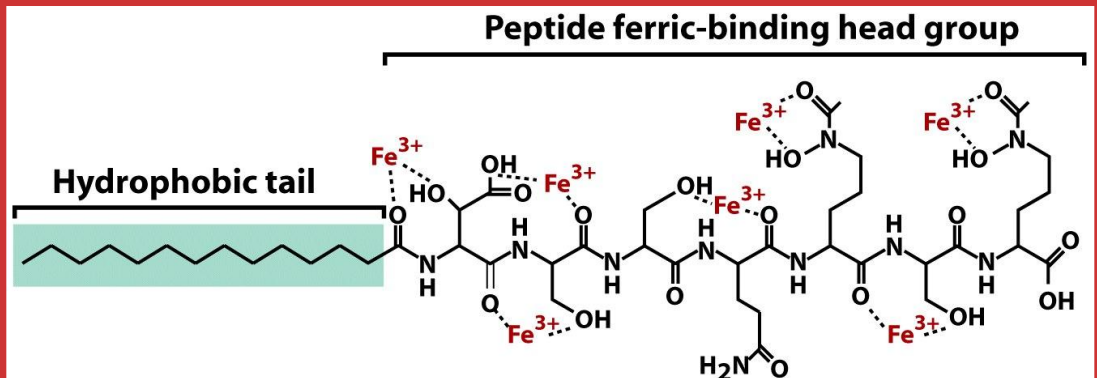


Table 5.1 Macronutrients in nature and in culture media

Element	Usual form of nutrient found in the environment	Chemical form supplied in culture media
Carbon (C)	CO ₂ , organic compounds	Glucose, malate, acetate, pyruvate, amino acids, hundreds of other compounds, or complex mixtures (yeast extract, peptone, and so on)
Hydrogen (H)	H ₂ O, organic compounds	H ₂ O, organic compounds
Oxygen (O)	H ₂ O, O ₂ , organic compounds	H ₂ O, O ₂ , organic compounds
Nitrogen (N)	NH ₃ , NO ₃ ⁻ , N ₂ , organic nitrogen compounds	<i>Inorganic:</i> NH ₄ Cl, (NH ₄) ₂ SO ₄ , KNO ₃ , N ₂ <i>Organic:</i> Amino acids, nitrogen bases of nucleotides, many other N-containing organic compounds
Phosphorus (P)	PO ₄ ³⁻	KH ₂ PO ₄ , Na ₂ HPO ₄
Sulfur (S)	H ₂ S, SO ₄ ²⁻ , organic S compounds, metal sulfides (FeS, CuS, ZnS, NiS, and so on)	Na ₂ SO ₄ , Na ₂ S ₂ O ₃ , Na ₂ S, cysteine, or other organic sulfur compounds
Potassium (K)	K ⁺ in solution or as various K salts	KCl, KH ₂ PO ₄
Magnesium (Mg)	Mg ²⁺ in solution or as various Mg salts	MgCl ₂ , MgSO ₄
Sodium (Na)	Na ⁺ in solution or as NaCl or other Na salts	NaCl
Calcium (Ca)	Ca ²⁺ in solution or as CaSO ₄ or other Ca salts	CaCl ₂
Iron (Fe)	Fe ²⁺ or Fe ³⁺ in solution or as FeS, Fe(OH) ₃ , or many other Fe salts	FeCl ₃ , FeSO ₄ , various chelated iron solutions (Fe ³⁺ EDTA, Fe ³⁺ citrate, and so on)

Micronutrients

Table 5.2 Micronutrients (trace elements) needed by living organisms^a

Element	Cellular function
Boron (B)	Present in an autoinducer for quorum sensing in bacteria; also found in some polyketide antibiotics
Chromium (Cr)	Required by mammals for glucose metabolism; no known microbial requirement
Cobalt (Co)	Vitamin B ₁₂ ; transcarboxylase (propionic acid bacteria)
Copper (Cu)	Respiration, cytochrome <i>c</i> oxidase; photosynthesis, plastocyanin, some superoxide dismutases
Iron (Fe) ^b	Cytochromes; catalases; peroxidases; iron-sulfur proteins; oxygenases; all nitrogenases
Manganese (Mn)	Activator of many enzymes; present in certain superoxide dismutases and in the water-splitting enzyme in oxygenic phototrophs (Photosystem II)
Molybdenum (Mo)	Certain flavin-containing enzymes; some nitrogenases, nitrate reductases, sulfite oxidases, DMSO-TMAO reductases; some formate dehydrogenases

Need very little amount but critical to cell function. Often used as enzyme cofactors

Nickel (Ni)	Most hydrogenases; coenzyme F ₄₃₀ of methanogens; carbon monoxide dehydrogenase; urease
Selenium (Se)	Formate dehydrogenase; some hydrogenases; the amino acid selenocysteine
Tungsten (W)	Some formate dehydrogenases; oxotransferases of hyperthermophiles
Vanadium (V)	Vanadium nitrogenase; bromoperoxidase
Zinc (Zn)	Carbonic anhydrase; alcohol dehydrogenase; RNA and DNA polymerases; and many DNA-binding proteins

^a Not every micronutrient listed is required by all cells; some metals listed are found in enzymes present in only specific microorganisms.

^b Needed in greater amounts than other trace metals.

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Growth factors

Organic compounds, required in very small amount and then only by some cells

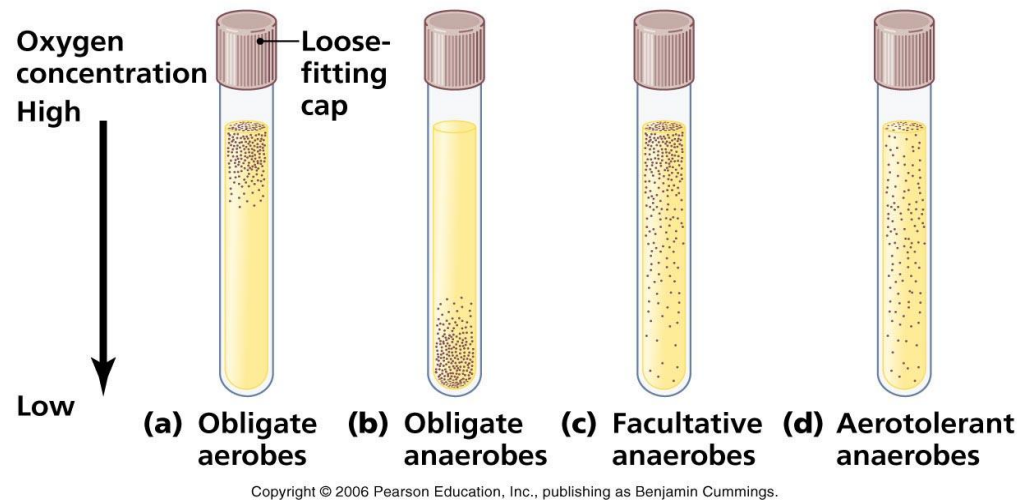
Table 5.3 Growth factors: Vitamins and their functions

Vitamin	Function
<i>p</i> -Aminobenzoic acid	Precursor of folic acid
Folic acid	One-carbon metabolism; methyl group transfer
Biotin	Fatty acid biosynthesis; β -decarboxylations; some CO ₂ fixation reactions
Cobalamin (B ₁₂)	Reduction of and transfer of single carbon fragments; synthesis of deoxyribose
Lipoic acid	Transfer of acyl groups in decarboxylation of pyruvate and α -ketoglutarate
Nicotinic acid (niacin)	Precursor of NAD ⁺ (see Figure 5.10); electron transfer in oxidation-reduction reactions
Pantothenic acid	Precursor of coenzyme A; activation of acetyl and other acyl derivatives
Riboflavin	Precursor of FMN (see Figure 5.15), FAD in flavoproteins involved in electron transport
Thiamine (B ₁)	α -Decarboxylations; transketolase
Vitamins B ₆ (pyridoxal-pyridoxamine group)	Amino acid and keto acid transformations
Vitamin K group; quinones	Electron transport; synthesis of sphingolipids
Hydroxamates	Iron-binding compounds; solubilization of iron and transport into cell

Classification of organisms based on O_2 utilization

- Utilization of O_2 during metabolism yields toxic by-products including O_2^- , singlet oxygen ($^1O_2$) and/or H_2O_2 .
- Toxic O_2 products can be converted to harmless substances if the organism has **catalase** (or **peroxidase**) and **superoxide dismutase** (SOD)
- SOD converts O_2^- into H_2O_2 and O_2
- Catalase breaks down H_2O_2 into H_2O and O_2
- Any organism that can live in or requires O_2 has SOD and catalase (peroxidase)

Classification of organisms based on O₂ utilization



- ❑ Obligate (strict) aerobes require O₂ in order to grow
- ❑ Obligate (strict) anaerobes cannot survive in O₂
- ❑ Facultative anaerobes grow better in O₂
- ❑ Aerotolerant organisms don't care about O₂
- ❑ Microaerophiles require low levels of O₂

Oxygen and Microbial Growth

□ **Aerobes :**

- **Obligate** : require oxygen to grow
- **Facultative** : can live with or without oxygen but grow better with oxygen
- **Microaerphiles** : require reduced level of oxygen

□ **Anaerobes :**

- **Aerotolerant anaerobes** : can tolerate oxygen but grow better without oxygen.
- **Obligate** : do not require oxygen. Obligate anaerobes are killed by oxygen

Table 6.4 Oxygen relationships of microorganisms

Group	Relationship to O ₂	Type of metabolism	Example ^a	Habitat ^b
Aerobes				
Obligate	Required	Aerobic respiration	<i>Micrococcus luteus</i> (B)	Skin, dust
Facultative	Not required, but growth better with O ₂	Aerobic respiration, anaerobic respiration, fermentation	<i>Escherichia coli</i> (B)	Mammalian large intestine
Microaerophilic	Required but at levels lower than atmospheric	Aerobic respiration	<i>Spirillum volutans</i> (B)	Lake water
Anaerobes				
Aerotolerant	Not required, and growth no better when O ₂ present	Fermentation	<i>Streptococcus pyogenes</i> (B)	Upper respiratory tract
Obligate	Harmful or lethal	Fermentation or anaerobic respiration	<i>Methanobacterium formicicum</i> (A)	Sewage sludge digestors, anoxic lake sediments

^a Letters in parentheses indicate phylogenetic status (B, *Bacteria*; A, *Archaea*). Representatives of either domain of prokaryotes are known in each category. Most eukaryotes are obligate aerobes, but facultative aerobes (for example, yeast) and obligate anaerobes (for example, certain protozoa and fungi) are known.

^b Listed are typical habitats of the example organism.

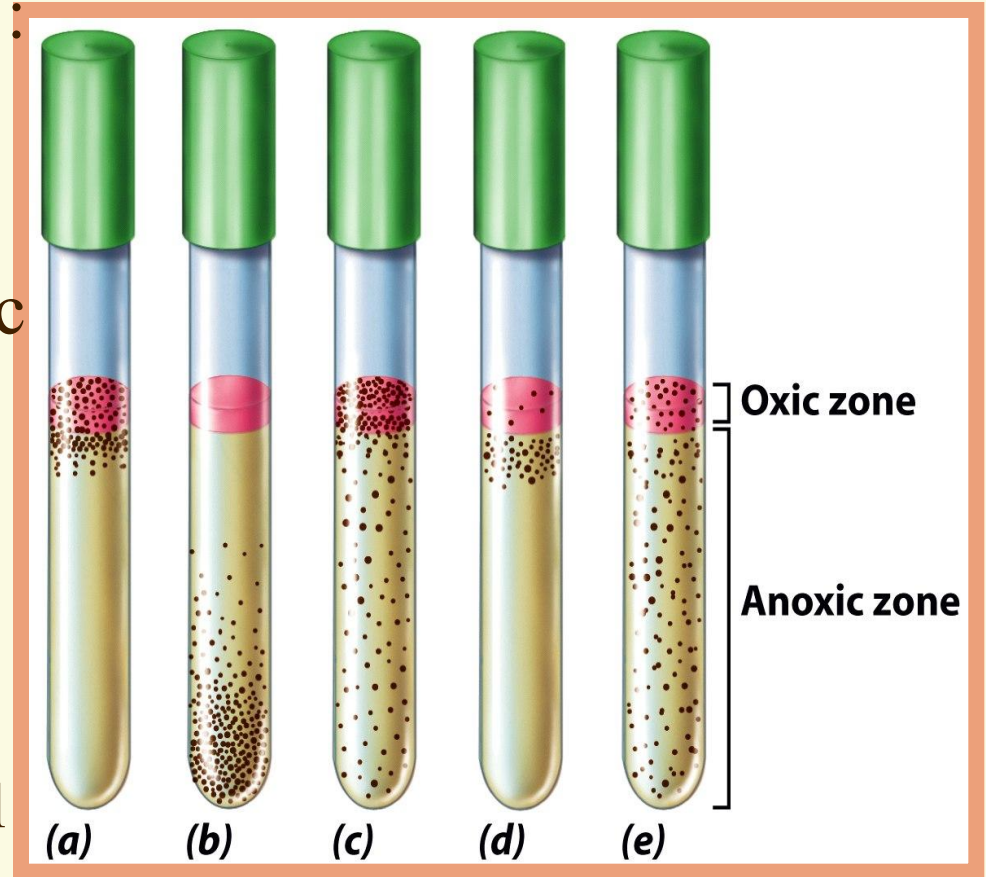
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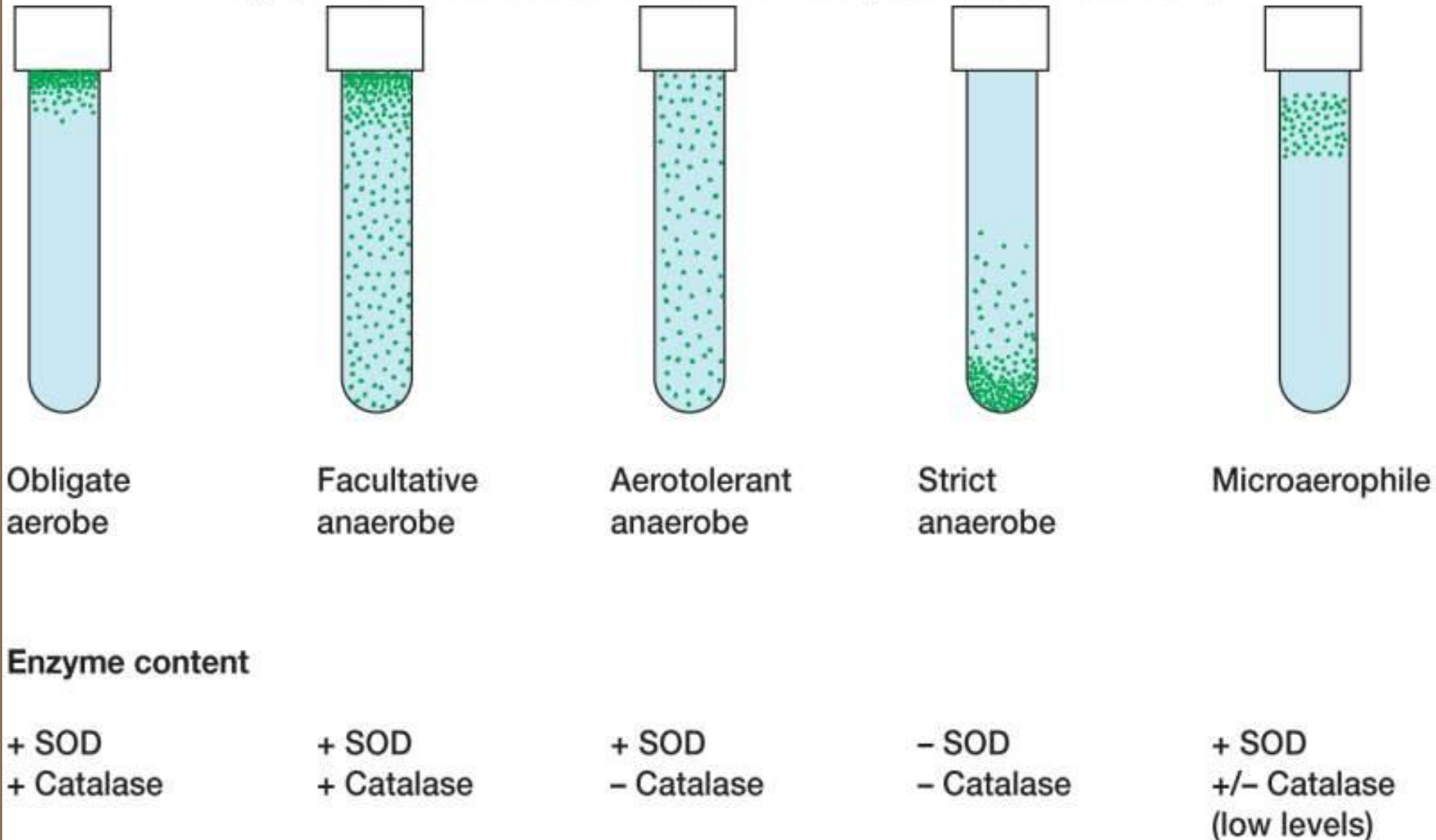
Test for Oxygen Requirements of Microorganisms

Thioglycolate broth :

contains a reducing agent and provides aerobic and anaerobic conditions

- a) Aerobic
- b) Anaerobic
- c) Facultative
- d) Microaerophil
- e) Aerotolerant





Toxic Forms of Oxygen and Detoxifying Enzymes

Hydrogen peroxide

(a) Catalase:



(b) Peroxidase:



Superoxide

(c) Superoxide dismutase:



(d) Superoxide dismutase/catalase in combination:



(e) Superoxide reductase:



Environmental factors and growth

4. Oxygen

anaerobes lack superoxide dismutase and/or catalase

anaerobes need high -, something to remove O_2

chemical: **thioglycollate**; pyrogallol + NaOH

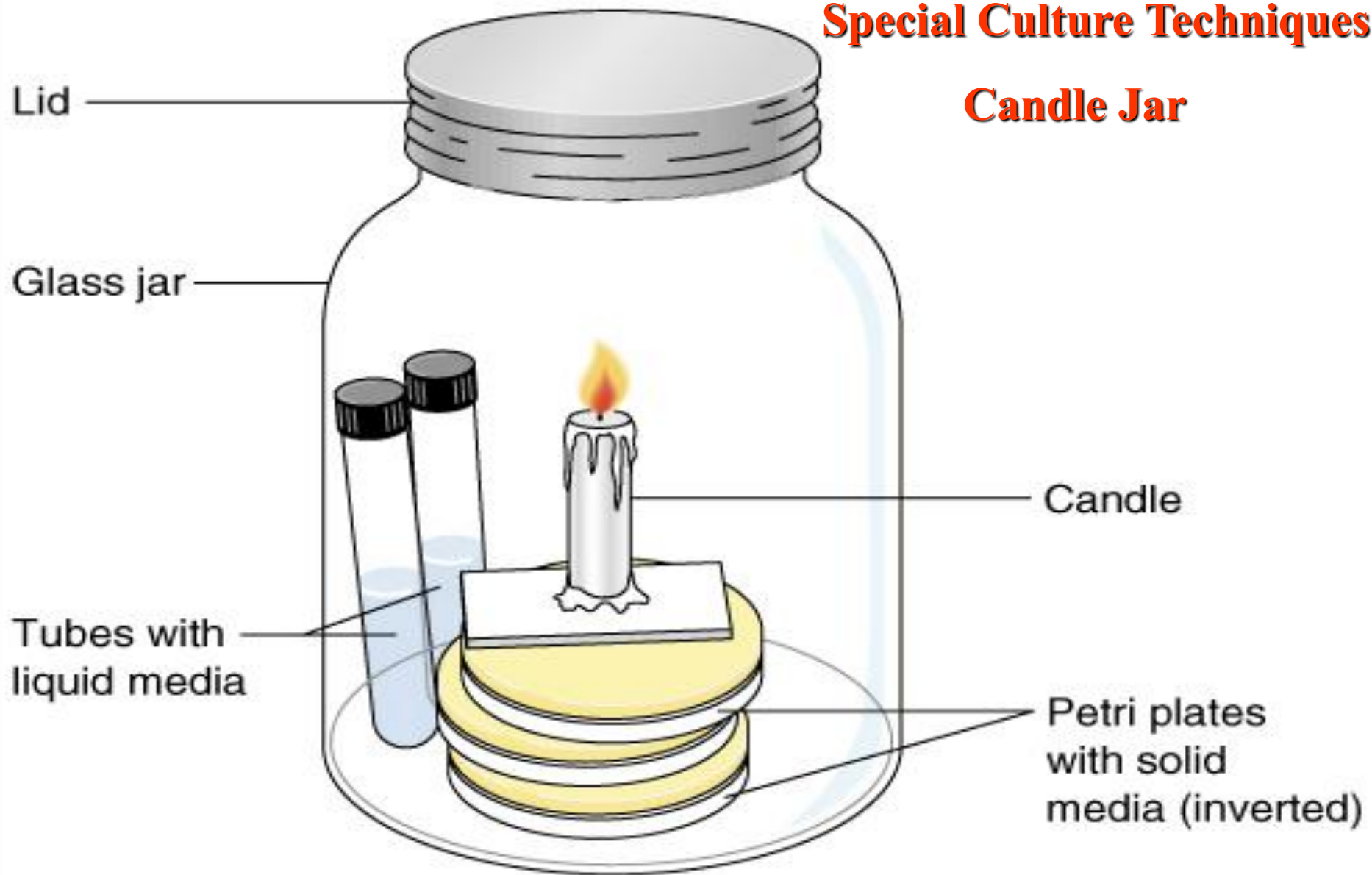
H_2 generator + catalyst

physical: removal/replacement



Special Culture Techniques

Candle Jar

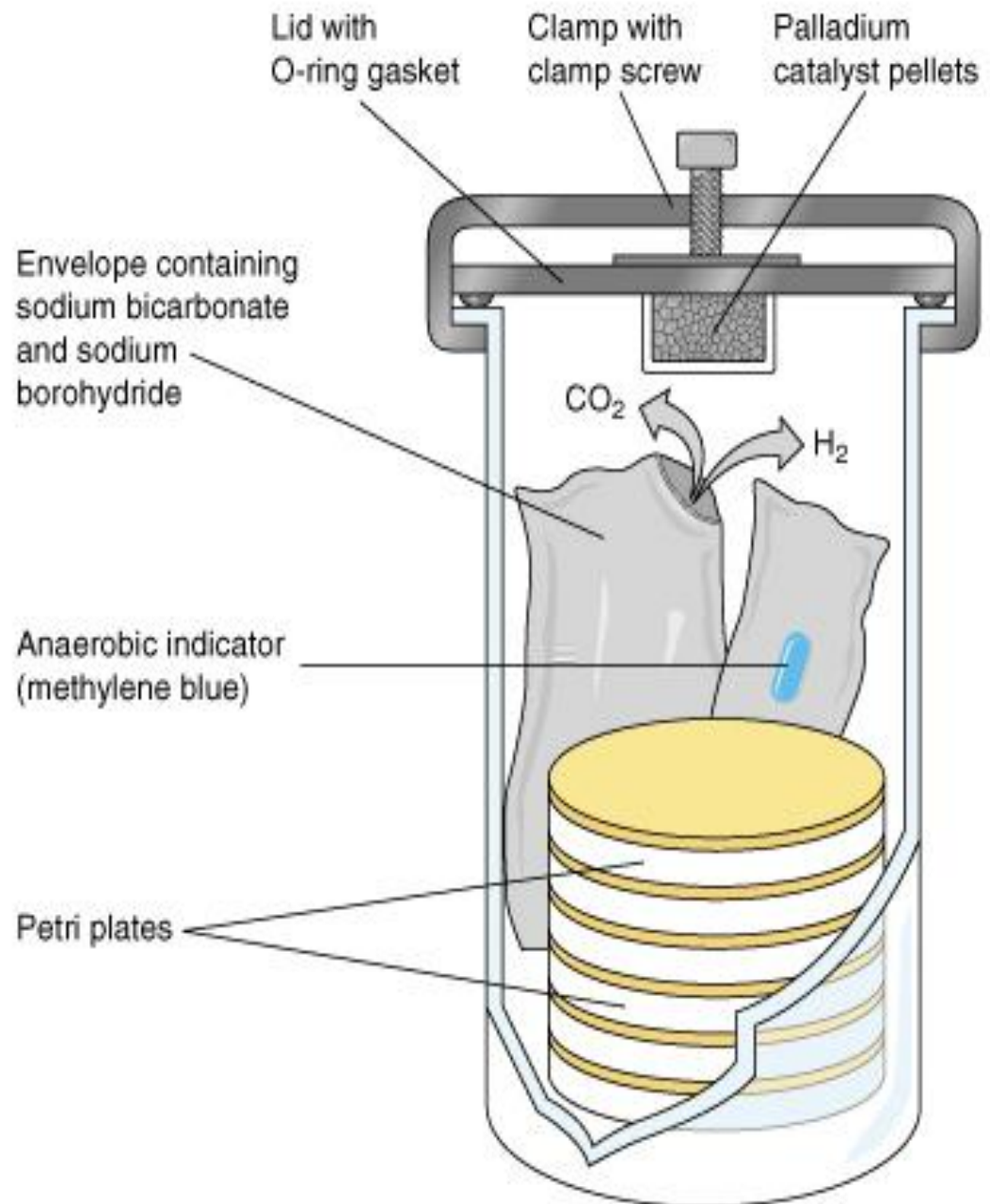


(a) Candle jar

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Special Culture Techniques

Gas Pack Jar Is Used for Anaerobic Growth



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Culture Media: Composition

- **Culture media** supply the nutritional needs of microorganisms (C ,N, Phosphorus, trace elements, etc)
 - **defined medium** : precise amounts of highly purified chemicals
 - **complex medium** (or undefined) : highly nutritious substances.
- **In clinical microbiology,**
 - **Selective** : contains compounds that selectively inhibit
 - **Differential**: contains indicator
 - terms that describe media used for the isolation of particular species or for comparative studies of microorganisms.

Types of Media

- Media can be classified on three primary levels
 1. Physical State
 2. Chemical Composition
 3. Functional Type

Physical States of Media

- Liquid Media
- Semisolid
- Solid (Can be converted into a liquid)
- Solid (Cannot be converted into a liquid)

Liquid Media

- ❑ Water-based solutions
- ❑ Do not solidify at temperatures above freezing / tend to be free flowing
- ❑ Includes broths, milks, and infusions
- ❑ Measure turbidity
- ❑ Example: Nutrient Broth, Methylene Blue Milk, Thioglycollate Broth

Semi-Solid Media

- Exhibits a clot-like consistency at ordinary room temperature
- Determines **motility**
- Used to localize a reaction at a specific site.
- Example: Sulfide Indole Motility (SIM) for hydrogen sulfide production and indole reaction and motility test.

Solid Media

- Firm surface for discrete colony growth
- Advantageous for isolating and culturing
- Two Types
 1. Liquefiable (Reversible)
 2. Non-liquefiable
- Examples: Gelatin and Agar (Liquefiable)
 Cooked Meat Media,
 Potato Slices (Non-liquefiable)

Chemical Composition of Culture Media

1. Synthetic Media

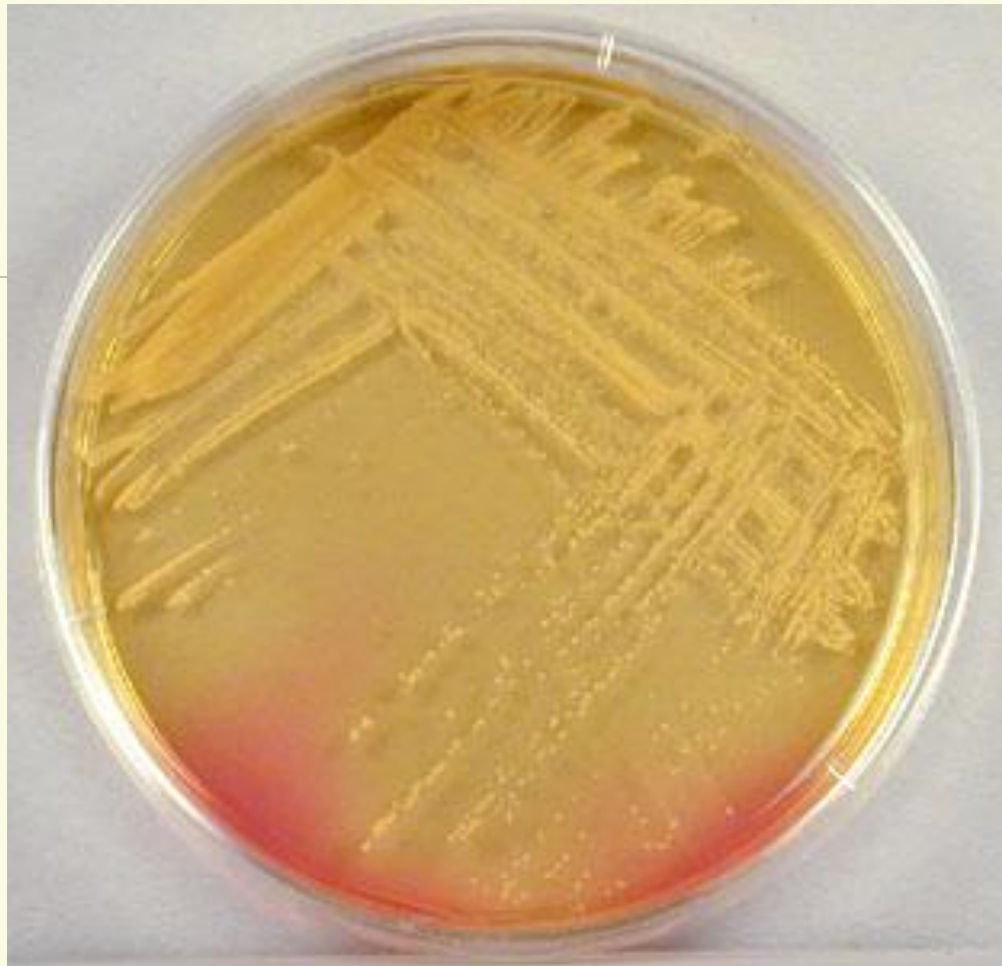
- Chemically defined
- Contain pure organic and inorganic compounds
- Exact formula (little variation)

2. Complex or Non-synthetic Media

- Contains at least one ingredient that is not chemically definable (extracts from plants and animals)
- No exact formula / tend to be general and grow a wide variety of organisms

Selective Media

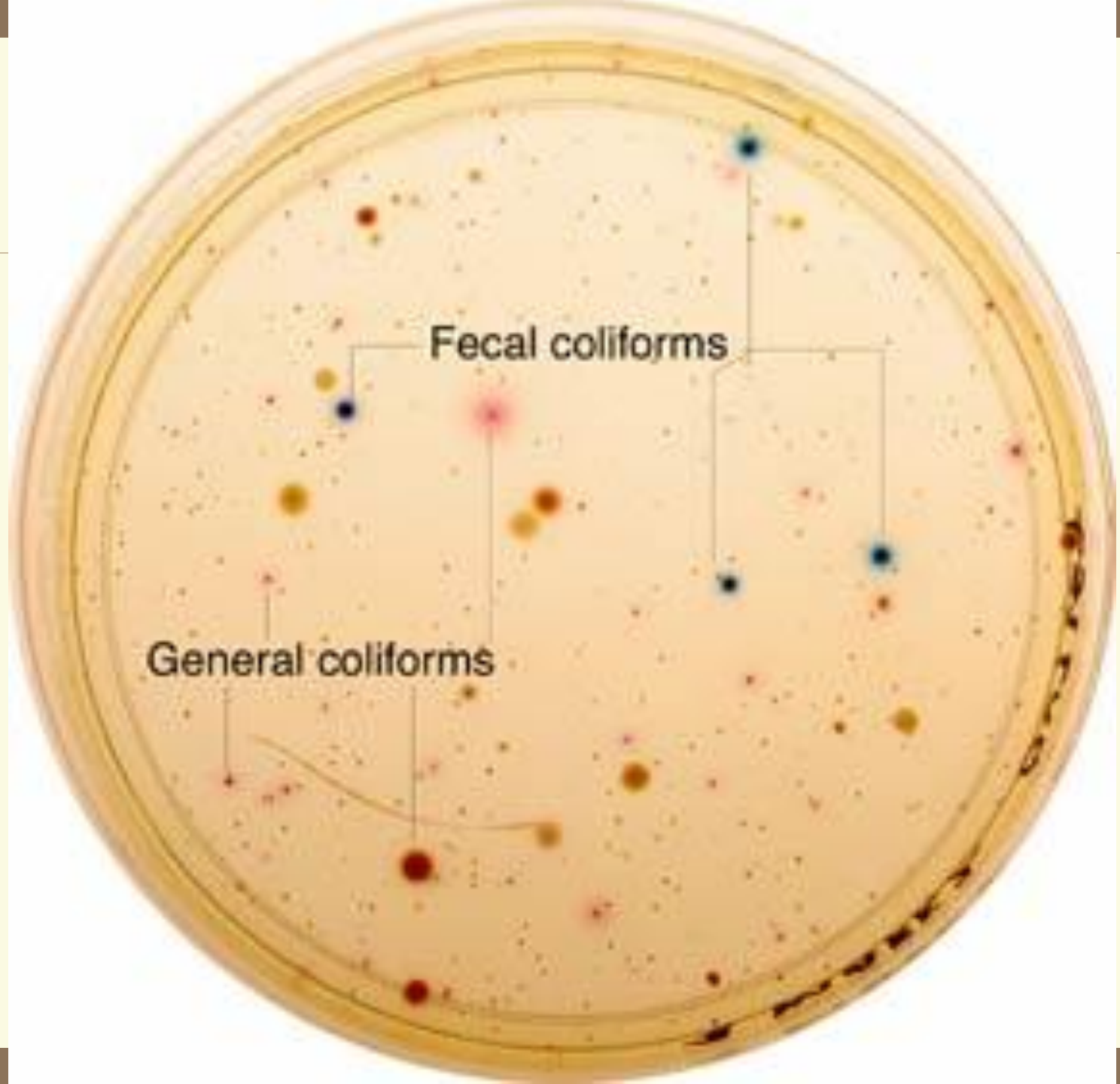
- Contains one or more agents that **inhibit** the growth of a certain microbe and thereby encourages, or **selects**, a specific microbe.
- Example: Mannitol Salt Agar [MSA] encourages the growth of *S. aureus*. MSA contain 7.5% NaCl which inhibit the growth of other Gram +ve bacteria



Growth of *Staphylococcus aureus* on Mannitol Salt Agar results in a color change in the media from pink to yellow.

Differential Media

- Differential shows up as **visible changes** or variations in colony size or color, in media color changes, or in the formation of gas bubbles and precipitates.
- Example: Spirit Blue Agar to detect the digestion of fats by lipase enzyme. Positive digestion (hydrolysis) is indicated by the dark blue color that develops in the colonies. Blood agar for hemolysis (α , β , and γ hemolysis), EMB, MacConkey Agar, ...etc.



Enrichment Media

- Is used to encourage the growth of a particular microorganism in a mixed culture.
- Ex. Manitol Salt Agar for *S. aureus*
- Blood agar , chocolate agar, Slenite F broth

Bacterial Colonies on Solid Media

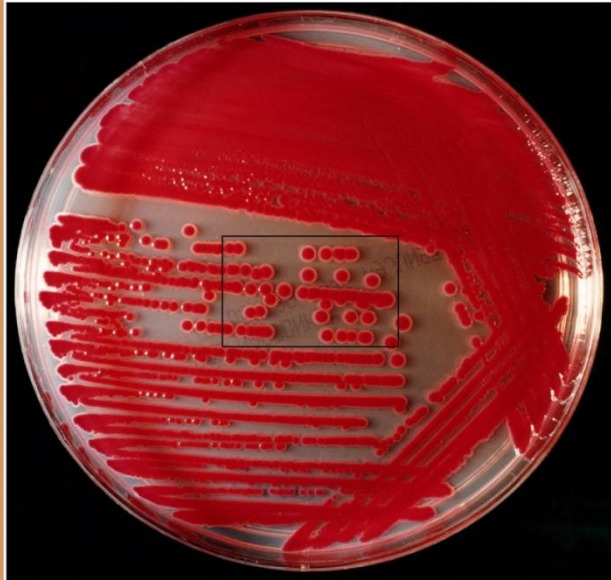


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James A. Shapiro, University of Chicago



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P. aeruginosa (TSA)

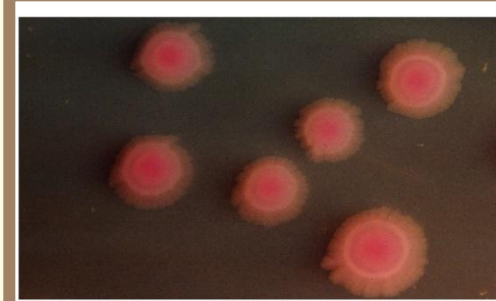


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S. Flexneri (Mac)

S. Marcescens (Mac)

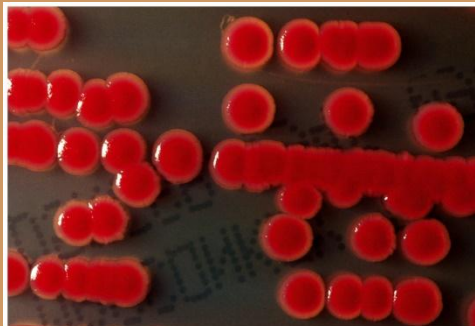
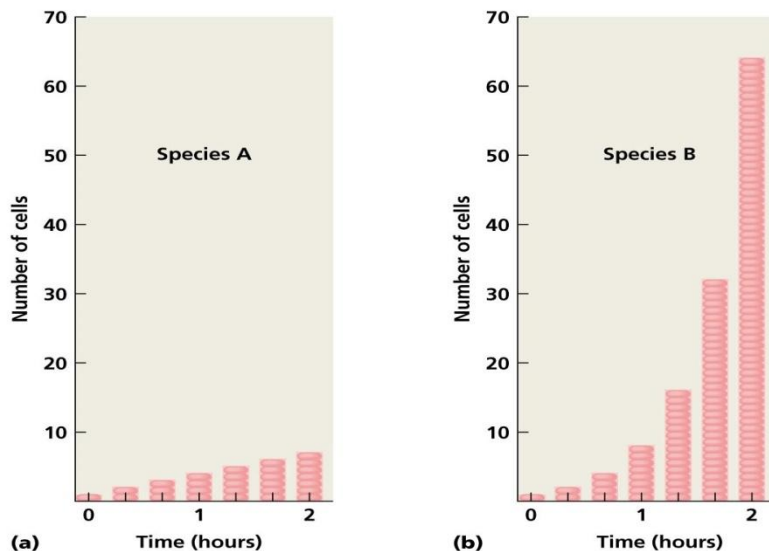


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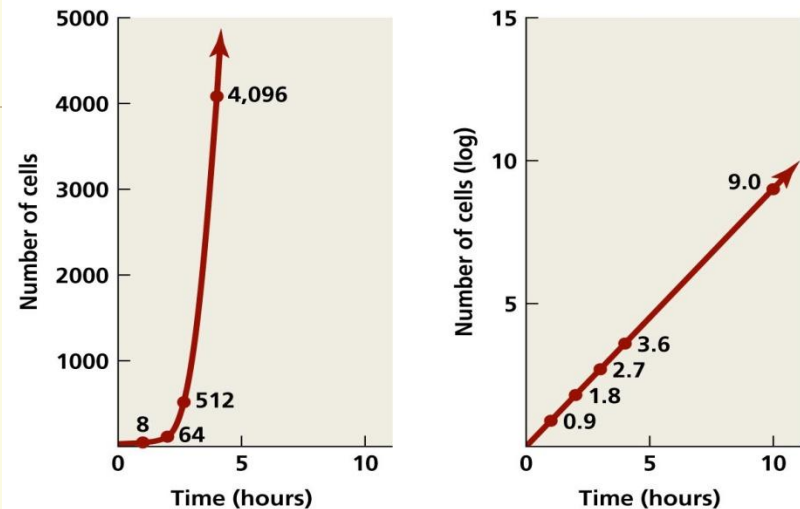
Laboratory Culture of Microorganisms

- Microorganisms can be grown in the laboratory in culture media containing the nutrients they require.
- Successful cultivation and maintenance of **pure cultures** of microorganisms can be done only if **aseptic technique** is practiced to prevent contamination by other microorganisms.

Microbial growth



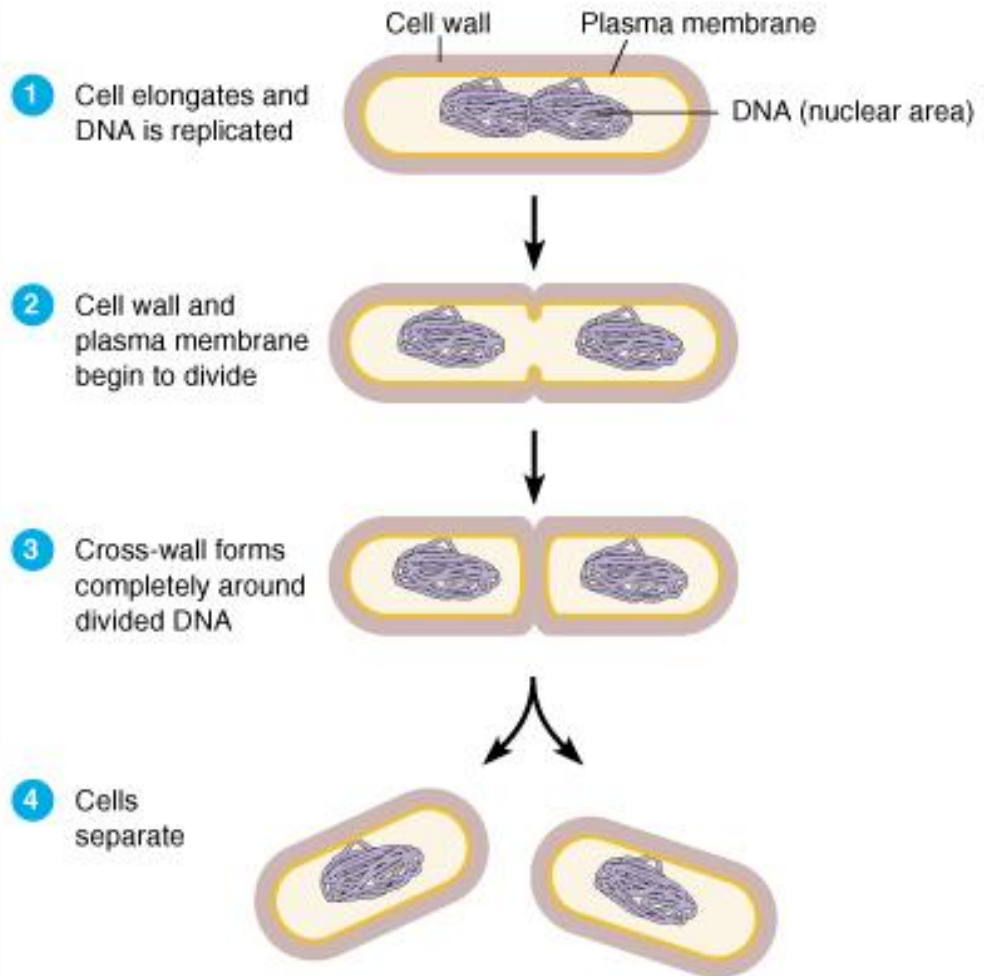
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- Microbes grow via binary fission, resulting in exponential increases in numbers
- The number of cell arising from a single cell is 2^n after n generations
- Generation time is the time it takes for a single cell to grow and divide

Binary Fission



(a)

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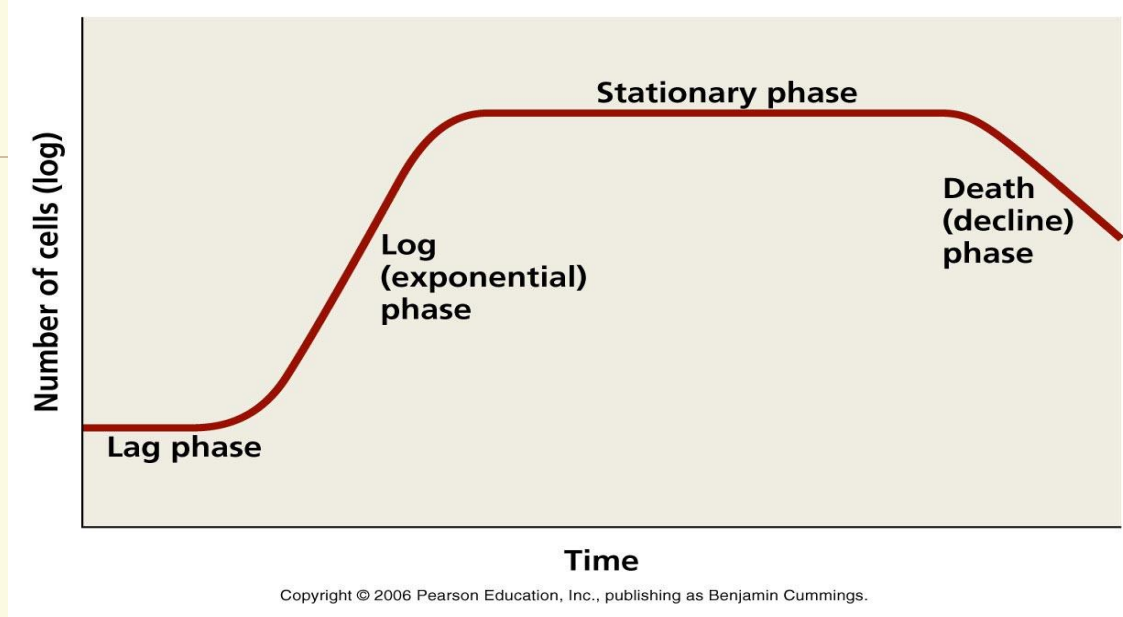
Rapid Growth of Bacterial Population

Arithmetic 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	● ●

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Growth curve

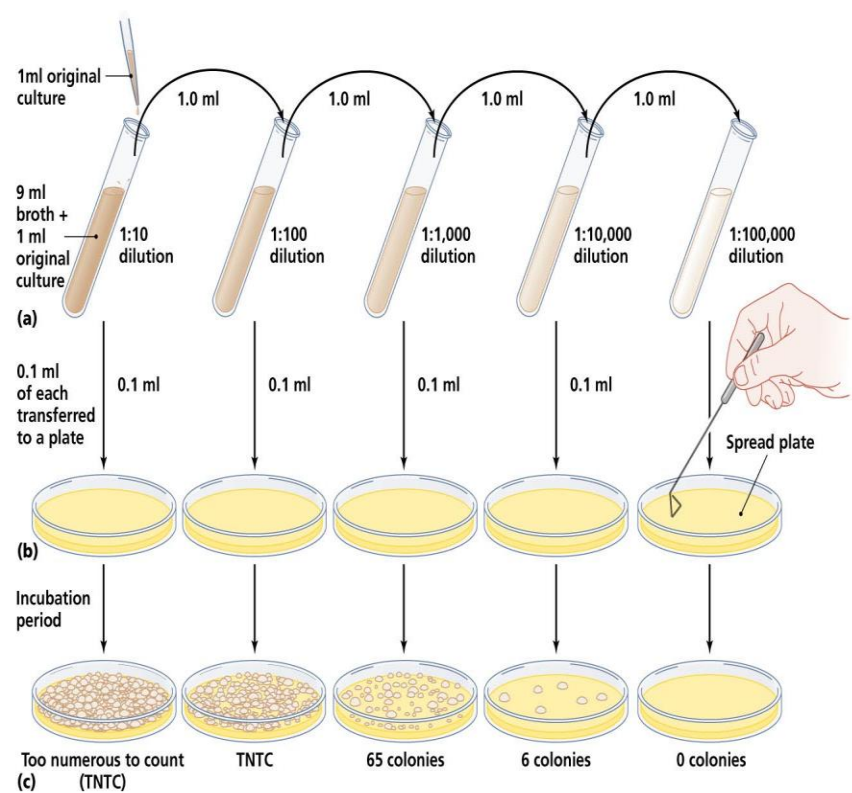


- During lag phase, cells are recovering from a period of no growth and are making macromolecules in preparation for growth
- During log phase cultures are growing maximally
- Stationary phase occurs when nutrients are depleted and wastes accumulate (Growth rate = death rate)
- During death phase death rate is greater than growth rate

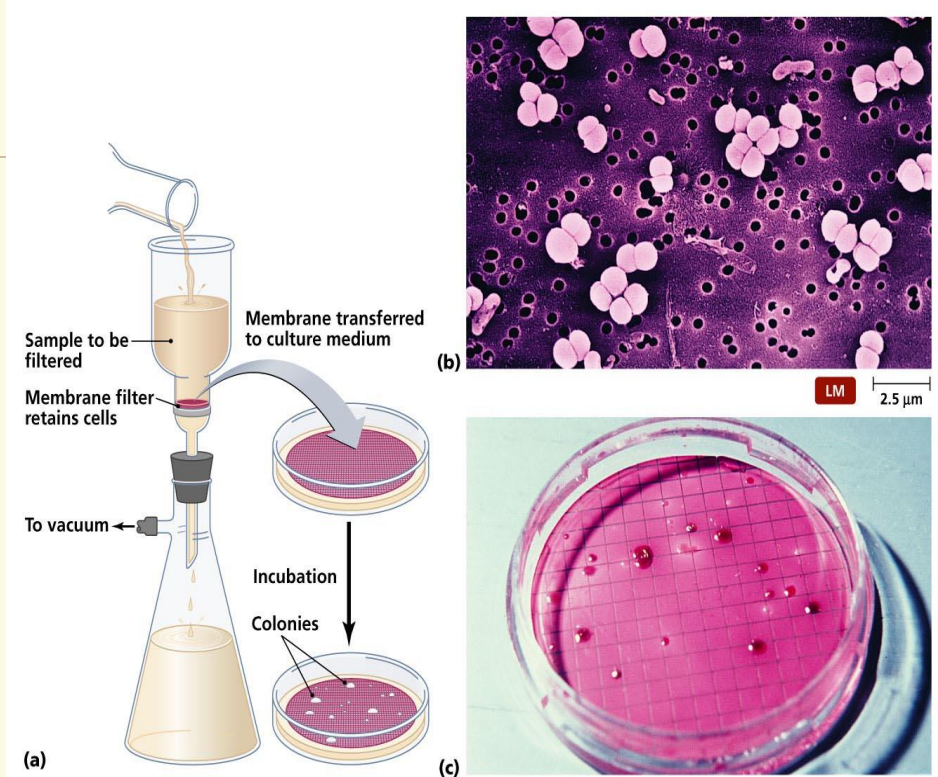
Methods used to measure microbial growth

- Count colonies on plate or filter (counts live cells)
- Microscopic counts
- Flow cytometry (FACS)
- Turbidity

Viable counts



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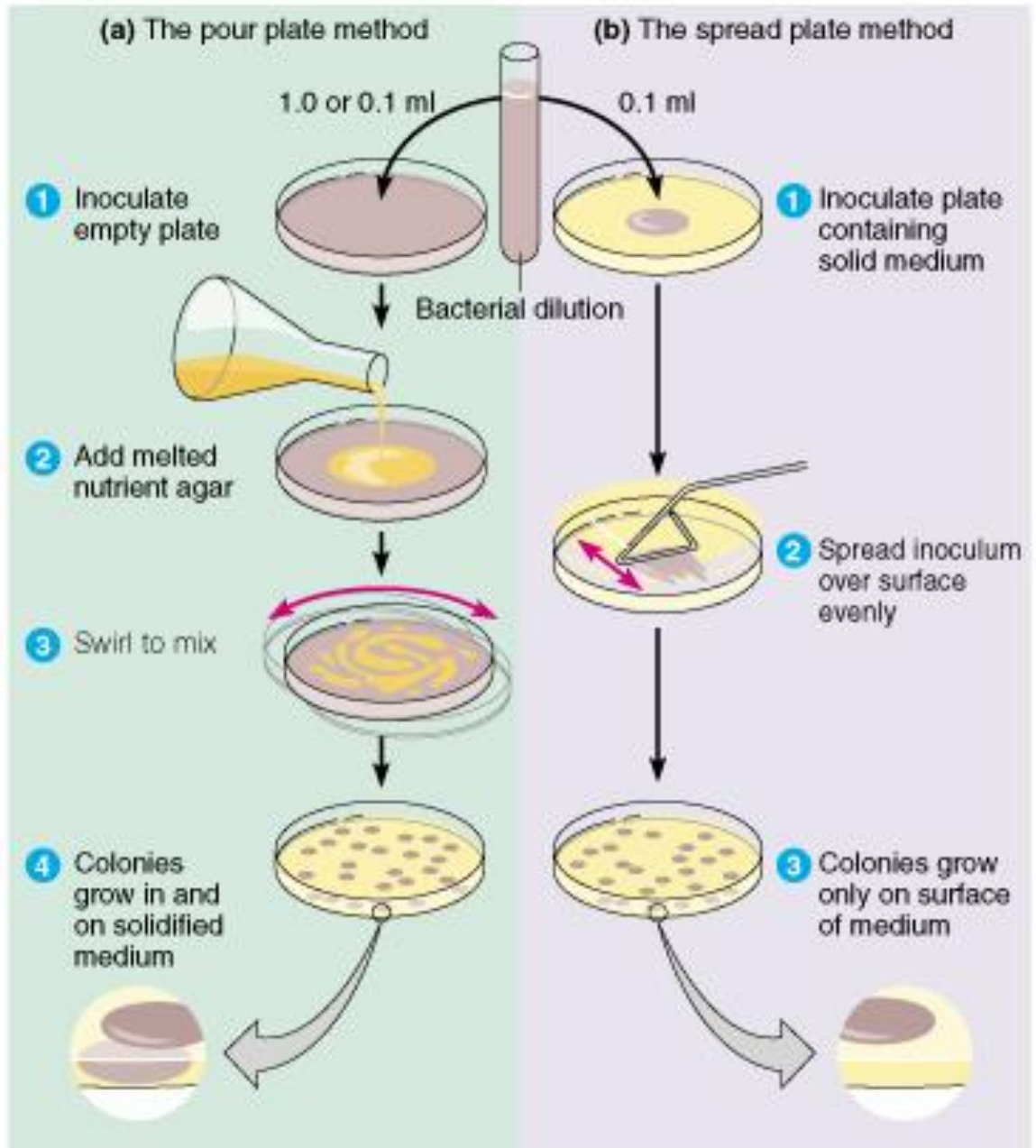


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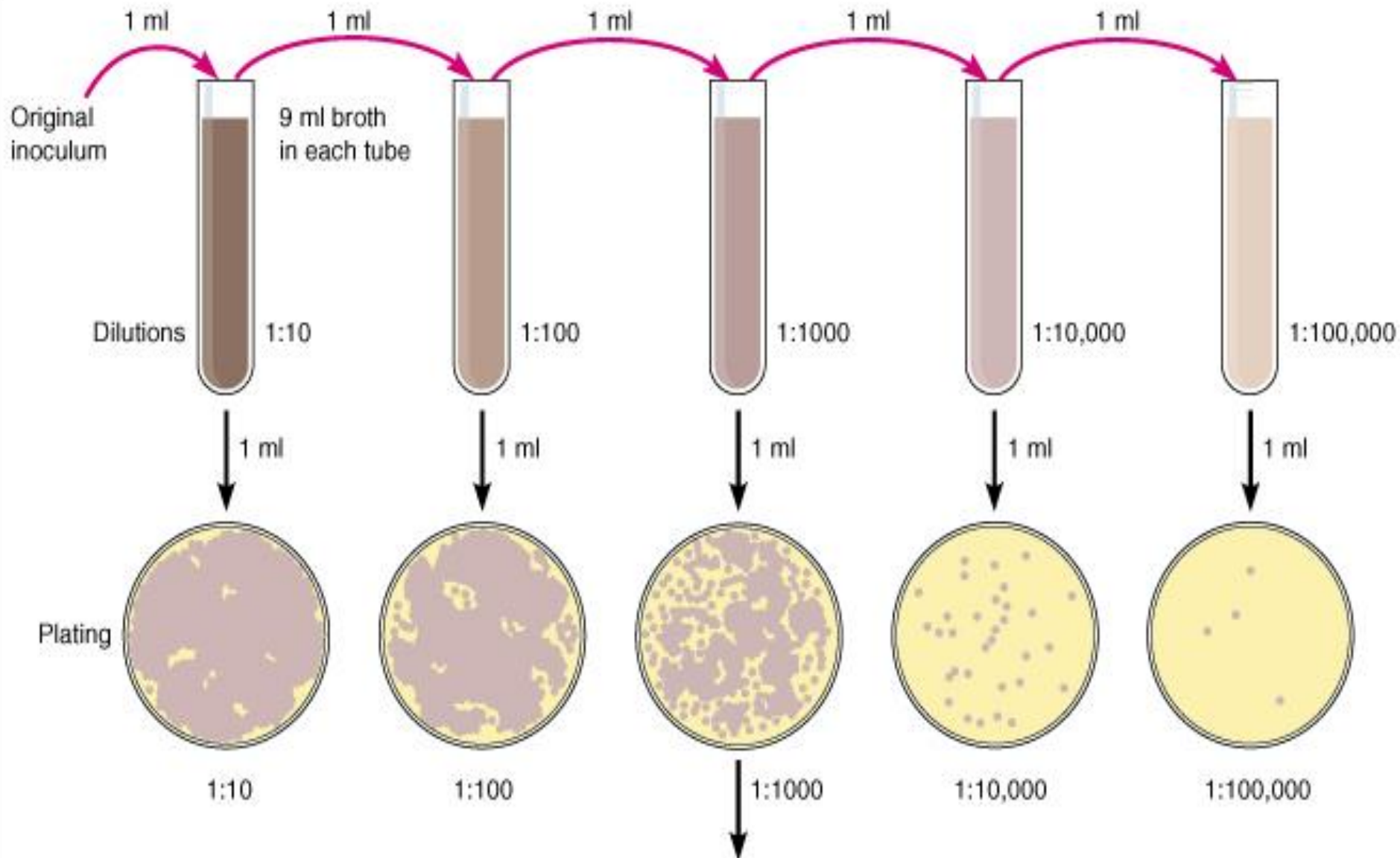
- Each colony on plate or filter arises from single live cell
- Only counting live cells

Direct Count

Pour Plate



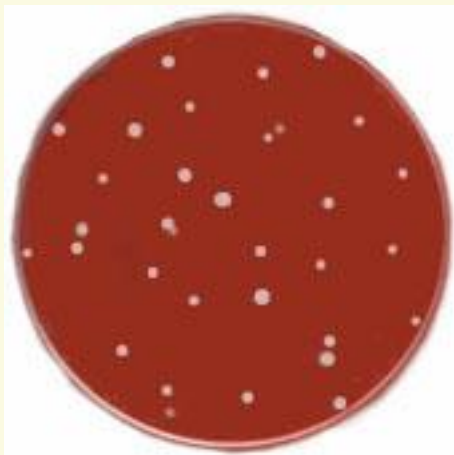
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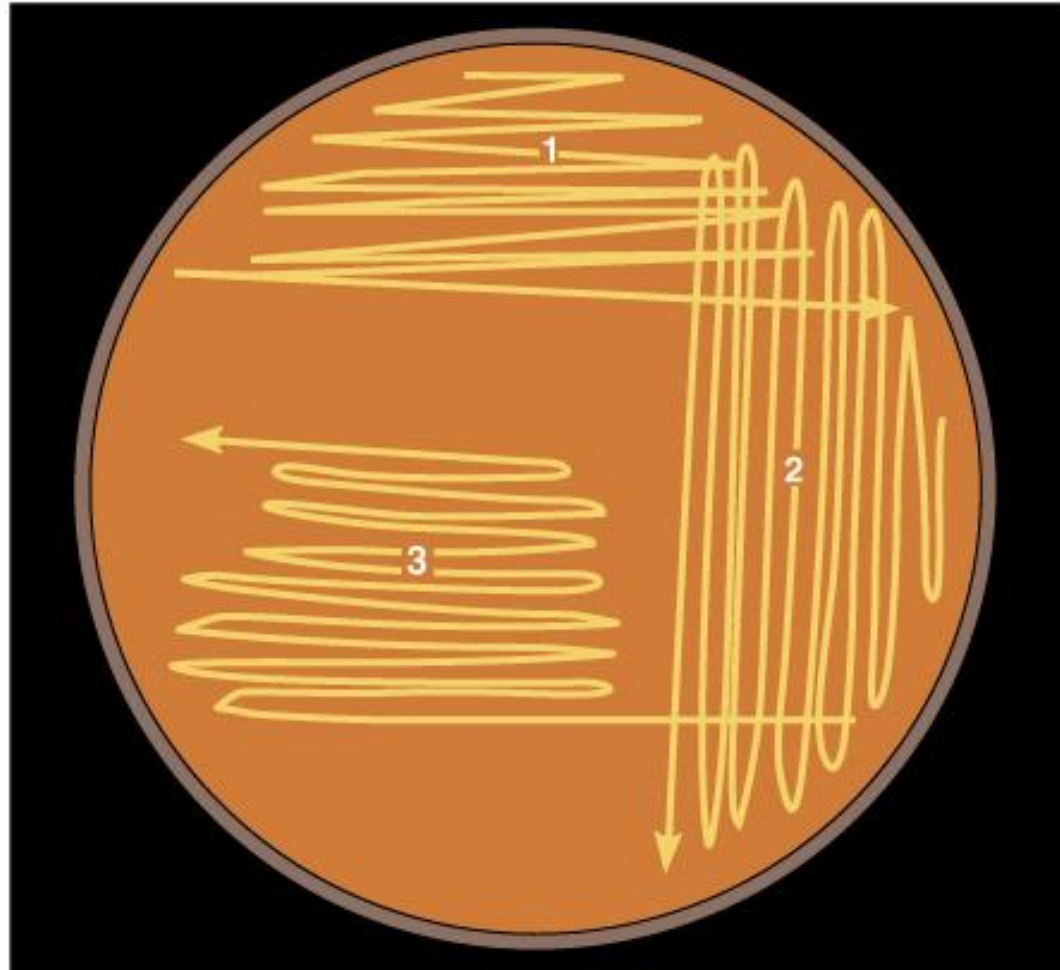
Calculation: Number of colonies on plate $\times$ reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $1/10,000$ dilution, then the count is $32 \times 10,000 = 320,000/\text{ml}$ in sample.)

Direct Count

Spread or Streak Plate

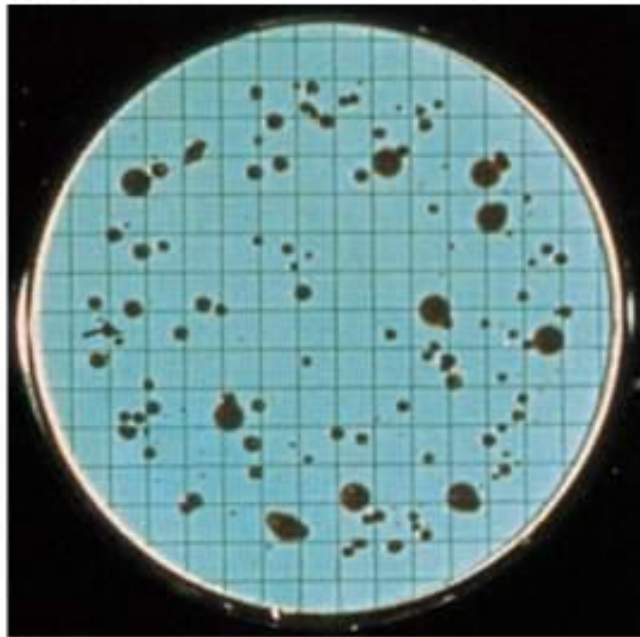


(a)

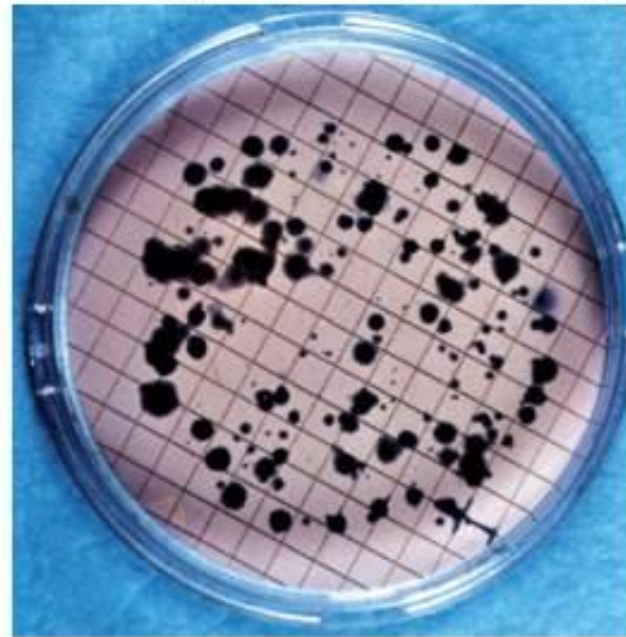


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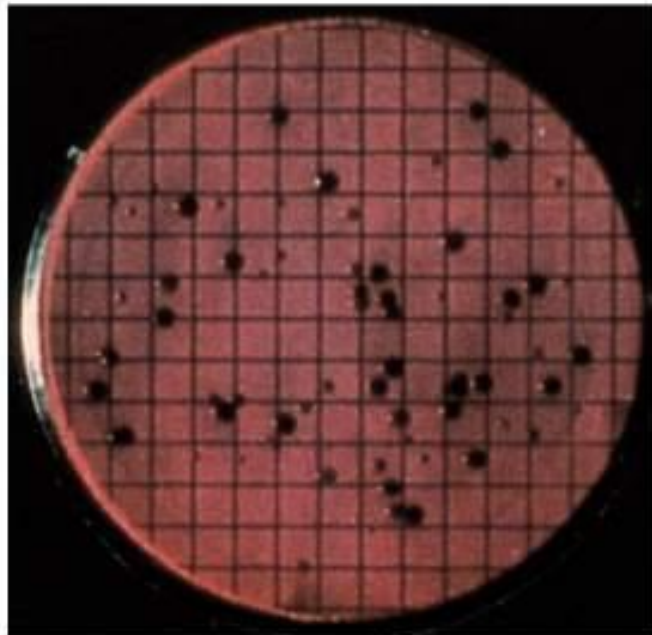
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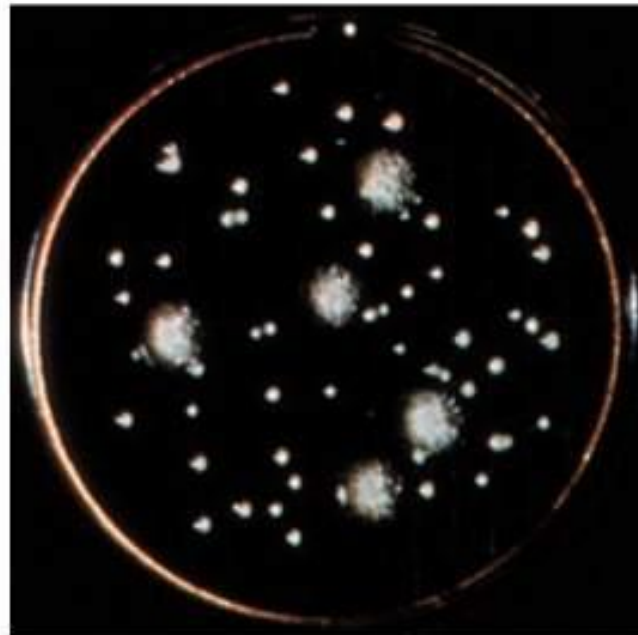
(a)



(b)

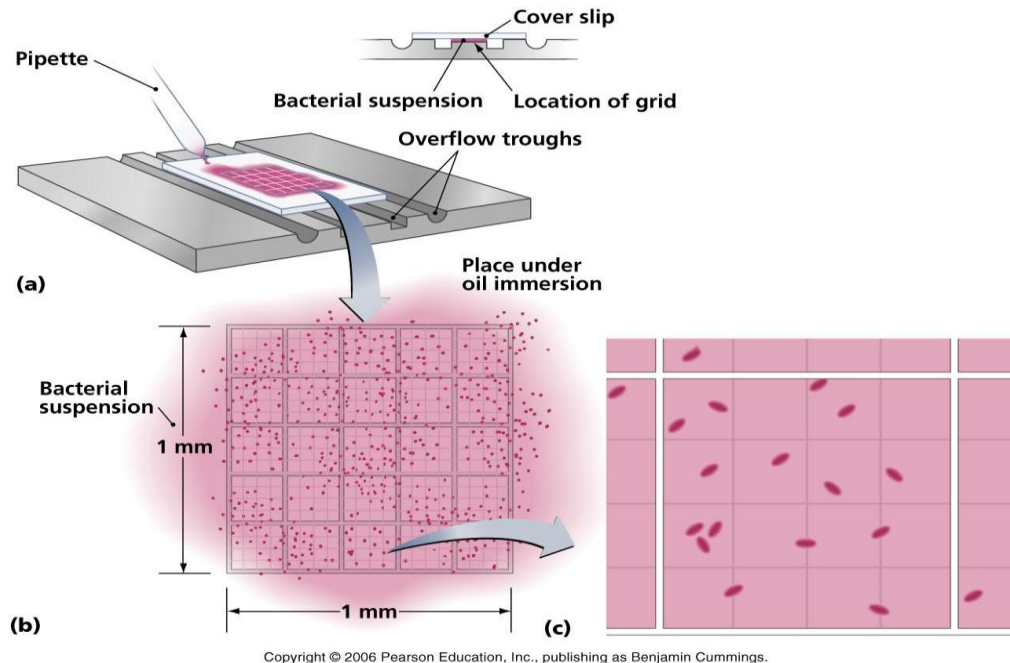


(c)



(d)

Microscopic counts

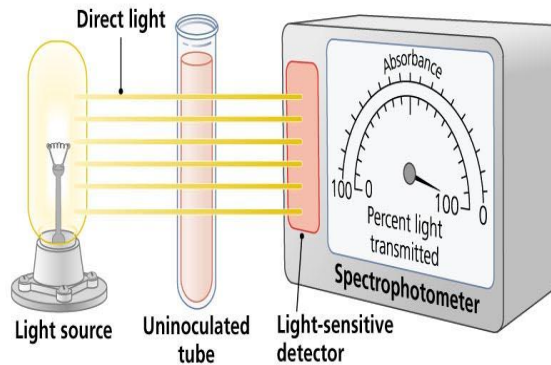


- ❑ Need a microscope, special slides, high power objective lens
- ❑ Typically only counting total microbe numbers, but differential counts can also be done

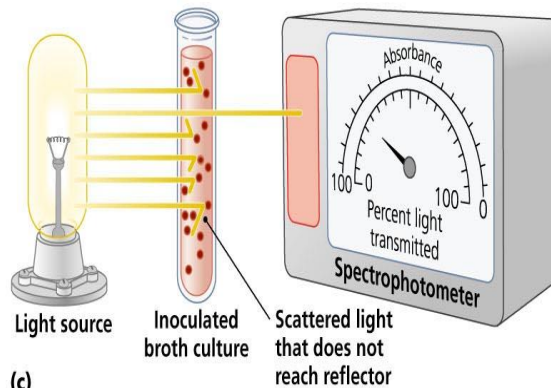
Turbidity



(a)



(b)



(c)

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- Cells act like large particles that scatter visible light
- A spectrophotometer sends a beam of visible light through a culture and measures how much light is scattered
- Scales read in either absorbance or % transmission
- Measures both live and dead cells

Inoculation

- Sample is placed on sterile medium providing microbes with the appropriate nutrients to sustain growth.
- Selection of the proper medium and sterility of all tools and media is important.
- Some microbes may require a live organism or living tissue as the inoculation medium.

Incubation

- An incubator can be used to adjust the proper growth conditions of a sample.
- Need to adjust for optimum temperature and gas content.
- Incubation produces a culture – the visible growth of the microbe on or in the media

Isolation

- The end result of inoculation and incubation is isolation.
- On solid media we may see separate colonies, and in broth growth may be indicated by turbidity.
- Sub-culturing for further isolation may be required.

Inspection

- Macroscopically observe cultures to note color, texture, size of colonies, etc.
- Microscopically observe stained slides of the culture to assess cell shape, size, and motility.

Identification

- Utilize biochemical tests to differentiate the microbe from similar species and to determine metabolic activities specific to the microbe.