

# Chapter 7 - Nutrition and Growth

## Topics

- Microbial Nutrition

- **Microbial Growth**

- Environmental Factors

# Microbial Growth

- Cellular growth
  - Binary fission
- Population growth
  - Generation time
  - Growth curve
- Enumeration of bacteria

# Cell growth - binary fission

- The division of a bacterial cell
  - Parental cell enlarges
  - Parental cell duplicates its DNA
  - Septum formation divides the cell into two separate “chambers”
- Complete division results in two identical cells

# Representation of the steps in binary fission of a rod-shaped bacterium.

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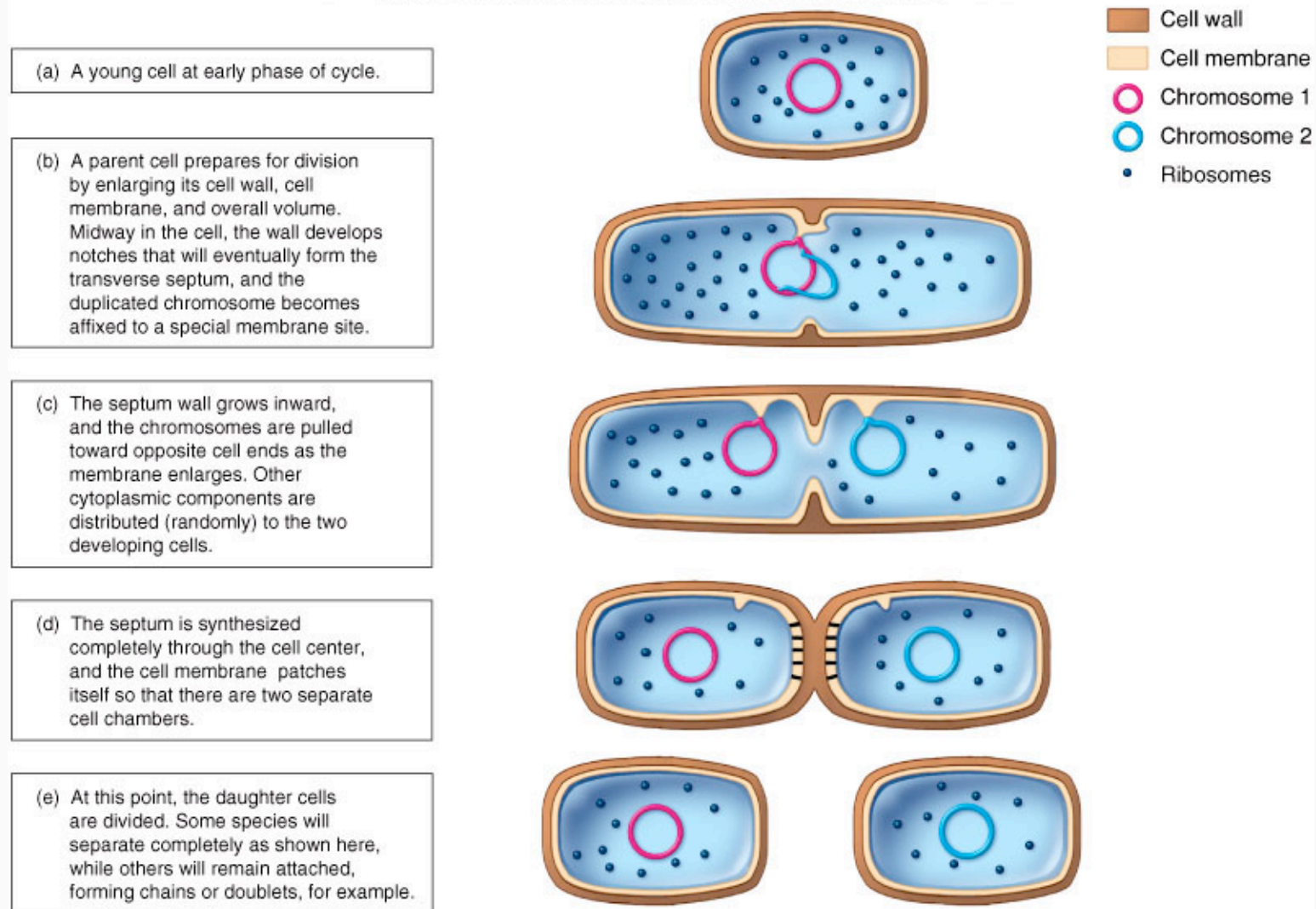
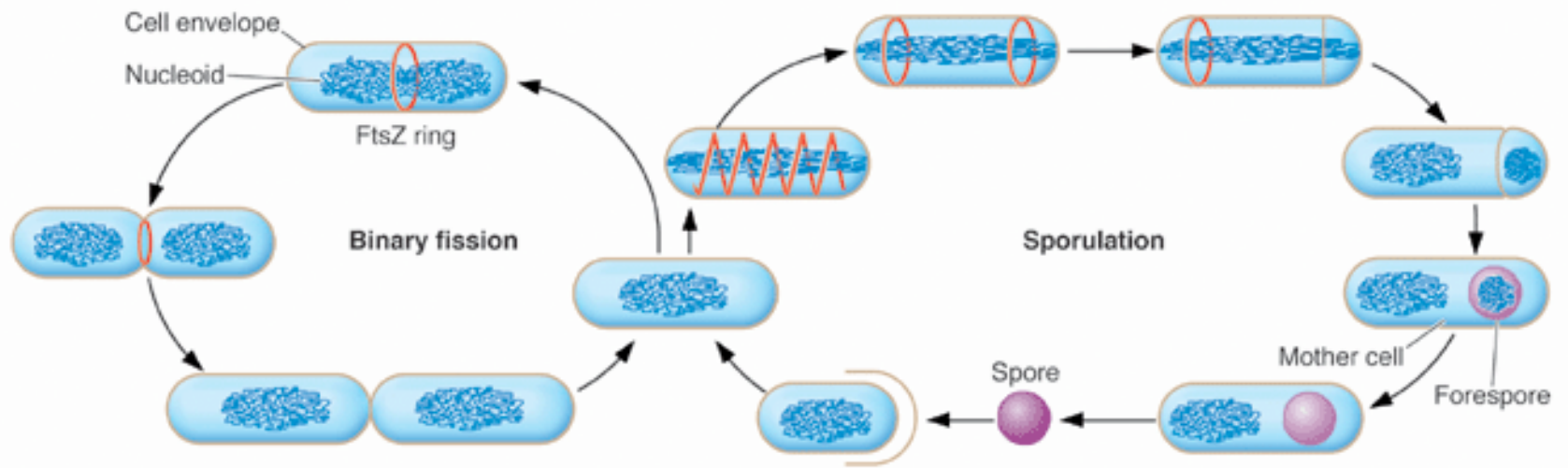
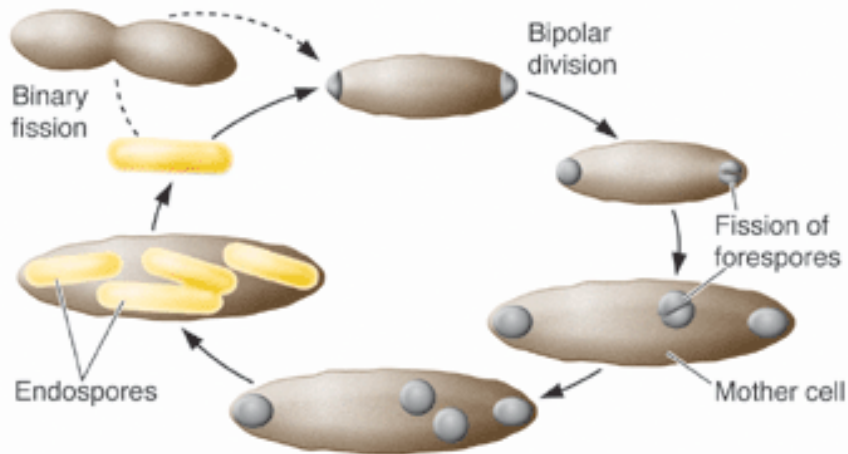


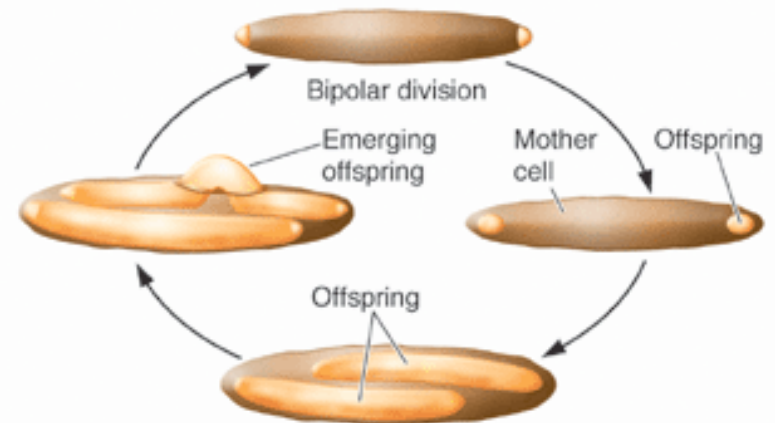
Fig. 7.13 Steps in binary fission of a rod-shaped bacterium.



**A** *Metabacterium polyspora*  
Limited binary fission; forms multiple endospores



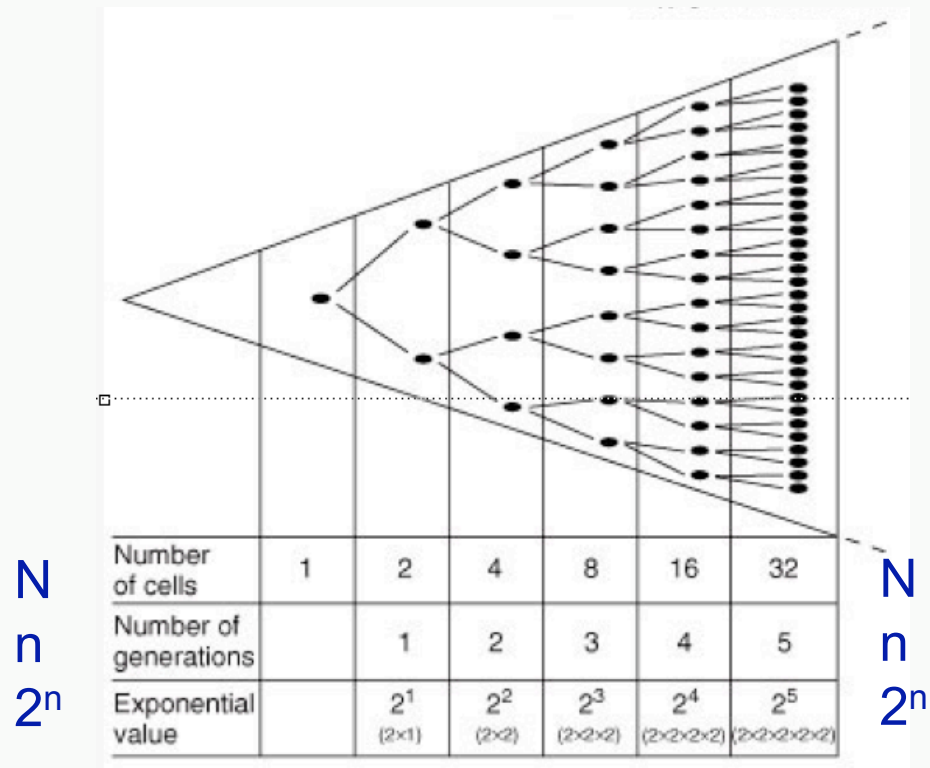
**B** *Epulopiscium* sp. type B  
No binary fission; shared mechanisms with sporulation



# Generation time

- The time required for a complete division cycle (doubling)
- Length of the generation time is a measure of the growth **rate**
- Exponentials are used to define the numbers of bacteria after growth

Representation of how a single bacterium doubles after a complete division, and how this can be plotted using exponentials.



$$N = N_0 \times 2^n \quad \text{Cell \#} = \text{initial cell \#} \times \text{product (generation \#)}$$

Fig. 7.14 The mathematics of population growth

# The Rate of Population Growth

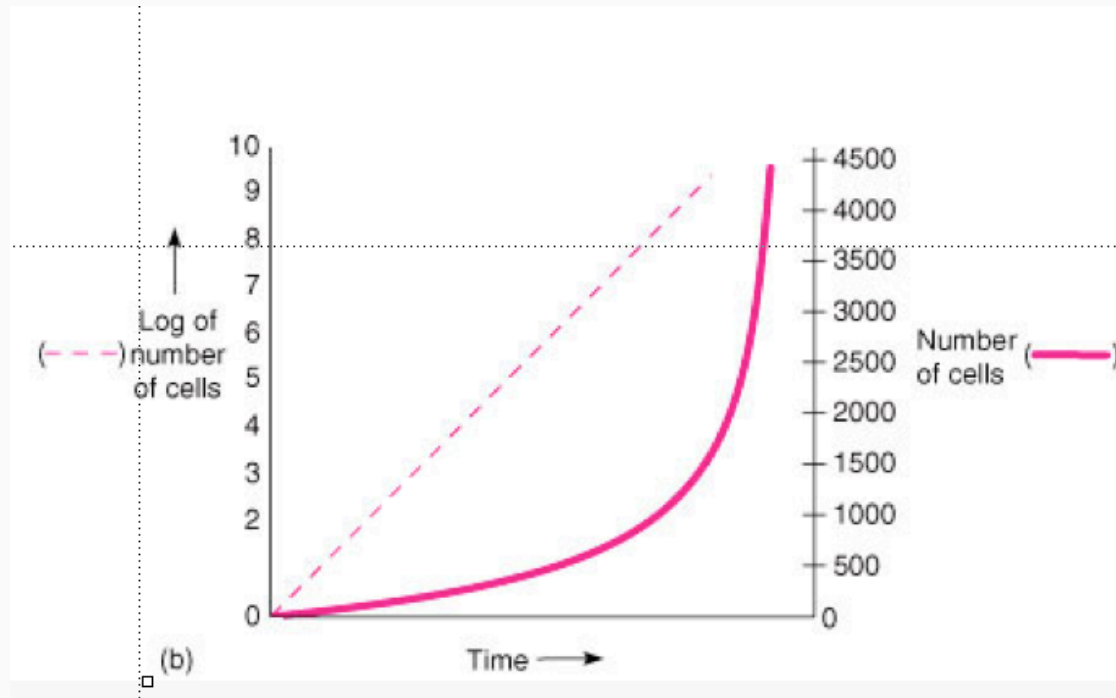
- **Generation or doubling time:** The time required for a complete fission cycle
- Each new fission cycle or generation increases the population by a factor of 2 (1, 2, 4, 8, 16, 32, 64, etc)
- As long as the environment is favorable, the doubling effect continues **at increasing rate (number/time)**
- The length of the generation time - a measure of the growth rate of an organism
  - Average generation time - 30 to 60 minutes under optimum conditions
  - Can be as short as 10 to 12 minutes
- This growth pattern is termed **exponential**



# Graphing Bacterial Growth

- The data from growing bacterial populations are graphed by plotting the number of cells as a function of time
  - If plotted logarithmically - a straight line
  - If plotted arithmetically - a constantly curved slope
- To calculate the size of a population over time:  
$$N_f = (N_i) \times 2^n$$
  - $N_f$  is the total number of cells in the population at some point in the growth phase
  - $N_i$  is the starting number
  - $n$  denotes the generation number

Representation of how a single bacterium doubles after a complete division, and how this can be plotted using exponentials.



$$N = N_0 \times 2^n \quad \text{Cell \#} = \text{initial cell \#} \times \text{product (generation \#)}$$

$$\log [N] = n \times \log [2] + \log [N_0] ; \text{ assuming } \mathbf{\text{constant generation time}}$$

Fig. 7.14 The mathematics of population growth

# The Population Growth Curve

- However, a population of bacteria does not maintain its potential growth rate and doubles endlessly ...
- Rate slows as generation time gets longer, because of
  - reduced resources
  - increased density (number/volume)
  - increased waste

$$K = (K_{\max} [S] / (K_s + [S]))$$

- A population displays a predictable growth pattern called **“growth curve”**, where the cell # is a function of time

$$\text{----> } N = N_0 \times e^{[k \times t]}$$

# The Population Growth Curve

- The plating method to observe the population growth pattern:
  - Place a tiny number of cells in a sterile liquid medium
  - Incubate this culture over a period of several hours
  - Sampling the broth at regular intervals during incubation
  - Plating each sample onto solid media
  - Counting the number of colonies present after incubation

# Population **Growth curve**

Data from an entire growth period typically produce a curve with a series of phases

- Lag phase
- Log phase
- Stationary phase
- Death phase

# The four main phases of growth in a bacterial culture.

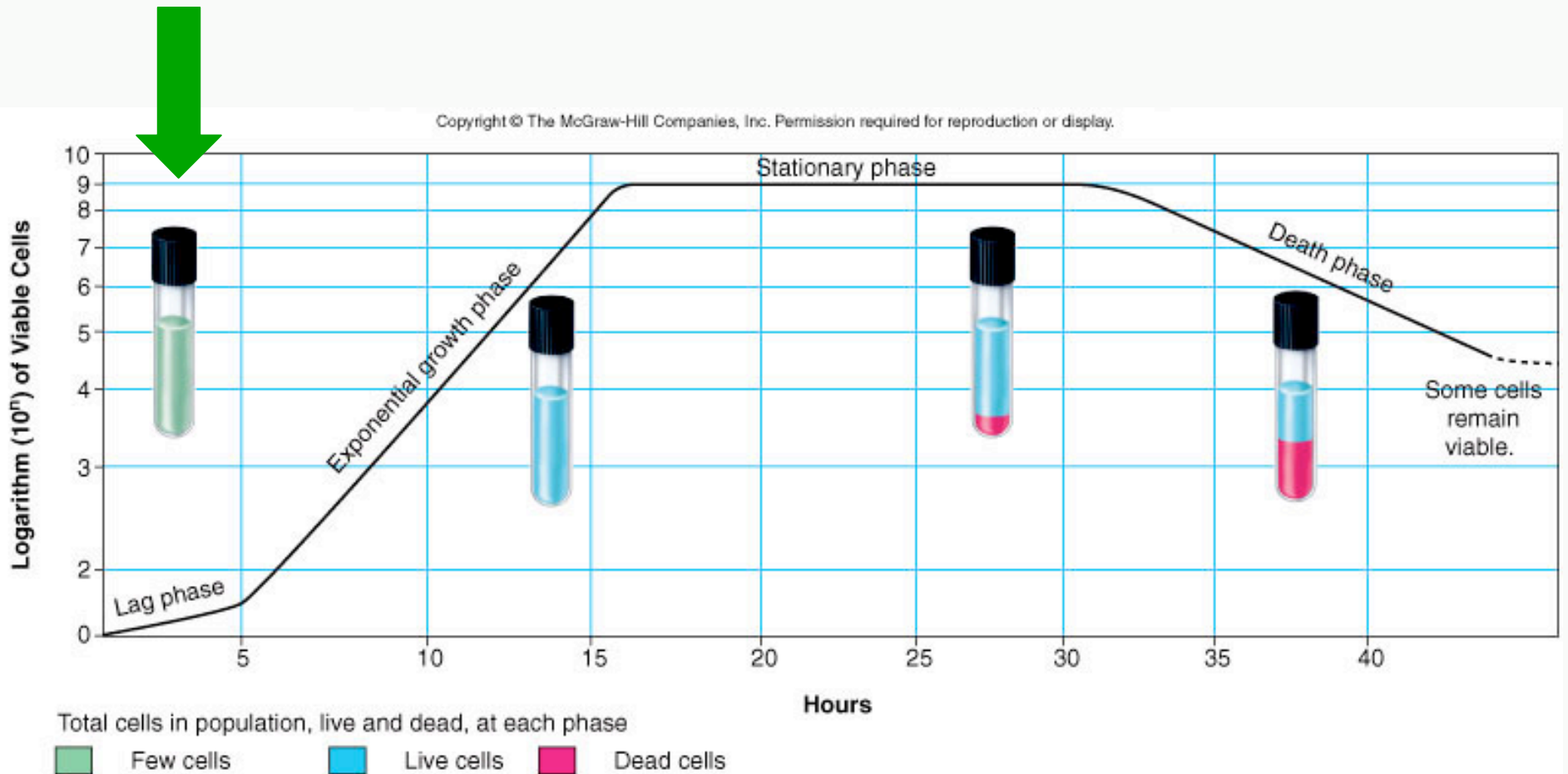


Fig. 7.15 The growth curve in a bacterial culture.

# Lag phase

- Cells are adjusting, enlarging, and synthesizing critical proteins and metabolites
- Not doubling at their maximum growth rate

# The four main phases of growth in a bacterial culture.

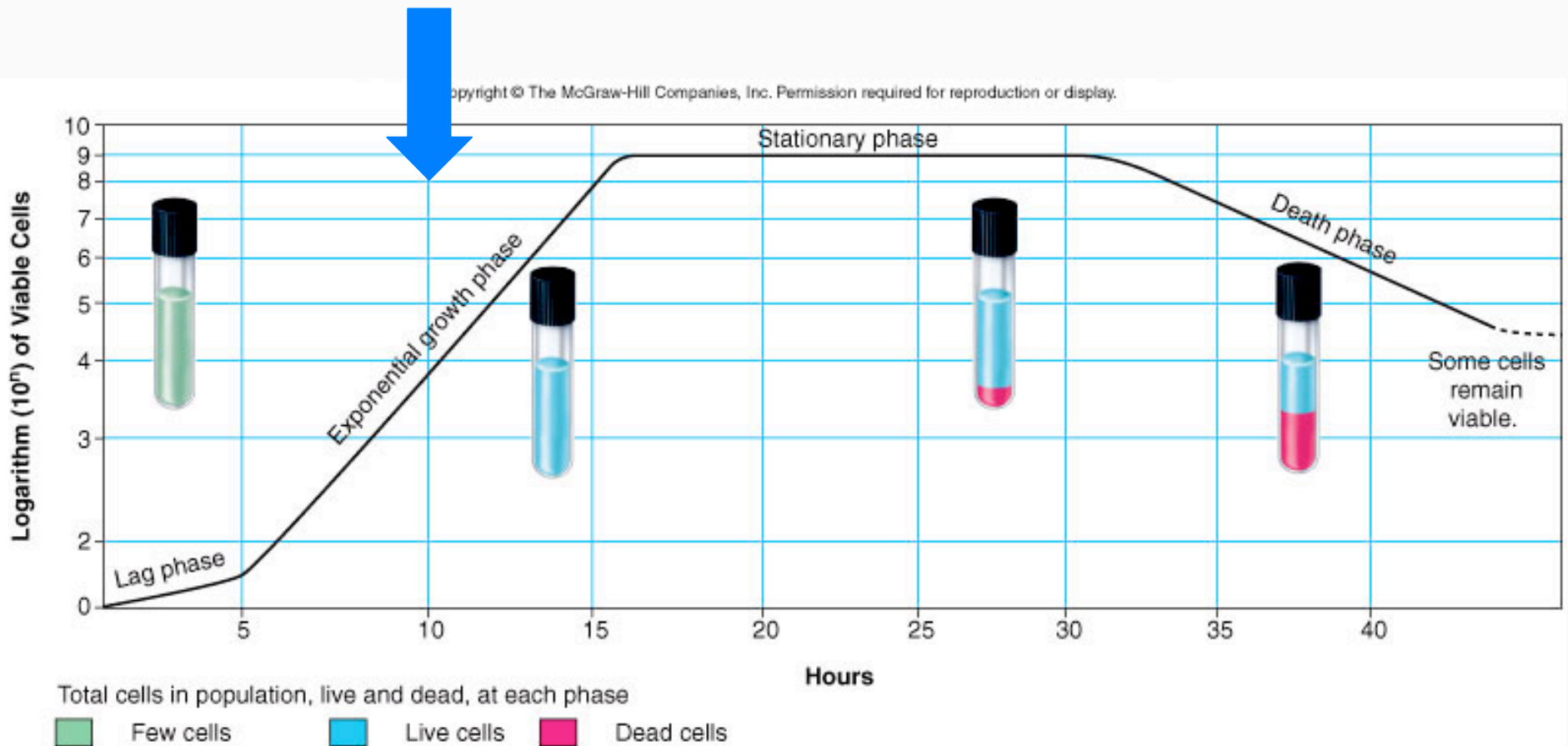


Fig. 7.15 The growth curve in a bacterial culture.



# Exponential (Log) phase

- Maximum growth rate of cell division
- Adequate nutrients
- Favorable environment

==> Proportional growth

# The four main phases of growth in a bacterial culture.

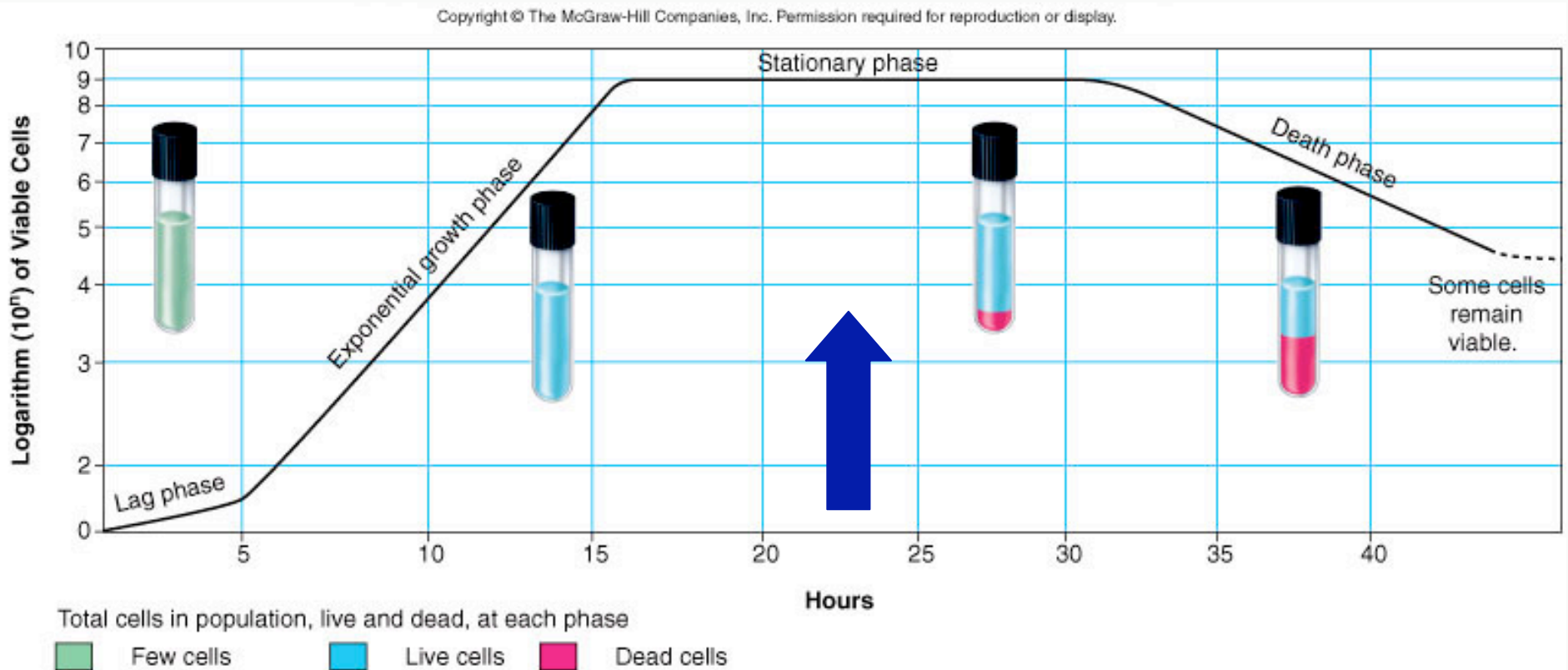


Fig. 7.15 The growth curve in a bacterial culture.

# Stationary phase

- Survival mode – depletion in nutrients, released waste can inhibit growth
- When the number of cells that stop dividing equal the number of cells that continue to divide

# The four main phases of growth in a bacterial culture.

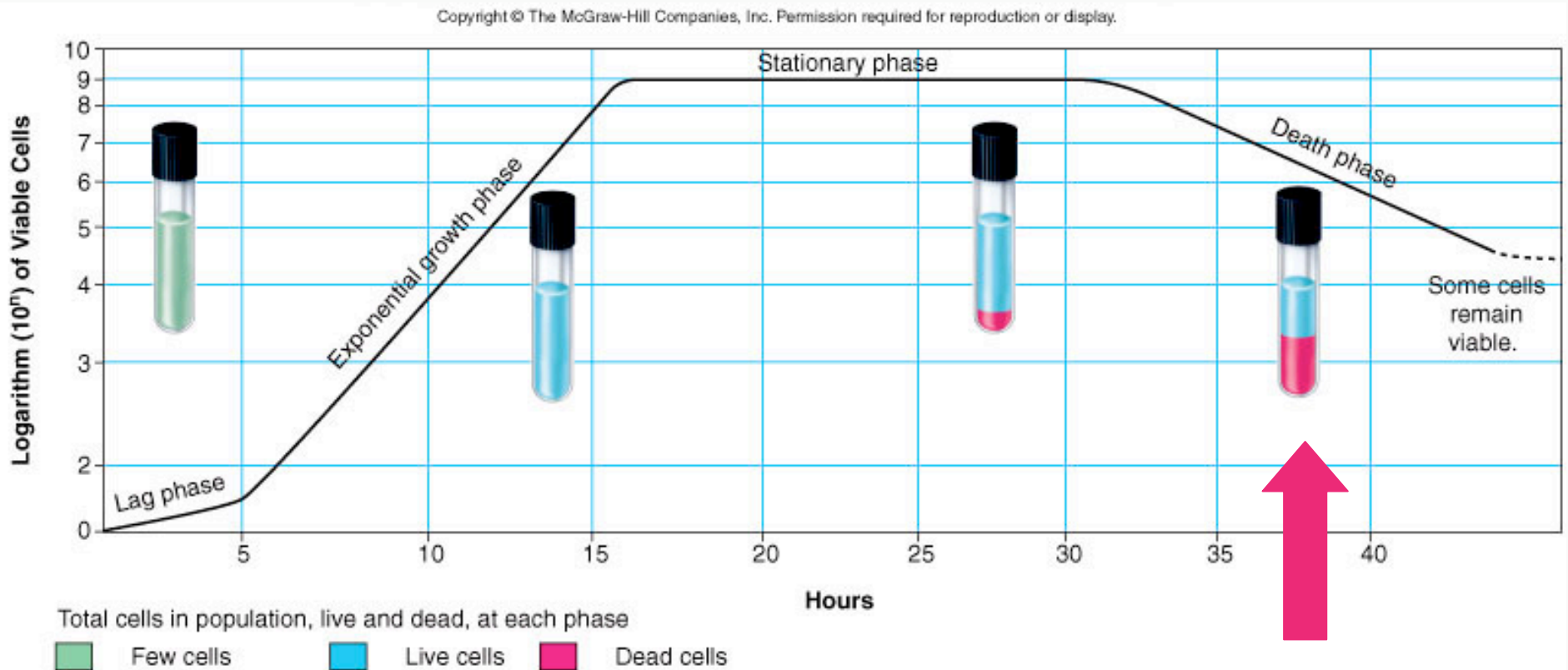


Fig. 7.15 The growth curve in a bacterial culture.

# Death phase

- A majority of cells begin to die exponentially due to lack of nutrients, effect of waste and lack of space

# Continuous culture

- A **chemostat** allows to “set” the growth rate at a particular level because it will :
  - provide a continuous supply of nutrients (influx)
  - removes a certain number of cells (efflux)
- can avoid that the culture reaches certain phase (i.e., stationary or death phases).

➔ Trophophase and Idiophase

# Enumeration of bacteria

- Turbidity
- Direct cell count
- Automated devices
  - Coulter counter
  - Flow cytometer
  - Real-time PCR

The greater the turbidity, the larger the population size.

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(a)



(b)

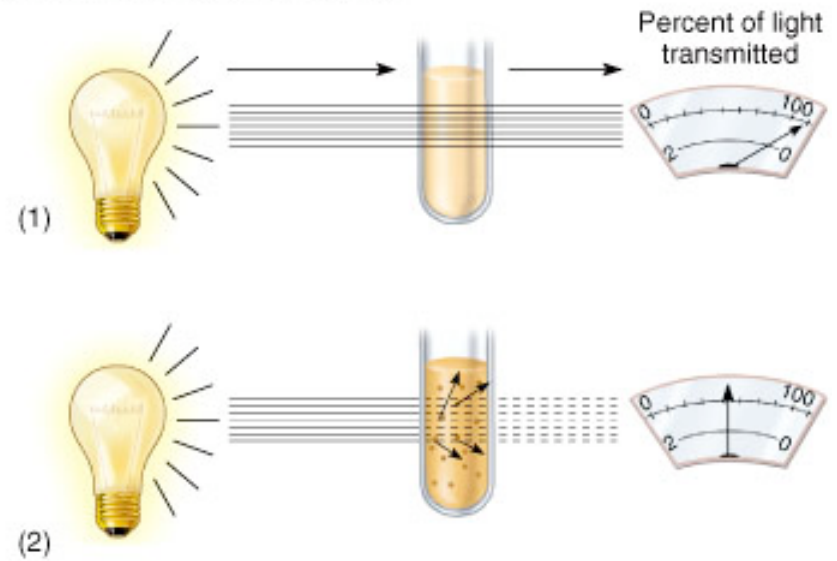


Fig. 7.16 Turbidity measurements as indicators of growth



The **direct cell counting method** counts the total dead and live cells in a special microscopic slide containing a premeasured grid (Petroff-Hauser cell).

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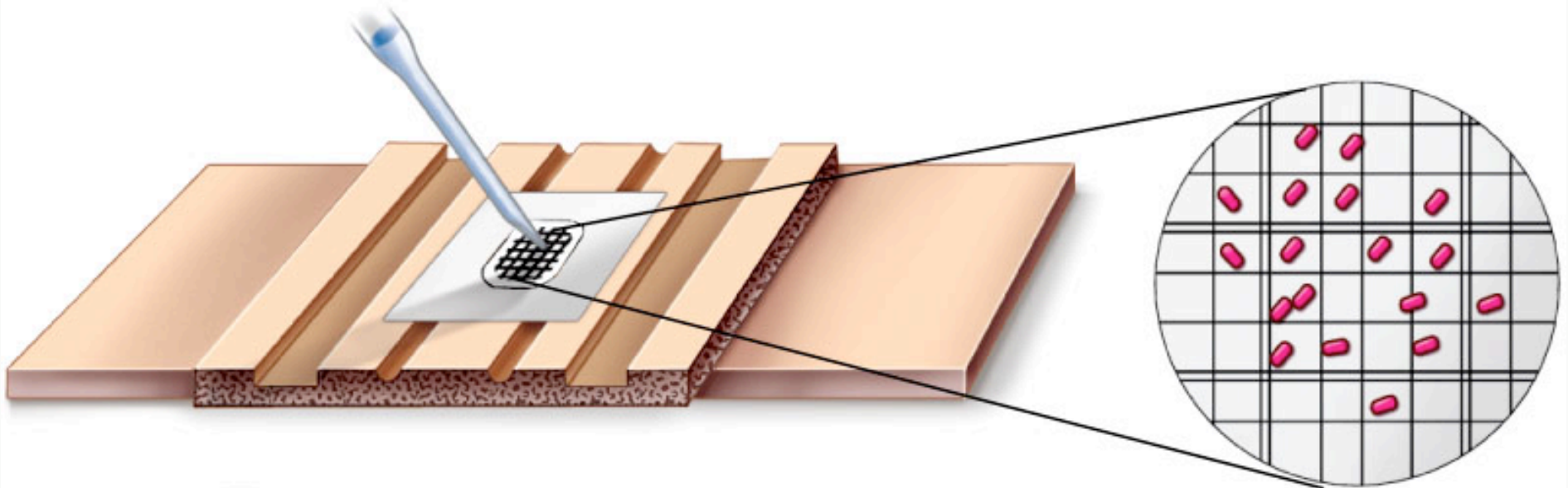


Fig. 7.17 Direct microscopic count of bacteria.

# Automated Counting

- Coulter counter- electronically scans a culture as it passes through a tiny pipette
- Flow cytometer using a LASER beam measures cell size and differentiates between live and dead cells
- Real-time Polymerase Chain Reaction allows to quantify bacteria and other microorganisms that are present in environmental or tissue samples without isolating or culturing them

A Coulter counter uses an electronic sensor to detect and count the number of cells.

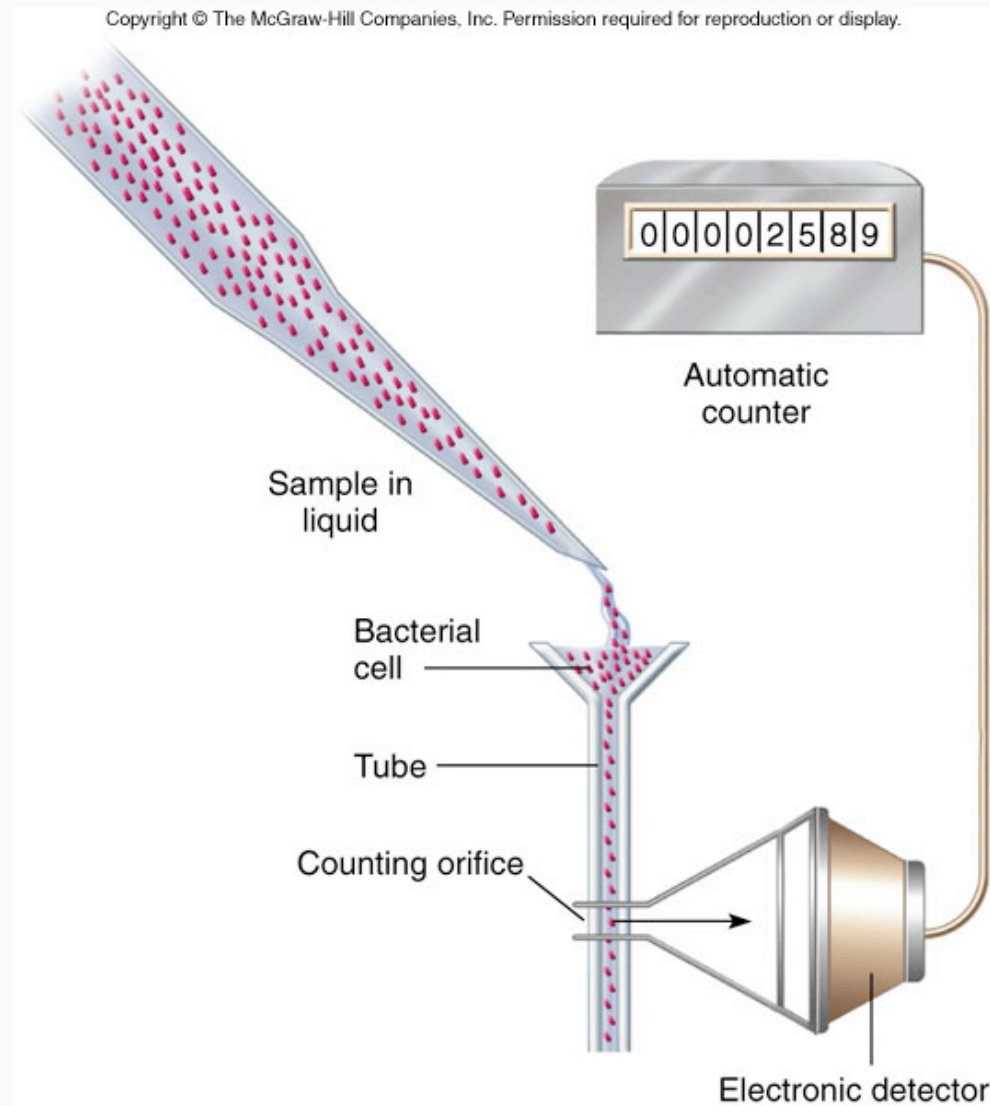


Fig. 7.18 Coulter counter

# Environmental Factors

- Temperature
- Gas (oxygen and CO<sub>2</sub>)
- pH
- Osmotic pressure
- Other factors
- Microbial association

# Temperature

- For optimal growth and metabolism
- Psychrophile - 0 to 15 °C
- Mesophile - 20 to 40 °C
- Thermophile - 45 to 80 °C
- Hyperthermophile >80 °C

==>Pasteurization (72 °C, 15 seconds)

Growth of different ecological groups based on ideal temperatures.

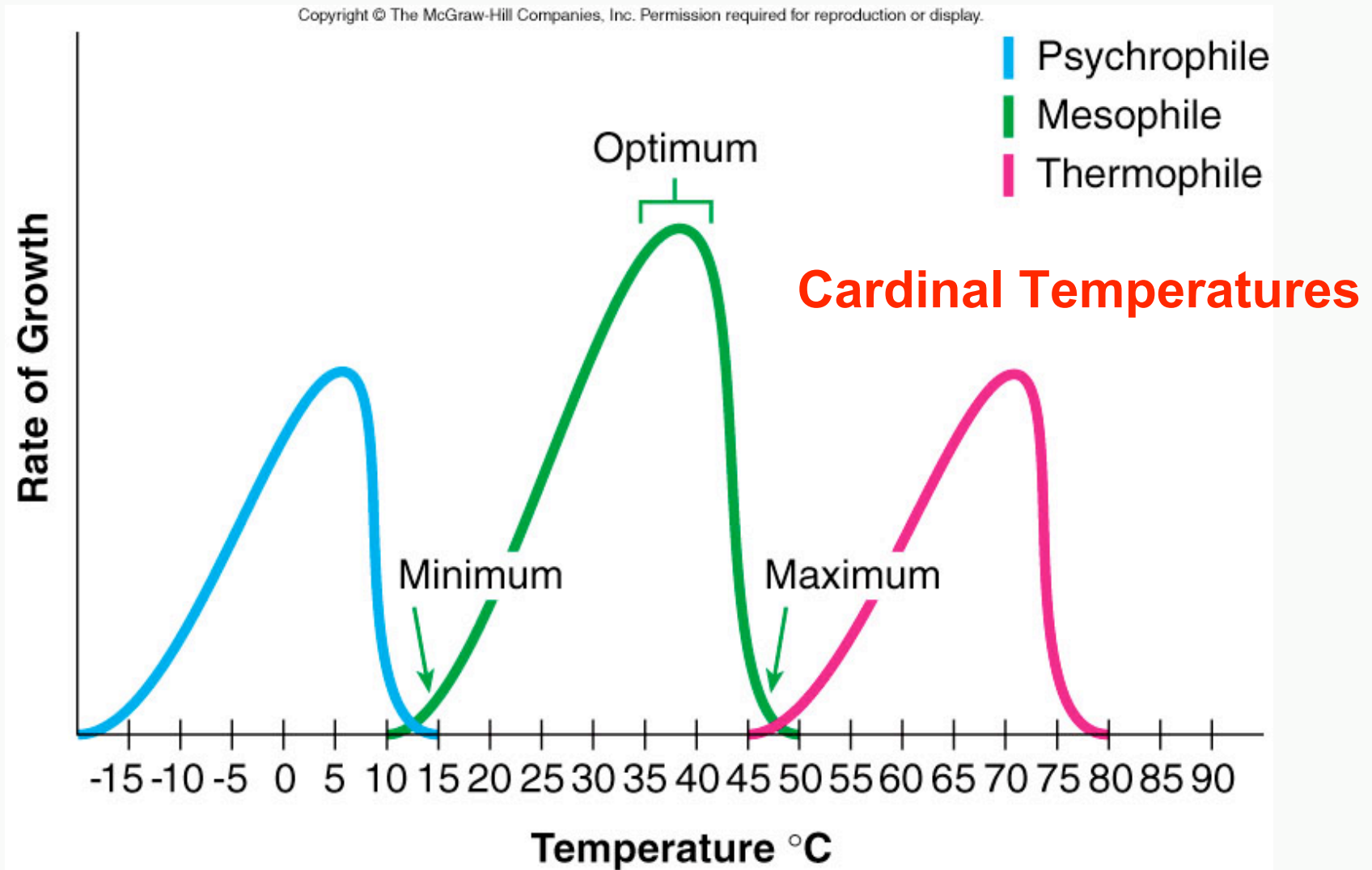


Fig. 7.8 Ecological groups organized by temperature

# Psychrophile

- A microorganism that has an optimum temperature below 15°C and is capable of growth at 0°C.
- True psychrophiles are obligate with respect to cold and cannot grow above 20°C.
- Psychrotrophs or facultative psychrophiles- grow slowly in cold but have an optimum temperature above 20°C.

# Mesophile

- An organism that grows at intermediate temperatures
- **Optimum growth temperature of most:**  
20°C to 40°C
- Temperate, subtropical, and tropical regions
- Most pathogens of mammals have growth optima between 30°C and 40°C



# Thermophile

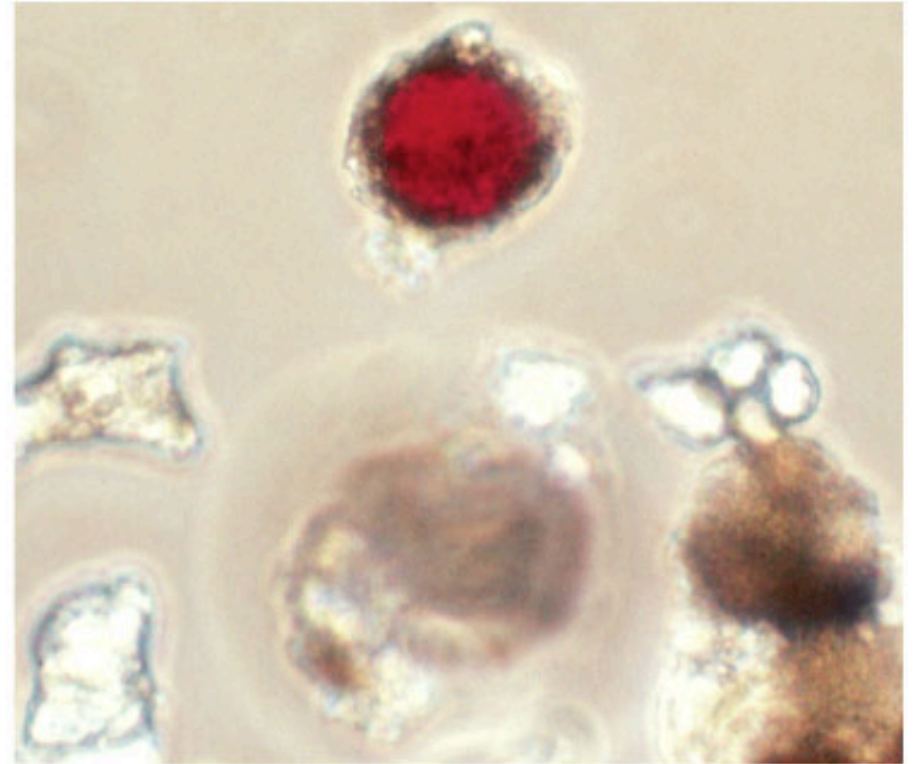
- A microbe that grows optimally at temperatures greater than 45°C
- Vary in heat requirements
- General range of growth of 45°C to 80°C
- Hyperthermophiles grow between 80°C and 120°C

Example of a psychrophilic photosynthetic Red snow organism.

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(a)



(b)

Fig. 7.9 Red snow

# Gas

- Two gases that most affect microbial growth
  - Oxygen
    - Oxidizing agent
    - Respiration
  - Carbon dioxide (carbon source)

# Oxidizing agent (electron acceptor)

- Oxygen metabolites are toxic
- These toxic metabolites must be neutralized for growth
- Categories of bacteria
  - Obligate aerobe
  - Microaerophiles
  - Facultative anaerobe
  - Obligate (strict) anaerobe

# Obligate aerobe

- Requires oxygen gas for metabolism
- Possesses enzymes that can neutralize the toxic (active) oxygen metabolites
  - Superoxide dismutase and catalase
- Effectively sequesters Iron
- Ex. Most fungi, protozoa, and bacteria

# Facultative anaerobe

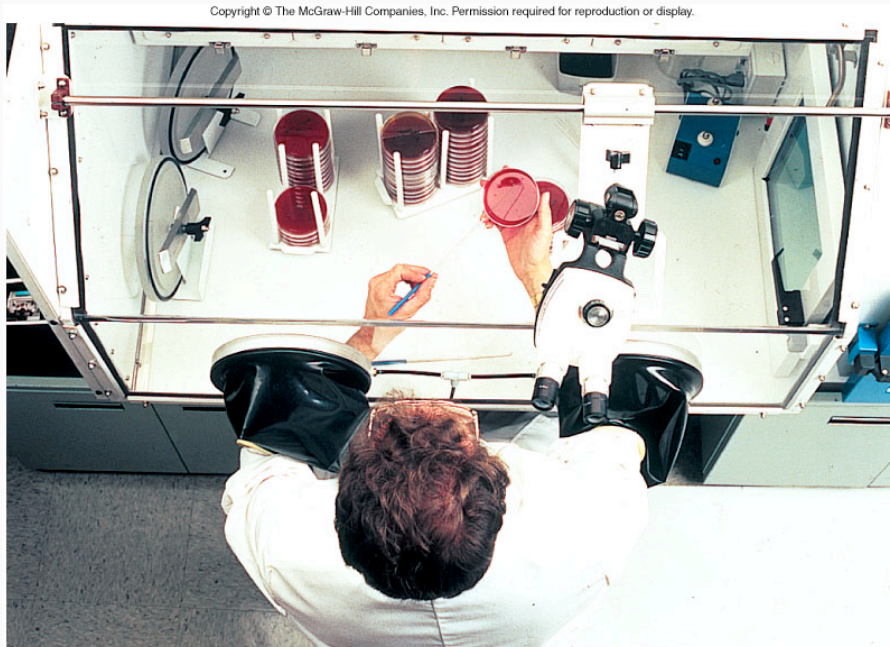
- Does not require oxygen for metabolism, but grows better in its presence
- Usually possess superoxide dismutase and catalase
- In the absence of oxygen, cells utilize other oxidants to dispose electrons (anaerobic respiration or fermentation occurs)
- Ex. Gram-negative pathogens

# Obligate (strict) anaerobes

- Does not use oxygen for metabolism
- Do not possess superoxide dismutase and catalase

==> The presence of oxygen is toxic to the cell ( $\text{H}_2\text{O}_2$ ,  $\text{O}_2^-$ ,  $\bullet\text{OH}$ )

Anaerobes must grow in an oxygen minus environment, because toxic oxygen metabolites cannot be neutralized.



(a)

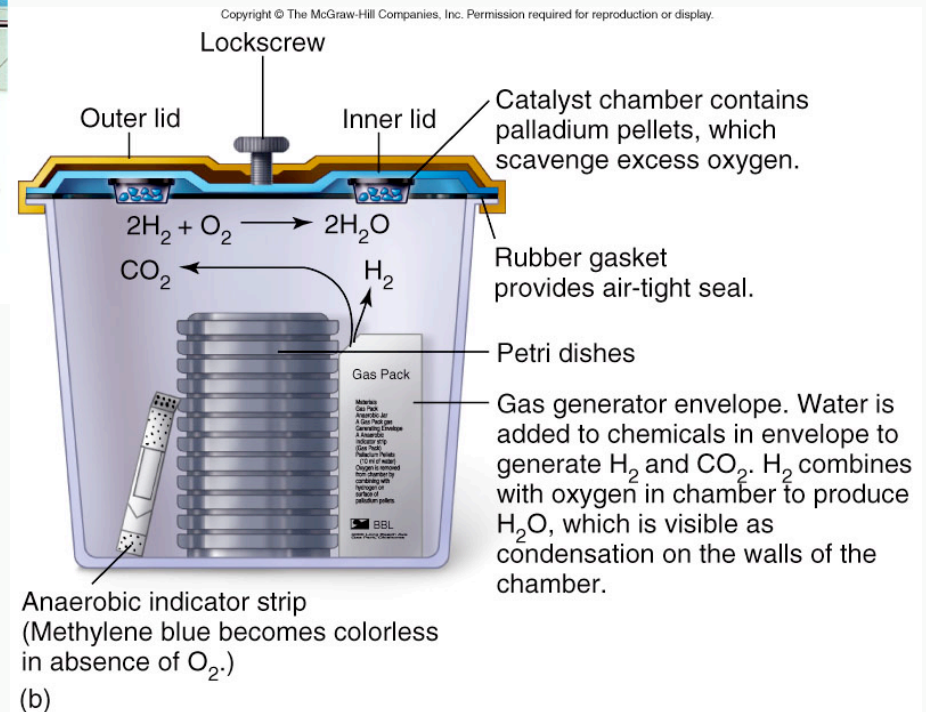


Fig. 7.10 Culturing technique for anaerobes



Thioglycollate broth enables the identification of aerobes(A), microaerophiles (B), facultative anaerobes(C), and obligate anaerobes (D).

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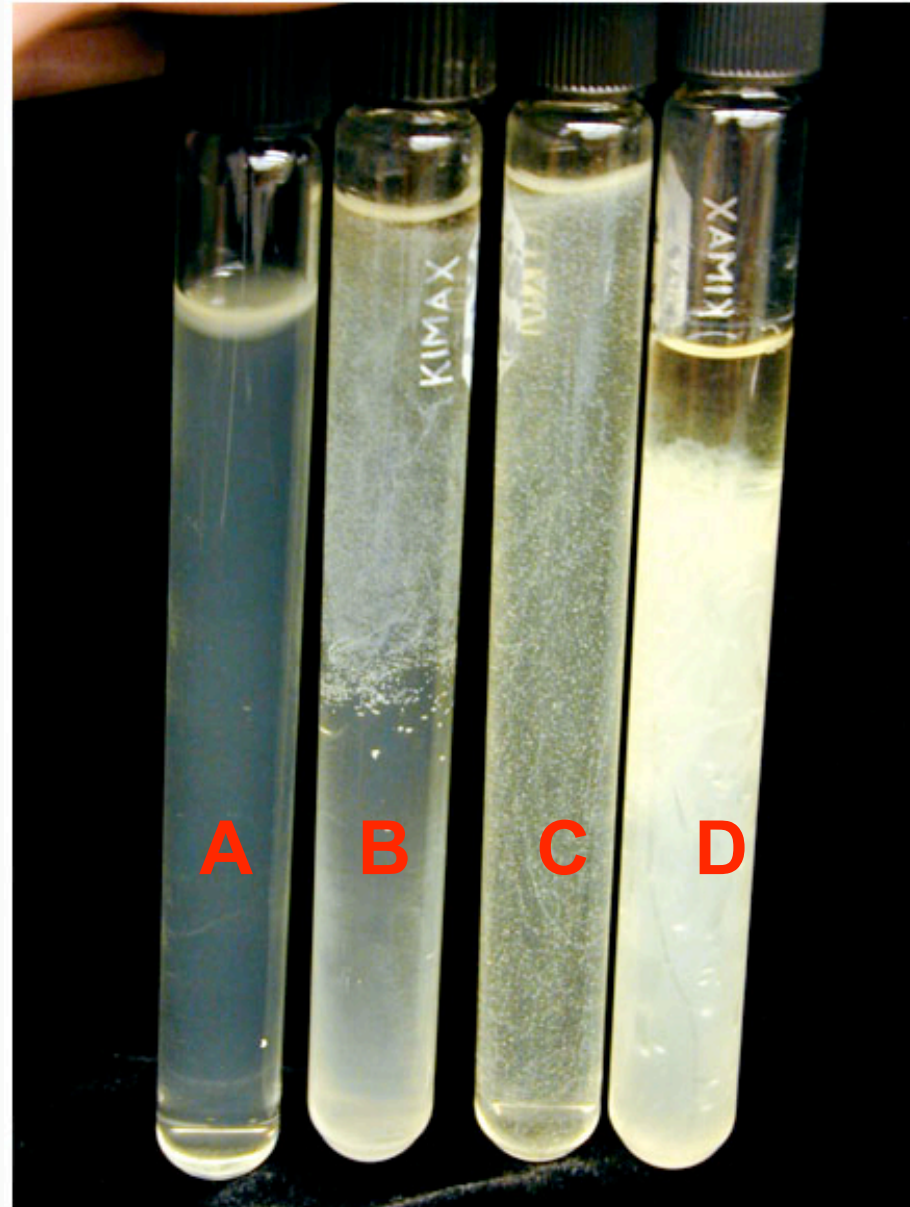


Fig. 7.11 Use of thioglycollate broth to demonstrate oxygen requirements.

# Carbon Dioxide

- All microbes require some carbon dioxide in their metabolism, some need it as their sole source of carbon
- Capnophiles grow best at a higher CO<sub>2</sub> tension than is normally present in the atmosphere and they REQUIRE CO<sub>2</sub>.
- Capnotrophs tolerate higher CO<sub>2</sub> concentrations.

# pH

- Cells grow best in the “temperate range” between pH 6-8
- Exceptions would be acidophiles (pH 0 -4), and alkali[no]philes (pH 9 -11).
- WHY is this important?????

# Water availability- Osmotic pressure

- Osmophiles (sugars, salts, etc.)
- Halophiles (salt)
  - Requires high salt/sugar concentrations
  - Osmophiles/halophiles withstand hypertonic conditions
    - Ex. *Halobacterium*
- Facultative halophiles
  - Can survive high salt conditions but salt is not required
    - Ex. *Staphylococcus aureus*
- Opposite is hypotonic conditions (maybe starvation)

# Other factors

- Hydrostatic Pressure - Barophiles withstand high pressures
- Radiation - pigmented or fortified walls withstand high energy (UV), heat (infrared)

====> Spores and cysts can survive dry habitats

# Ecological association

- Influence microorganisms have on other microbes
  - Symbiotic relationship
  - Non-symbiotic relationship

# Symbiotic

- Organisms that live in close nutritional relationship (syntrophy)
- Types
  - Mutualism – both organism benefit
  - Commensalism – one organisms benefits without disadvantage to the other
  - Parasitism (host/microbe relationship) - one organism benefits on the expense of the other

An example of commensalism, where *Staphylococcus aureus* provides vitamins and amino acids to *Haemophilus influenzae*.

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*Staphylococcus aureus* growth

*Haemophilus* satellite colonies

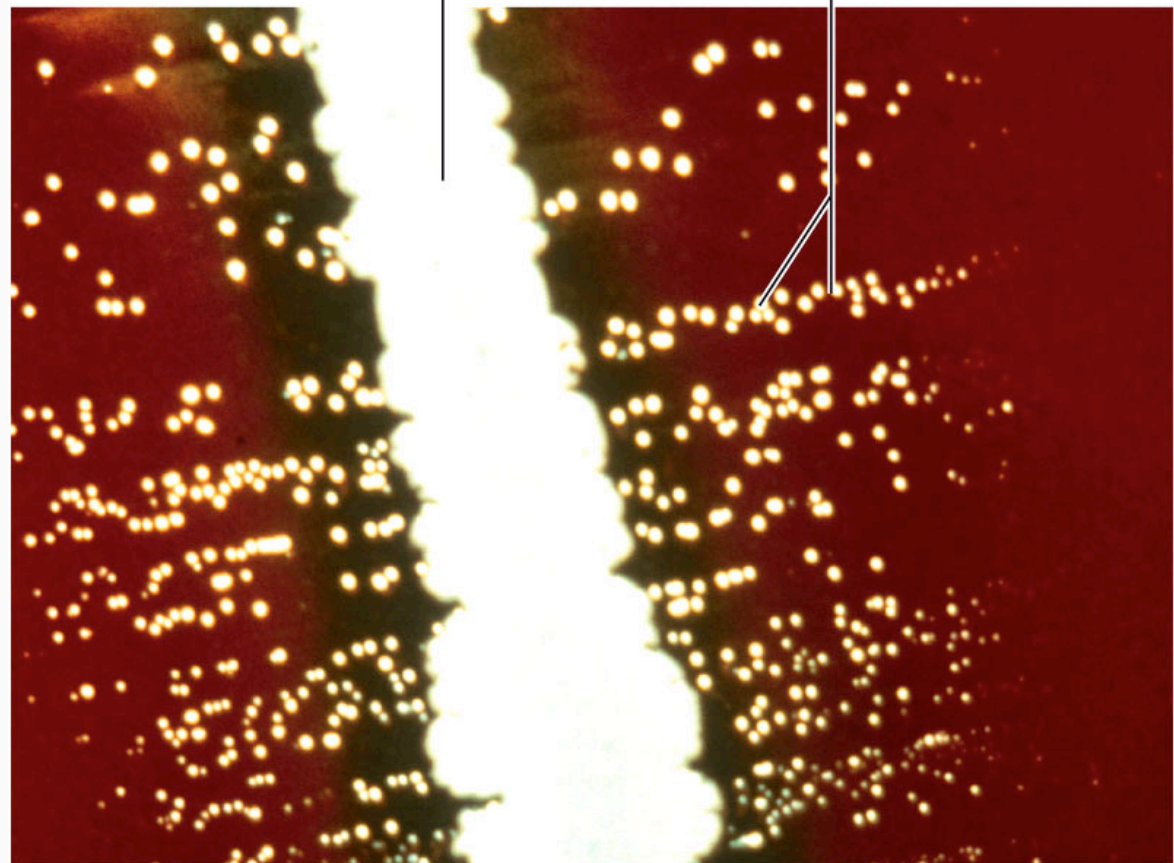


Fig. 7.12 Satellitism, a type of commensalism



# Non-symbiotic

- Organisms are free-living, and do not rely on each other for survival
- Types
  - Synergism – shared metabolism, not required
  - Antagonism - competition between microorganisms (to each other's detriment)

# Interrelationships between microbes and humans

- Can be commensal, parasitic, or synergistic
  - Ex. *E. coli* produce vitamin K for the host