

# Chapter 6 NUTRITION and GROWTH of BACTERIA

## I. Bacterial Nutrition

### Types of Bacteria

	Energy	Electron	Carbon
Photo Autotrophs (photo lithotrophs)	Light	Inorganic Molecule	CO <sub>2</sub> (plants & cyanobacteria)
Chemo heterotrophs (Chemo organotrophs)	Organic molecule reduced carbon such as glucose	Organic molecule H <sup>+</sup> ions stripped from carbon	Organic (animals, most bacteria)
Photo Heterotrophs (Photo organotrophs)	Light	Organic molecule alcohol, fatty acids, & organic acid.	Organic (unique to some bacteria) Green non-sulfur bacteria
Chemo Autotrophs (chemo lithotrophs)	Inorganic molecule uses reduced inorganic compounds	Inorganic molecule (H <sub>2</sub> S, S, NH <sub>3</sub> , NO <sub>2</sub> <sup>-</sup> , Fe <sup>+2</sup> , CO.)	CO <sub>2</sub> (unique to bacteria) Some bacteria are know to require organic carbon

B. Carbon sources, energy sources and nutritional classes of organisms.

Remember: CHNOPS

1. The requirements for Macronutrients & Micronutrients

Macronutrients: Carbon(C), Hydrogen(H), Oxygen(O), Nitrogen(N), Phosphorous (P)  
Sulfur(S)

Micronutrients: Phosphorous (P), Potassium (K), Calcium (Ca), Magnesium (Mg),  
& Iron (Fe).

### *Requirement for nitrogen, phosphorus and sulfur and growth factors*

a) **Carbon** Basic structural component of compounds

b) **Hydrogen** Constituent of organic compounds; electrons of hydrogen atoms are used in redox reactions.

c) **Nitrogen** is a component of molecules of purines, pyrimidines, amino acids and cell wall peptidoglycan. Nitrogen source depends on the need of the microbe. It can be organic nitrogen only, ammonia, or nitrate, nitrite or nitrogen gas which are all converted to ammonia by the microbe as first step in its assimilation and usage in making organic nitrogenous compounds for the cell.

d) **Oxygen** Component of many organic and inorganic compounds;  $O_2$  is the final electron acceptor in aerobic respiration.

e) **Phosphorus element** always occurs as phosphate ionic form, outside, or in the cell. The cell takes up phosphate from the medium. Phosphate is a component of phosphoproteins, phospholipids, nucleic acids, and nucleotides.

f) **Sulfur** is present and needed for amino acids methionine and cysteine and some coenzymes and vitamins. Sulfur for microbial use may be sulfate, sulfite, thio-sulfate or sulfide (all inorganic), or it may be organic sulfur in amino acids or other organic sulfur containing molecules.

## 2. Trace elements

Chloride(Cl), Sodium(Na), Zinc(Zn) Manganese(Mn), Molybdenum(Mo), Nickel(Ni), Chromium(Cr), Copper(Cu), Cobalt(Co), Tungsten(W), Vanadium (V)

## 3. Growth factors

Vitamins, biotin, cobalamin ( $B_{12}$ ) Pantothenic acid, Riboflavin, Thiamine ( $B_1$ ) Vitamin  $B_6$ , Vitamin K group, Hydroxamates

**Growth factors** are organic molecules required by the cell which can not be synthesized by the cell itself and need to be provided in the growth medium. In the natural environment some other nearby bacteria may provide these growth factors. Growth factors may be vitamin-like molecules that the cell can not synthesize.

**Transport** of nutrients and waste by bacteria (Usually small molecules: ions, amino-acids, sugars, purines and pyrimidines, vitamins, organic acids and alcohols, etc.

## II. Environmental Effects on the Growth of Bacteria

Microbial Adaptations to various types of environments

1) Temperatures; 2) Solution pH ; 3) salinity; 4) Oxygen requirements (Aerobic/Anaerobic)

### A. Temperature relations

#### 1. Psychrophiles and psychrotrophs

a. Microbes that reproduce and grow best at low temperatures,

1) range  $-10$  to  $20^{\circ}\text{C}$  ( $14$  to  $68^{\circ}\text{F}$ ).

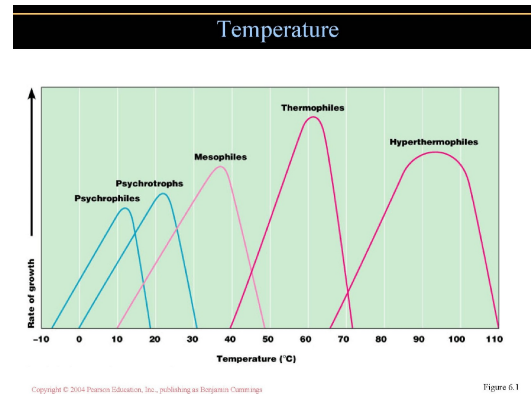
- b. Thrive and found in the Arctic and Antarctic oceans
- c. Enzymes adapted to function at lower temperatures and are denatured at moderate temperatures.
  - 1) They also exhibit polyunsaturated fatty acids in their lipids.

## 2. Mesophiles

- a. can survive at low temperatures but grow more slowly

## 3. Thermophiles and extreme (hyper) thermophiles

- a. Microbes that reproduce and grow in the temperature range 40 to 70°C (104 to 158°F).



- b. key to adaptation -

- 1) Heat stable proteins.
  - a) These proteins are more densely packed to exclude internal water
  - b) high temp proteins are more hydrophobic,
  - c) high temp proteins have more salt bridges
- 2) Membrane lipids have more saturated and longer chained fatty acids
- 3) The DNA has a greater C to G ratio than Mesophilic bacteria

- c. Archaea have ether-linked, branched chain fatty acids

- 1) And more hydrophobic
- 2) Capable of thriving at even higher temperatures than thermophiles

## B. Solution pH

### 1. Acidophiles

- a. Can live in pH values are in the range 1 to 5.
- b. Evolved ability ability to pump hydrogen ions out of their cells at a constantly high rate.
- c. result: internal pH of about 6.5 compared with a typical external pH of about 2.
- d. Examples: unicellular red alga *Cyanidium caldarium* and the green alga *Dunaliella acidophila*..

### 2. Neutrophiles

- a. Most bacteria.
  - 1) pH 5 to 8

### 3. Alkalophiles

- a. Bacteria and archaea, which thrive in highly alkaline environments
  - 1) Soda lakes and carbonate-rich soils,

a) pH values range from about 9 to 11.

- b. Intracellular pH of about 8 amid surroundings of much higher pH  
1) by continuously pumping hydrogen ions across their cell membranes into their cytoplasm

### C. Osmolarity and water activity

#### 1. Normal organisms

- a. Microbes die or become dehydrated in high salt concentration

#### 2. Osmo-tolerant and halo-tolerant organisms

- a. Halo-tolerant can tolerate some increase salt percentage  
1) Sea water is roughly 3% salt concentration  
2) Microbes adapted to sea water take up sodium to offset osmotic tension
- b. Osmo-tolerant can grow in high sugar concentrations

#### 3. Facultative halophiles

- a. Do not require high salt concentration  
1) Able to grow in salt concentrations up to 2%

#### 4. Extreme halophiles

- a. Requires high salt concentrations  
b. Dead sea microbes live in 30% salt concentration

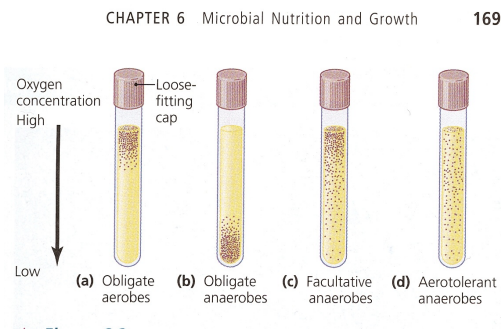
### D. The Roles of &The Nature of Oxygen

#### 1. Obligate aerobes

- a. Requires  $O_2$  to live final electron acceptor for respiration  
b. can not grow in absents of  $O_2$   
c. Use Superoxide dismutase catalase enzyme complex to detoxify  $O_2$  radicals

#### 2. Facultative anaerobes

- a. Can grow in low  $O_2$  environment  
b. During low  $O_2$  condition microbe use fermentation or anaerobic pathway for energy generation.  
c. Overall efficiency decreased  
d. Many are fermenters of carbohydrates forming lactic acid  
1) Lactic acid inhibits the growth of competitor microbes  
2) lacto bacilli - food fermentation type bacteria  
e. Tolerate an  $O_2$  environment by producing **SOD**  
f. Example: *E. coli*



### 3. **Micro aerophiles**

- a. Does not tolerate high O<sub>2</sub> environment (damage by 21% O<sub>2</sub>)
  - 1) Produce superoxide free radicals and peroxides in an O<sub>2</sub> rich environment
- b. grows best in low O<sub>2</sub> environments (2 - 10% O<sub>2</sub>)
- c. Uses Superoxide reductase catalase complex to detoxify O<sub>2</sub> radicals

### 4. **Obligate anaerobes**

- a. Unable to grow in O<sub>2</sub> environment
- b. O<sub>2</sub> is considered a poison
- b. Exhibits no means to detoxify O<sub>2</sub> radicals

## **Role of Oxygen**

### **A. Singlet oxygen (O<sub>2</sub>)**

- 1. Unreactive O<sub>2</sub> boosted to a higher energy state
- 2. This oxygen molecule is very reactive to biological systems

### **B. Superoxide free radicals (O<sub>2</sub><sup>-</sup>)**

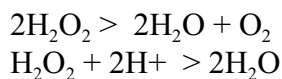
- 1. Formed in small amounts during respiration processes
- 2. Toxic to cell component & membranes
  - a. Aerobes, facultative anaerobes, aerotolerant anaerobes produce (SOD)



H<sub>2</sub>O<sub>2</sub> (hydrogen peroxide) is also very toxic to cells - - -> can produce O<sub>2</sub><sup>2-</sup> peroxide free radicals

### **C. Defense against H<sub>2</sub>O<sub>2</sub>**

- 1. Catalase decomposes
- 2. Peroxidase decomposes



### 3. Hydroxyl radical

**OH<sup>-</sup>** formed in the cytoplasm in very tiny amounts

## **III. Culture Media terms**

### **A. Culture media**

- 1. Nutrient material for microbial growth
  - a. Some bacteria are generalist growing on most media
  - b. Other bacteria require a specific set of substances with in a specific media to facilitate growth

## 2. **Innoculum**

- a. Introduced microbes to media

## 3. **Culture**

- a. Growth of the microbes on a nutrient substance

## 4. **Sterile media**

- a. no microbes

### **B. Make up of Media**

#### 1. Agar: a polysaccharide complex derived from sea weed algae

- a. Agar characteristics

- 1) Melts at 100 C
- 2) Very few microbes can degrade agar
- 3) At sea level agar remains liquid until 40°C
  - a) Microbes can be mix into liquid agar without harm

#### 2. Type of containers used for agar

- a. Petri dish (top and bottom) - when filled called a *plate*. b. Or, test tubes at an angle called *slants*

### **IV. Culture media for the growth of bacteria**

#### A. Design of laboratory culture media

Media may be solid (has agar) or liquid. Media must provide all nutrients necessary for growth

#### B. Synthetic (chemically-defined) media synthetic or defined is medium where the precise composition down to the formulae and concentrations is known.

#### C. Complex media

Complex medium may have organic or inorganic components precise concentration or composition may not be known. Trypticase Soy Agar (TSA) plate or TSB (broth are example of such media.

### **V. Media may be classified based on their usage**

#### D. A general purpose media

Media that grows most common bacteria. Examples are Trypticase Soy Agar (TSA) or Trypticase Soy Broth (TSB).

#### E. Enrichment media

Enriched media may have extra nutrients which allow growth of more fastidious microbes

## F. Selective media

Selective media prevent growth of some bacteria while allowing others to grow.

## G. Differential media

Allows some visual differences among the colonies growing on a solid medium.

# VI. Isolation Methods for microbes

## A. Streak plate:

On solid medium specimens that contain one or more bacteria are streaked for isolation.

## B. Spread plate:

A suspension of bacteria suitably diluted can be spread on surface of agar to grow well isolated colonies.

## C. Pour plate:

A suspension of bacteria suitably diluted can be mixed with melted agar and poured into plate to grow well-isolated colonies (some are inside the agar).

1. The isolated colony need to be placed on TSA slant before work can proceed.
2. Colony appearance gives clues as to the identity of the microbe, and has to be carefully analyzed.
3. Gram staining of a smear prepared from an isolated colony should give uniform-looking bacteria under oil immersion lens.

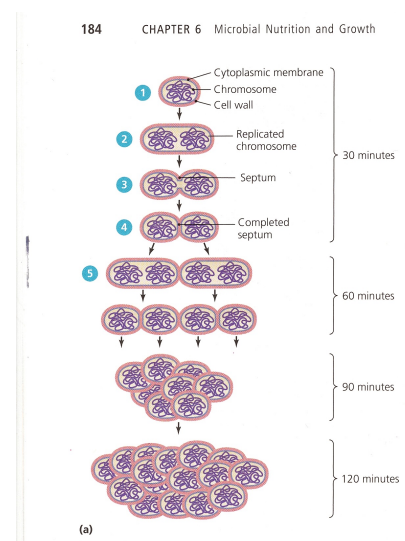
# VII. Cell Division and growth of bacterial populations

## A. Mechanisms of bacterial cell division

Growth is the orderly increase in quantity of all cellular components and structures. The growth of an individual cell leads to an increase in size and is generally followed by cell division

**Vegetative cell** - one that is actively growing and dividing.

Cell division in bacteria usually occurs by **binary fission**, in which the cell divides into two new (approximately equal and identical) cells.



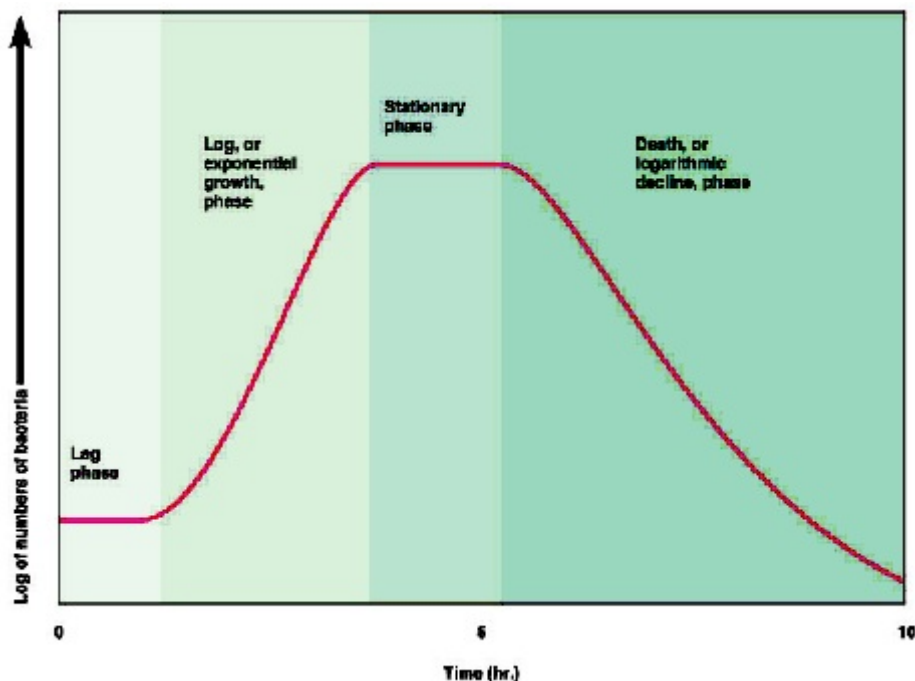
**Cell division by budding**, in which the new cell develops as a small outgrowth from the surface of the existing (parent) cell, occurs in some bacteria and in yeast.

Other bacteria may reproduce by fragmentation or by aerial spore formation. Although it does occur under favorable conditions as well, spore formation (**sporulation**) generally serves to allow the organism to withstand long periods of unfavorable conditions such as extreme temperatures or dryness.

## B. Methods for measurement of cell growth

Microbial growth is assayed as an increase in cell number or mass of a **population** of cells.

1. Measurements of cell mass
  - a. direct microscopic count: count a given number of bacteria on a slide section using a grid square method.
  - b. Estimation of numbers by indirect method
    1. Turbidity measurement
      - a. light sensitive detector of spectrophotometer
2. Measurements of cell numbers
  - a. Plate Count - Counts the number of viable cell. Disadvantage: takes 24 hours for colonies to form for count - often referred to as colony-forming units (CFU).



Growth of bacterial populations

The bacterial growth cycle and the typical bacterial growth curve



**Lag phase** -cells are metabolically active but are not dividing. Acquiring the environment phase of living. This is a period when the cells are synthesizing novel enzymes, coenzymes, etc., necessary for growth and division.

**Exponential growth phase** - bacteria are growing and dividing at an exponential, or logarithmic, rate. This is the period of fastest growth; the generation time is maximal and constant. All nutrients and molecules needed for growth are in good supply.

**Stationary phase** - at this point, the medium is becoming depleted in some nutrients, and toxic quantities of waste materials may be accumulating. The number of new cells produced is offset by the number of cells that are dying; thus, the total number of viable cells remains approximately constant.

**Exponential Death phase** - conditions are becoming less and less conducive to cell growth. Cells are dying more rapidly than new ones are being formed, resulting in a logarithmic decrease in the number of cells.

b. Generation time

**Generation time** (doubling time) - the time it takes for an individual cell to divide or for a population of cells to double. Bacterial growth follows a **logarithmic** (exponential) progression, e.g., 2 cell > 4 > 8 > 16 cells. . . . etc.....

### **Generation time**

If 100 cells over 5 hours produces 1720320 cells

$$\text{Number of generations} = \frac{\text{Log number cells (end)} - \log \text{number of cells (beginning)}}{0.301}$$

$$\text{Generation time} = \frac{60 \text{ min} \times \text{hours}}{\text{Number of generations}} = 21 \text{ minutes/generation}$$

### **Predicting The Number of cells over time**

Predicting the number of cells that will arise during a long growth period is base on a relatively simple concept.

$$N_f = (N_i)2^n$$

$N_f$  is the total number of cells in the population at some point in the growth phase

$N_i$  is the starting number of cells

$n$  is the exponent denoting the generation number of cells

$2^n$  represents the number of cells in the generation

Example: Assume that *Staphylococcus aureus* has a 20 minute generation time based on a 30°C time. We start with 10 *S. aureus* cell on an egg sandwich. Over 4 hours how many bacteria cells can we expect to find?

$N_f = 10$     Generation of 20 minutes

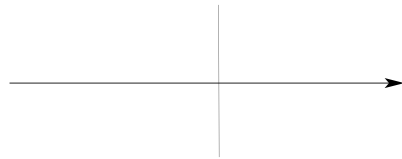
4 hours = 240 minutes - - - there are 12 cycles of cell division during  
this time  $(240/20) = 12$

$N_f = 10 \times 2^{12} = 40,960$  bacterial cells predicted in the warm sandwich.

# Tonicity and Diffusion of Water

**Hypotonic**

> % of water  
< % of solutes



**Hypertonic**

< % of water  
> % of solutes

**Water diffuses from greatest percentage to lowest percentage**

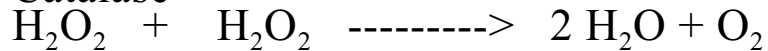
***Electron reduction of oxygen in a stepwise manner***

Reaction	Reaction type
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$O_2 + e^- \rightarrow O_2^-$	<b>Superoxide</b>
$O_2^- + e^- + 2H^+ \rightarrow H_2O_2$	<b>Hydrogen peroxide</b>
$H_2O_2 + e^- + H^+ \rightarrow H_2O_2 + OH\cdot$	<b>Hydroxyl radical</b>
$OH\cdot + e^- + H^+ \rightarrow H_2O$	<b>Water</b>

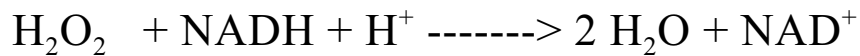
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***Enzyme required to carry out reaction:***

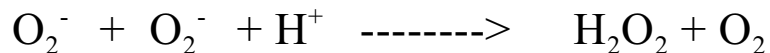
Catalase



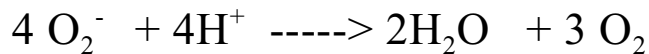
Peroxidase



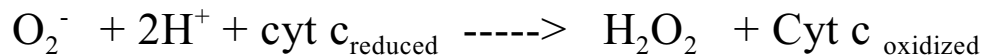
Superoxide dismutase



Superoxide dismutase/catalase in combination



Superoxide reductase



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Medium	Selective Agent(s)	Organism Encouraged to Grow
Brilliant green agar	Brilliant green	Gram-negative rods*
Eosin-methylene blue agar	Eosin Y, methylene blue	Grams-negative rods
Hektoen enteric agar	Bile salts	Grams-negative rods
MacConkey agar	Bile salts, crystal violet	Grams-negative rods
Mannitol-salt agar	Sodium chloride	<i>Staphylococcus aureus</i>

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\* this medium is not used for the isolations of *Salmonella typhi*.

A selective medium is defined as one that permits the growth of certain organisms while preventing or retarding the growth of others. Selection, in general, can be carried out through (1) control of ingredients of the medium, (2) alteration of atmospheric components, or (3) adjustment of incubation temperature.

Selective media may contain selective agents that inhibit the growth of one or more unwanted organisms in a specimen without preventing the growth of the wanted organism... i.e...Different nutrient rich media or Anti-biotic media impregnated media.

Summary of Reactions Associated with Selected Differential (D) and Selective Differential (SD) Media Used for Isolation and/or Identification.

Medium	Substrates(s)	Type of medium	Reaction and descriptions
Blood agar	Hemoglobin	D	<ol style="list-style-type: none"> <li>1. Alpha hemolysis (green zones around colonies)</li> <li>2. Beta hemolysis (clear zones around colonies)</li> <li>3. Gamma hemolysis (no zone around colonies)</li> </ol>
brilliant green agar	Lactose, sucrose	SD	<ol style="list-style-type: none"> <li>1. Lactose-fermenter (yellow-green colonies)</li> <li>2. Non-lactose fermenter (pink to white colonies Surrounded by brilliant red zones)</li> </ol>
Eosin-methylene blue agar	Lactose, sucrose	SD	<ol style="list-style-type: none"> <li>1. Lactose-fermenter (dark purple colonies or colonies With dark centers and transparent colorless borders)</li> <li>2. Non-lactose or non-sucrose fermenters (colorless colonies.</li> </ol>
Hektoen enteric agar	Lactose, sucrose salicin, and amino acids containing sulfur	SD	<ol style="list-style-type: none"> <li>1. Lactose-fermenter (salmon-pink colonies)</li> <li>2. Non-lactose-fermenters (green, most colonies)</li> <li>3. Salicin-fermenters (pink zones around colonies)</li> <li>4. Non-salicin-fermenters (no change)</li> <li>5. H<sub>2</sub>S producers (colonies with black centers)</li> </ol>
MacConkey agar	Lactose	SD	<ol style="list-style-type: none"> <li>1. Lactose-fermenter (pink-red colony is surrounded by pink zones due to precipitated bile).</li> <li>2. Non-lactose fermenter (colorless and translucent colonies)</li> </ol>
Mannitol-salt agar	Mannitol	SD zone.	<ol style="list-style-type: none"> <li>1. Mannitol fermenter (colonies surrounded by yellow</li> <li>2. Non-mannitol fermenter (small colonies with no color yellow change)</li> </ol>