Microbial Growth

Lecture 10

Dr. Samer Y. Alqaraleh

PhD. Nanobiotechnology (Microbiology)

Faculty of Dentistry

2025-2026

Microbial Growth Curve

- Bacteria are easy to grow in the lab.
- It has been determined that in a **closed system** or **batch culture** (no food added, no wastes removed) bacteria will grow in a predictable pattern.
- resulting in a **growth curve** composed of **four distinct phases of growth**:

1. **Lag phase,**
2. **Exponential** or **Log phase**,
3. **Stationary phase**,
4. **Death** or **decline phase**.

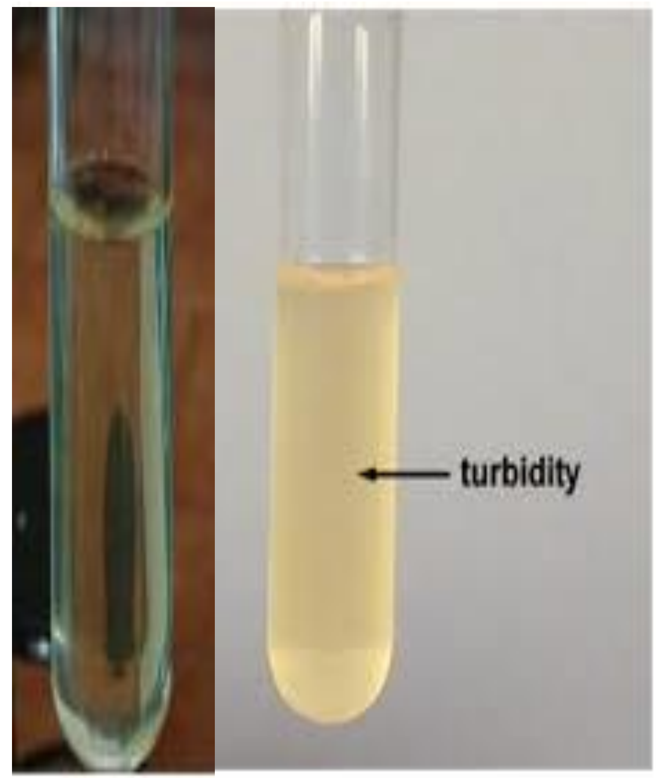
- Growth curve can yield **generation time** for a particular organism – <u>the amount of time it takes for the population to double</u>.

1) Bacterial Growth

❖ **Indicated by:**

I. **Turbidity of the fluid media**

II. **Colonies on solid media(Macroscopic product)**



13min (*V.cholerae*)

24 hrs (*M.tuberculosis*)

Single bacterium

On solid media

After 20-30 division

Binary fission

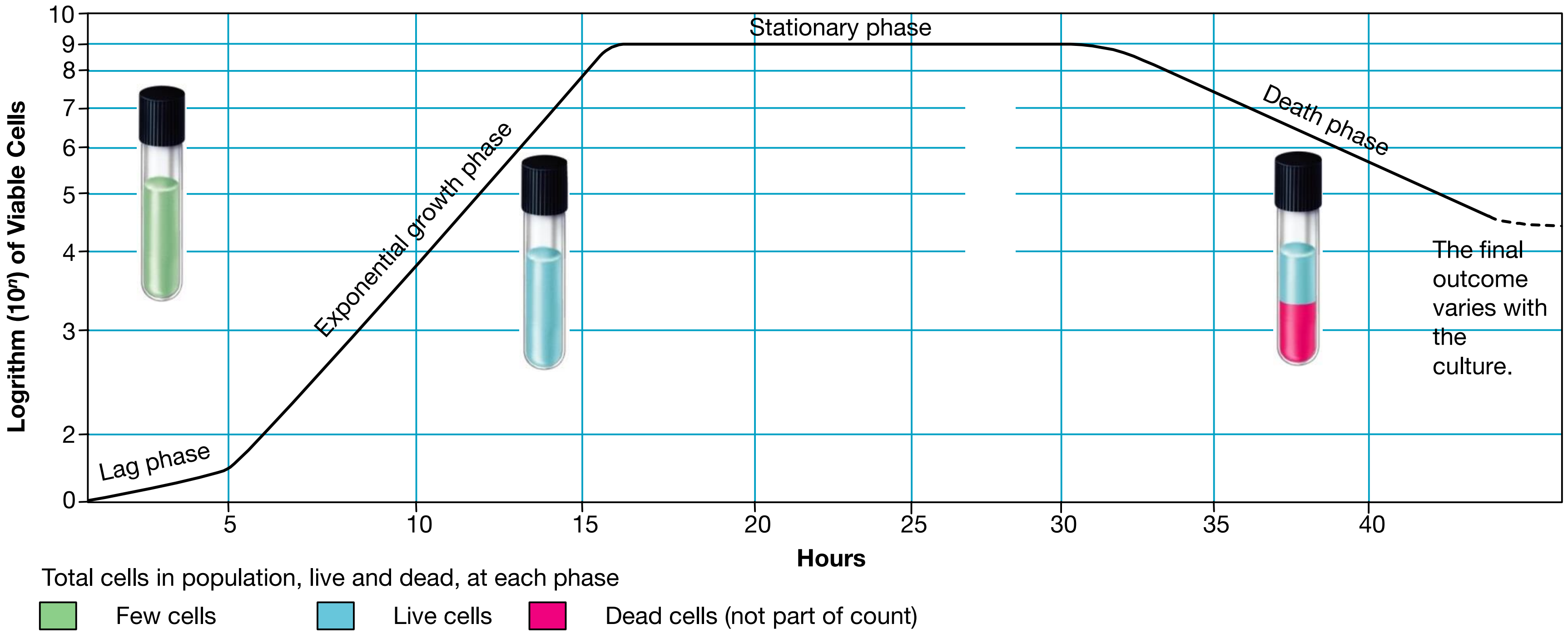
Colony

1 Million (2^{20})

The Population Growth Curve

1. Lag Phase

- When cells are introduced into fresh medium, **no immediate cell division** occurs.
- Cells are **metabolically active**—synthesizing: (New enzymes, ATP, cofactors, and ribosomes Components needed for growth and division).
- Causes for lag phase:
    - Cells may be old or depleted of nutrients.
    - Adjustment to a new environment (different medium).
    - Need for synthesis of new metabolic pathways.



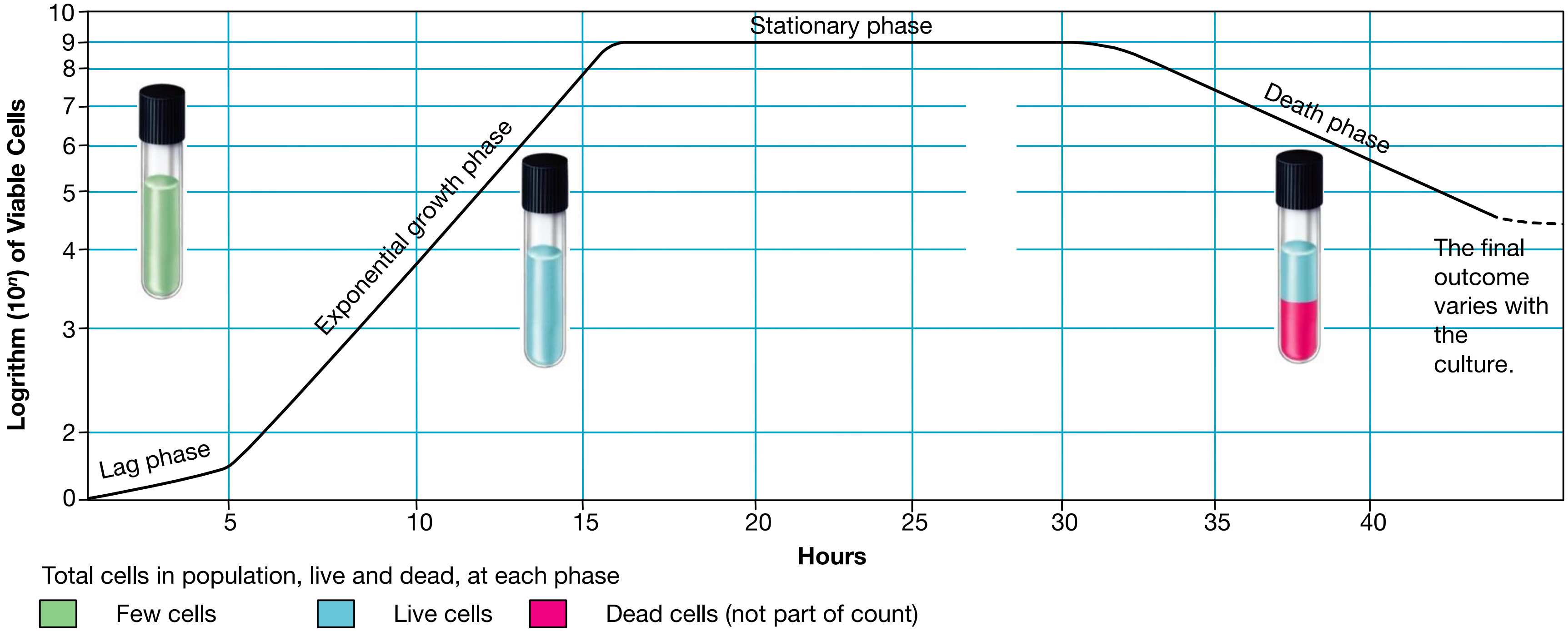
The Population Growth Curve

1. **Lag Phase**
2. **Exponential (Log) Phase**

- Cell **divide at their maximal rate**.
- Population **doubles at regular intervals** → produces a **smooth exponential growth curve**. Produce primary metabolites. Which are essential to microorganisms for proper growth.

•**Balanced Growth**:

- All cell components are synthesized at constant rates relative to each other.
- Occurs when environmental conditions are stable.



Examples of Unbalanced Growth

- **Shift-up**: transfer from nutrient-poor → nutrient-rich medium.
  - Cells first make new ribosomes → increase in protein and DNA synthesis.

- **Shift-down**: transfer from nutrient-rich → nutrient-poor medium.
  - Protein and RNA synthesis slow; cells reorganize metabolically.

- After adjustment, cells return to **balanced exponential growth**.

The Population Growth Curve

1. **Lag Phase**
2. **Exponential (Log) Phase**
3. **Stationary Phase**

- Growth rate = Death rate.

- Causes of stationary phase: (Nutrient limitation, Oxygen limitation, Accumulation of waste products).

- Cells remain **metabolically active**, producing compounds like acids or secondary metabolites, these products do not play a role in growth, development, and reproduction, and are formed during the end of the stationary phase.

- **Starvation responses** occur: (Reduced cell size and metabolic activity, **starvation proteins** (increase resistance), DNA protection proteins (e.g., **Dps**)
- Some bacteria (e.g., *Salmonella enterica*) become more virulent and resistant in this phase.

The Population Growth Curve

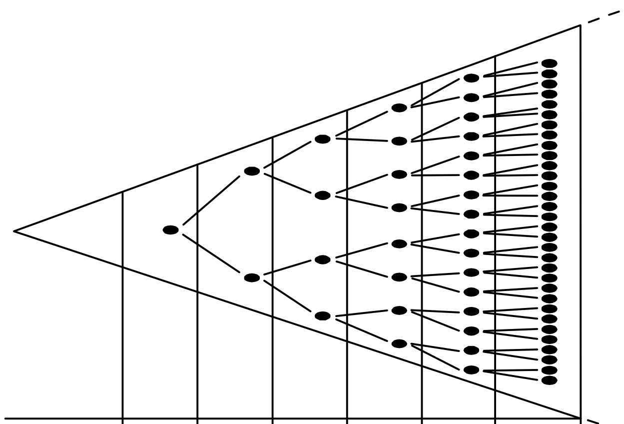
1. **Lag Phase**
2. **Exponential (Log) Phase**
3. **Stationary Phase**
4. **Death Phase**
   - Showing a decline in viable cell number.
   - Two main hypotheses:
     - ✓ **Programmed Cell Death (PCD):**
     - ✓ **Viable but Nonculturable (VBNC) State:**
     - Cells are **alive but cannot be cultured**.
     - Can “revive ” when conditions improve (e.g., temperature or host passage).

Phase of Prolonged Decline

- ✓ A **slow, long-term decline** where subpopulations adapt genetically.
- ✓ New **<u>mutants arise</u>** that are more efficient at using scarce nutrients and tolerating toxins.
- ✓ Leads to **genetic diversity** within the population.
- ✓ Demonstrates **natural selection** acting within a single culture.

Rate of Population Growth

- Time required for a complete fission cycle is called the **generation**, or **doubling** time
- Each new fission cycle increases the population by a factor of 2 – **exponential** growth
- Generation times vary from minutes to days



Number of cells	1	2	4	8	16	32
Number of generations		1	2	3	4	5
Exponential value		2^1 (2×1)	2^2 (2×2)	2^3 (2×2×2)	2^4 (2×2×2×2)	2^5 (2×2×2×2×2)

Rate of Population Growth

- Equation for calculating population size over time:

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

N_f is total number of cells in the population

N_i is starting number of cells

Exponent n denotes generation time

2^n number of cells in that generation

Concept Check:

Escherichia coli has a doubling time of 20 minutes. If there are 5 cells at the beginning of the experiment, how many will there be in 24 hours?

Generation time (doubling time)

What is the time need for growth of single *V. cholerae* on solid media to get a visible colony

13*20= 260

260/60min= 4.3 hours

Generation time (doubling time)

- What is the time need for growth of single (M.T) on solid media to get a visible colony

20*1/1 day = 20 day

or

30*1/1day = 30 day

Environmental Factors That Influence Microbes

- Environmental factors affect the function of metabolic enzymes
- Factors include:
  - Temperature
  - Oxygen requirements
  - pH
  - Osmotic pressure
  - Barometric pressure

Cardinal Temperatures

- **Minimum temperature** – lowest temperature that permits a microbe's growth and metabolism
- **Maximum temperature** – highest temperature that permits a microbe's growth and metabolism
- **Optimum temperature** – promotes the fastest rate of growth and metabolism.
- How to maintain T.M?

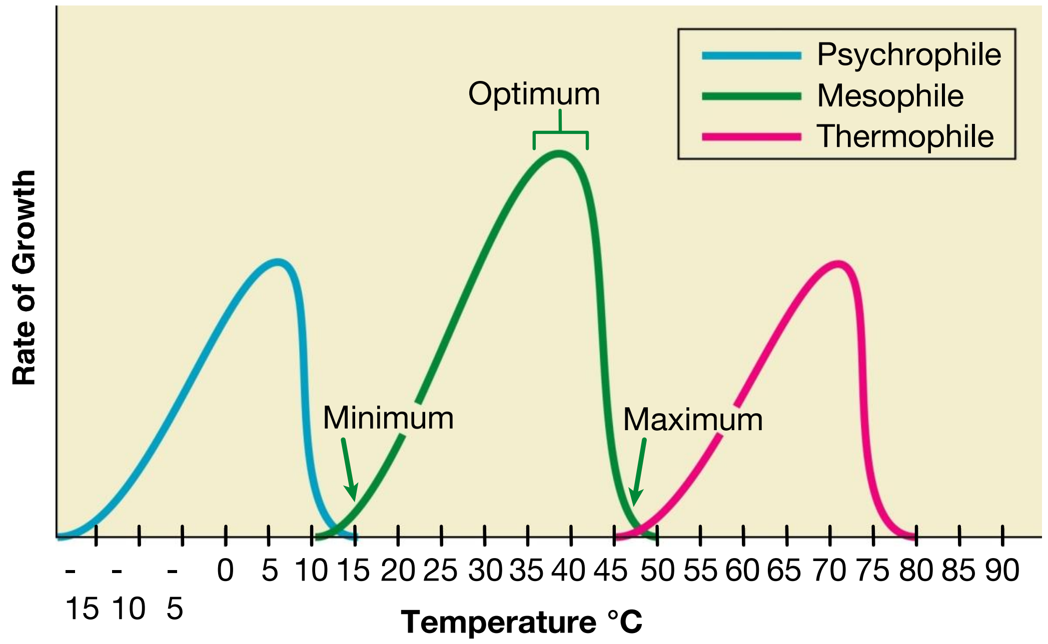
1. Maintain plasma membrane.
2. Controlling structural stability of proteins
3. Controlling Enzyme activities

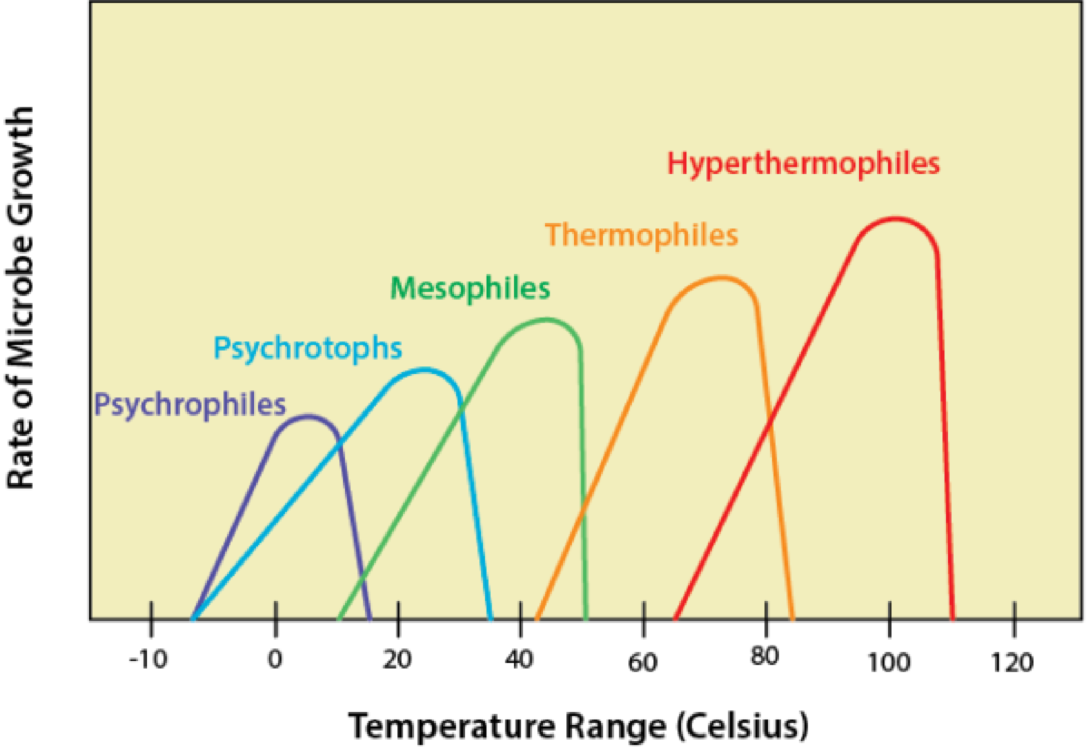
Temperature Adaptation Groups

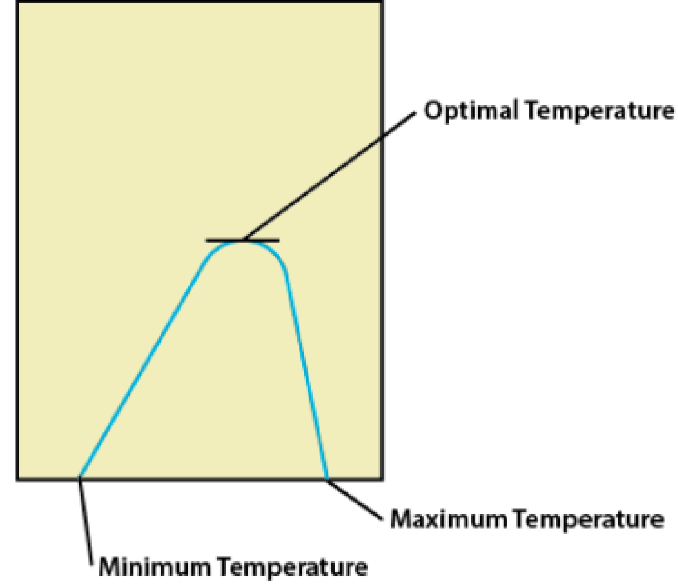
Psychrophiles – optimum growth temperature 0-20^{o}C

Mesophiles – optimum temperature 30^{o}-37^{o}C; most human pathogens

Thermophiles – optimum temperature greater than 45^{o}C (50-60)







Psychrophiles 0-20

Psychrotrophs 20-30 → Food spoilage

Mesophiles 25-45 → Disease causing

Thermophiles 45-70

Hyperthermophiles 70-110

Gas Requirements

Oxygen

- As oxygen is utilized it is transformed into several toxic products:
  - Singlet oxygen ($^1O_2$), superoxide ion (O_2^-), peroxide (H_2O_2), and hydroxyl radicals (OH^-)
- Most cells have developed enzymes that neutralize these chemicals:
  - Superoxide dismutase, catalase
- If a microbe is not capable of dealing with toxic oxygen, it is forced to live in oxygen free habitats

Categories of Oxygen Requirement

- **Aerobe** – utilizes oxygen and can detoxify it (*P. aeruginosa*)
- **Obligate aerobe** – cannot grow without oxygen.
- **Facultative anaerobe** – utilizes oxygen but can also grow in its absence
- **Microaerophilic** – requires only a small amount of oxygen (*Campylobacter jejuni and H. pylori*)
- **Anaerobe** – does not utilize oxygen
- **Obligate anaerobe** – lacks the enzymes to detoxify oxygen so cannot survive in an oxygen environment (*Bacteroides fragilis*)
- **Aerotolerant anaerobes** – do not utilize oxygen but can survive and grow in its presence (*Clostridium perfringens*). Its lack some Enzymes.

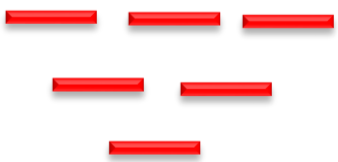
Respiration

- ❑ Glucose catabolism
- ❖ Energy production
- ✓ Aerobic respiration
  (O_2)
- ✓ Anaerobic respiration
  (No O_2)

1- Obligate aerobes (Aerobic respiration)

Presence of O_2

Only



Growth

Absence of O_2

No growth

e.g. Pseudomonas aeruginosa

1- Obligate aerobes (Aerobic respiration)

Aerobic respiration

↓

For production Energy (ATP)

↓

Glucose catabolism (glycolysis)

1- Obligate aerobes (Aerobic respiration)

Glucose +ADP → **2 pyruvate** **+ 2 ATP**

($2NAD^+$ → $2NADH$)

2 pyruvate —Kreb's Cycle→ **$2FADH_2$ +<u>8NADH</u>** **+ 2 ATP**

Oxidative phosphorylation

O_2 → **Carry $2H^+$** → **H_2O** **+ 34 ATP**

38 ATP

1- Obligate aerobes

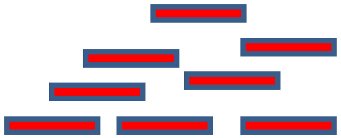
Highly toxic molecules

Superoxide ($O_2^{\cdot}$) — **Superoxide dismutase**

(H_2O_2) — **Catalase**

2- Obligate anaerobes

Presence of O2 | **Absence of O2**



Only

No growth | **Growth**

Bacteroides fragilis

2- Obligate anaerobes (Anaerobic respiration)

Glucose +ADP → 2 pyruvate **+ 2 ATP**

($2NAD^+$ → 2NADH)

2 pyruvate —Kreb's Cycle→ $2FADH_2$ +<u>8NADH</u> **+ 2 ATP**

4 ATP

✗ O_2 ➡ Other pathway

Lack Superoxide dismutase &Catalase

2- Obligate anaerobes (Anaerobic respiration)

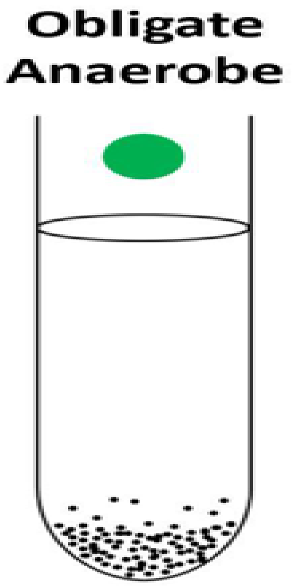
The organism used inorganic molecules

- Nitrate
- Sulfate
- Co2

} Carry H^+

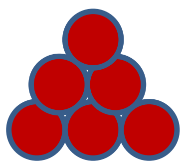
13 ATP + 4
17 ATP

Lack
Superoxide dismutase
Catalase



3- Facultative anaerobes

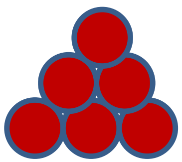
Presence of O_2



Growth

Rate of growth

Absence of O_2



Growth

Most bacteria

3- Facultative anaerobes

Anaerobes

Fermentation

Glucose → glycolysis → **2 Pyruvate + 2ATP**

No Kreb's cycle & other pathway

(Absence of carriers)

Acid & Alcohol

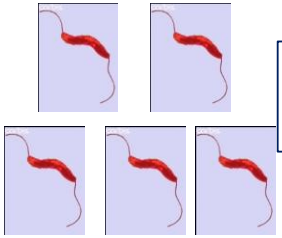
4- Micro-aerophilic

Presence of O_2

superoxide & H_2O_2

No growth

Low O_2



2-10% O_2

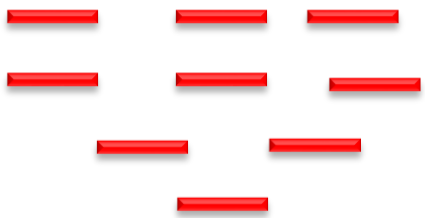
Growth

- *Campylobacter*
- *Helicobacter*

Low superoxide dismutase & catalase

5- Aero-tolerant anaerobes

Low O2

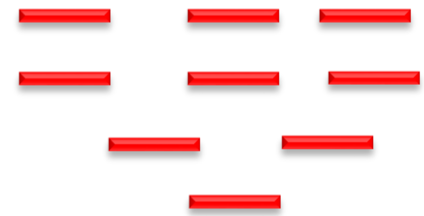


Superoxide dismutase

- $O_2^{\bullet-} \xrightarrow{SOD} \uparrow H_2O_2$

Tolerate

Absence of O2



Growth

- ***C.perfringens***

Carbon Dioxide Requirement

All microbes require some carbon dioxide in their metabolism

- **Capnophile** – grows best at higher CO_2 than normally present in the atmosphere

- Can use CO2 as their sole source of carbon.
- Cellular building blocks
- **Growth requirements:** human gut microbes

Effects of pH

- Majority of microorganisms grow at a pH between 6 and 8 (**neutrophiles**)
- **Acidophiles** – grow at extreme acid pH
- **Alkalinophiles** – grow at extreme alkaline pH

Osmotic Pressure

- Most microbes exist under hypotonic or isotonic conditions
- **Halophiles** – require a high concentration of salt
- **Osmotolerant** – do not require high concentration of solute but can tolerate it when it occurs

Other Environmental Factors

- **Barophiles** – can survive under extreme pressure and will rupture if exposed to normal atmospheric pressure

Concept Check:

Chlamydomonas nivalis grows on Alaskan glaciers and it's photosynthetic pigments give the snow a red crust. This organism would be best described as a

A. Psychrophile
B. Alkalinophile
C. Microaerophile
D. Osmotolerant
E. Barophile

Image courtesy Nozomu Takeuchi

(a)

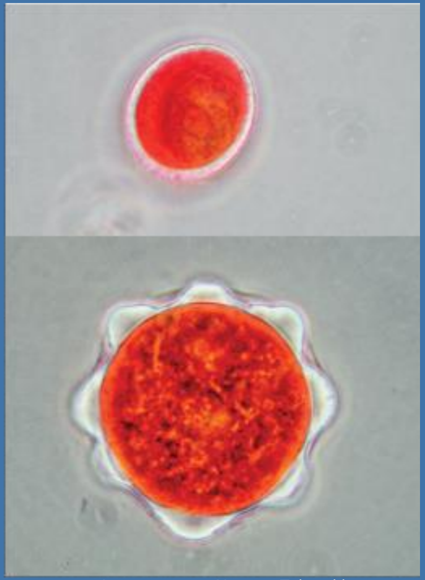


Image courtesy Nozomu Takeuchi

(b)

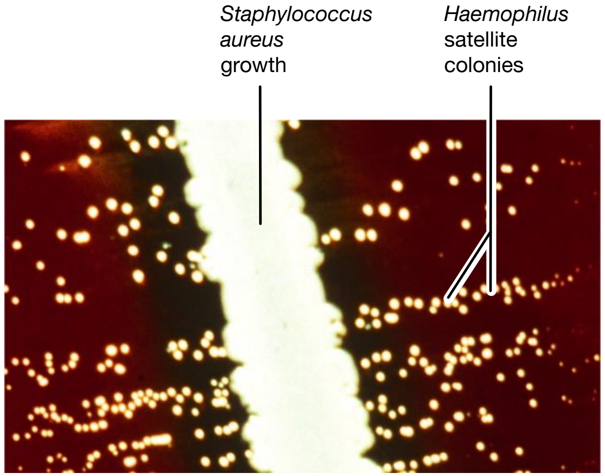
Ecological Associations Among Microorganisms

Microbial Associations

- **Symbiotic**
  Organisms live in close nutritional relationships; required by one or both members.
  - **Mutualism**
    Obligatory, dependent; both members benefit.
  - **Commensalism**
    The commensal benefits; other member not harmed.
  - **Parasitism**
    Parasite is dependent and benefits; host harmed.
- **Nonsymbiotic**
  Organisms are free-living; relationships not required for survival
  - **Synergism**
    Members cooperate and share nutrients.
  - **Antagonism**
    Some members are inhibited or destroyed by others.

Ecological Associations

- Symbiotic – two organisms live together in a close partnership
  - **Mutualism** – obligatory, dependent; both members benefit
  - **Commensalism** – commensal member benefits, other member neither harmed nor benefited
  - **Parasitism** – parasite is dependent and benefits; host is harmed



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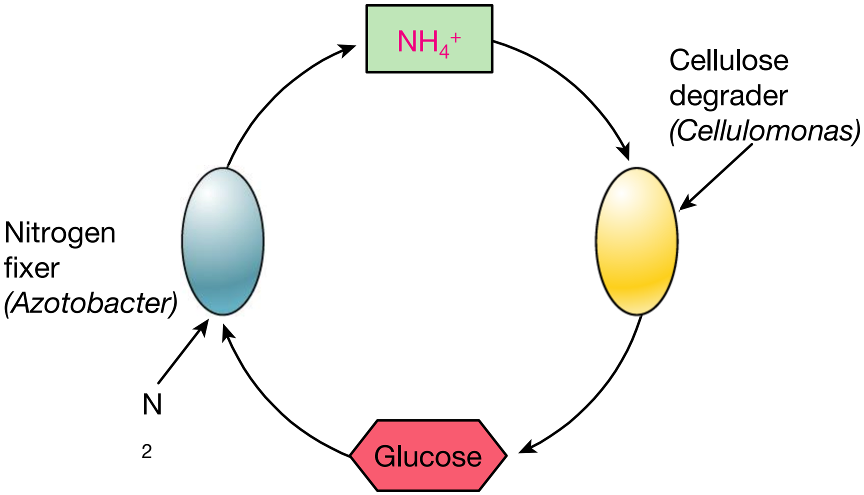
Courtesy Arthur Hauck (Germany)

Ecological Associations

- **Non-symbiotic** – organisms are free-living; relationships not required for survival
  - **Synergism** – members cooperate to produce a result that none of them could do alone
  - **Antagonism** – actions of one organism affect the success or survival of others in the same community (competition)
    - Antibiosis



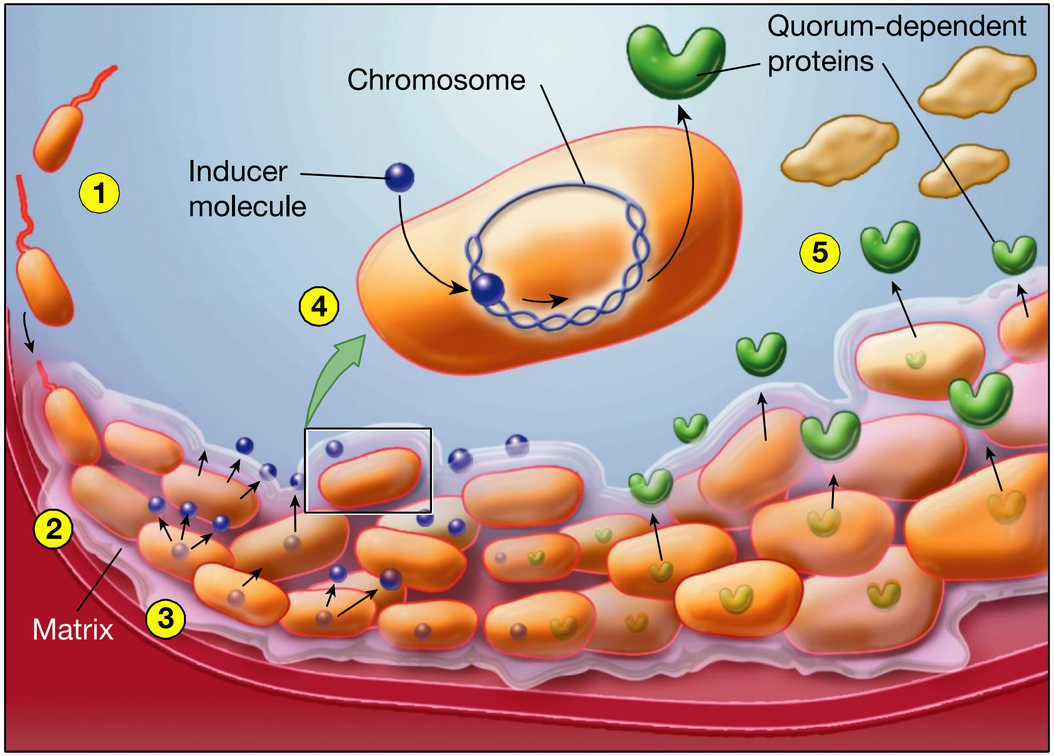
Interrelationships Between Microbes and Humans

- Human body is a rich habitat for symbiotic bacteria, fungi, and a few protozoa – called **normal microbial flora**
- Commensal, parasitic, and synergistic relationships – all exist or can exist on the human body

Microbial Biofilms

- **Biofilms** result when organisms attach to a substrate by some form of extracellular matrix that binds them together in complex organized layers
- Dominate the structure of most natural environments on earth
- Communicate and cooperate in the formation and function of biofilms – **quorum sensing**

Biofilm Formation and Quorum Sensing



1. Free-swimming cells settle on a surface and remain there.
2. Cells synthesize a sticky matrix that holds them tightly to the substrate.
3. When biofilm grows to a certain density (quorum), the cells release inducer molecules that can coordinate a response.
4. Enlargement of one cell to show genetic induction. Inducer molecule stimulates expression of a particular gene and synthesis of a protein product, such as an enzyme.
5. Cells secrete their enzymes in unison to digest food particles.