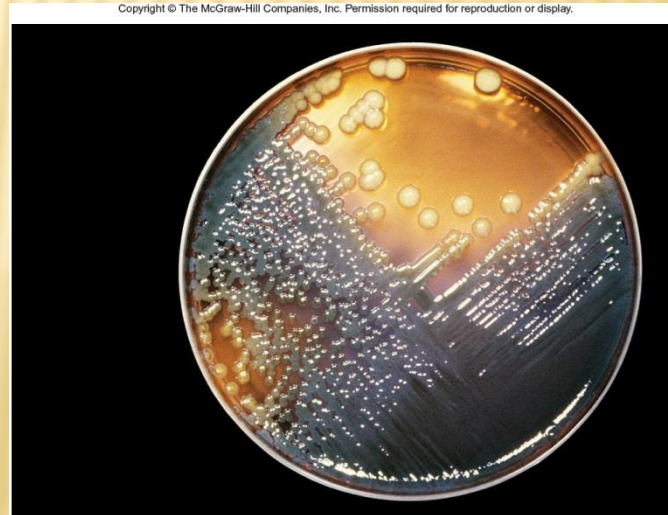


# Chapter 04

## Lecture

# A GLIMPSE OF HISTORY

- ✕ German physician Robert Koch (1843–1910)
  - + Studied disease-causing bacteria; Nobel Prize in 1905
  - + Developed methods of cultivating bacteria
    - ✕ Worked on methods of solid media to allow single bacteria to grow and form colonies
    - ✕ Tried potatoes, but nutrients limiting for many bacteria
- Solidifying liquid nutrient media with gelatin helped
- Limitations: melting temperature, digestible
- In 1882, Fannie Hess, wife of associate, suggested agar, then used to harden jelly





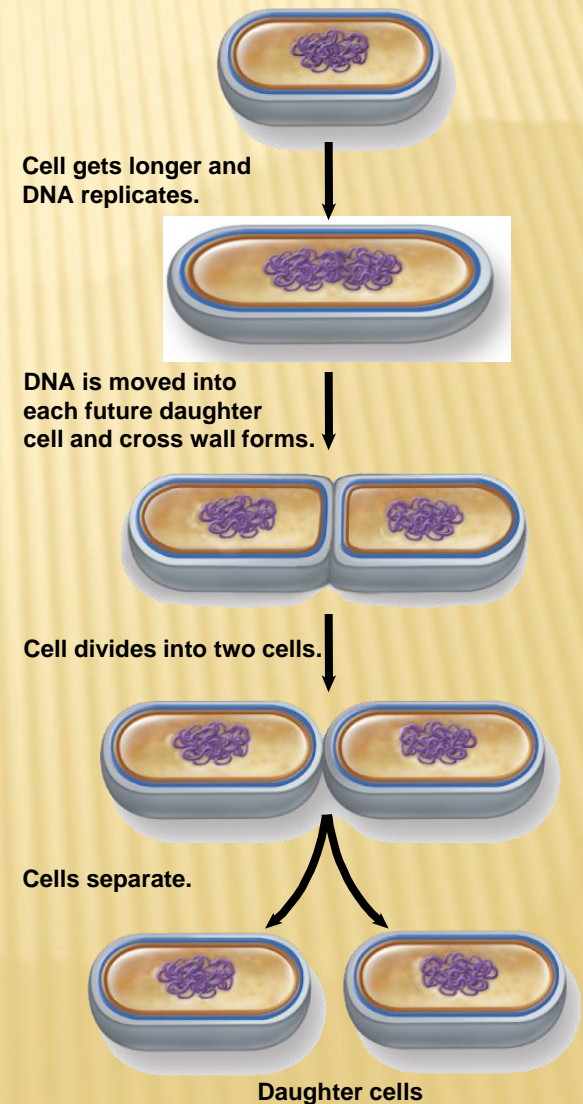
# INTRODUCTION

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- ✖ Prokaryotes found growing in severe conditions
  - + Ocean depths, volcanic vents, polar regions all harbor thriving prokaryotic species
  - + Many scientists believe that if life exists on other planets, it may resemble these microbes
- ✖ Individual species have limited set of conditions
  - + Also require appropriate nutrients
- ✖ Important to grow microbes in culture
  - + Medical significance
  - + Nutritional, industrial uses

# 4.1. PRINCIPLES OF BACTERIAL GROWTH

- ✗ Prokaryotic cells divide by binary fission
  - + One cell divides into two, two into four,  $4 \rightarrow 8$ ,  $8 \rightarrow 16$ , etc...
  - + Exponential growth: population doubles each division
  - + Generation time is time it takes to double
    - ✗ Varies among species
    - ✗ Environmental conditions
  - + Exponential growth has important consequences
    - ✗ 10 cells of food-borne pathogen in potato salad at picnic can become 40,000 cells in 4 hours



# 4.1. PRINCIPLES OF BACTERIAL GROWTH

## ✗ Growth can be calculated

+  $N_t = N_0 \times 2^n$

✗  $N_t$  = number of cells in population at time  $t$

✗  $N_0$  = original number of cells in population

✗  $n$  = number of divisions

+ Example: pathogen in potato salad at picnic in sun

✗ Assume 10 cells with 20 minute generation time

✗  $N_0$  = 10 cells in original population

✗  $n$  = 12 (3 divisions per hour for 4 hours)

✗  $N_t = N_0 \times 2^n = 10 \times 2^{12}$

✗  $N_t = 10 \times 4,096$

✗  $N_t$  = 40,960 cells of pathogen in 4 hours!



# 4.1. PRINCIPLES OF BACTERIAL GROWTH

- ✖ The power of exponential growth
  - + Rapid generation time with optimal conditions can yield huge populations quickly
  - + Remember that generation time depends on species and growth conditions

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**TABLE 4.1**

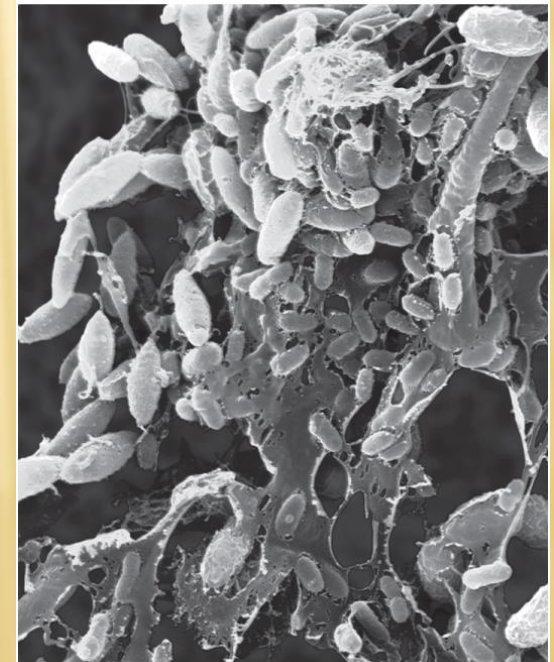
**Example of Exponential Growth**

Time in Minutes (t)	Initial Population ( $N_0$ )	Number of Generations (n)	$2^n$	Population $N_0 \times 2^n$
0	10	0		10
20	10	1	$2^1 (= 2)$	20
40	10	2	$2^2 (= 2 \times 2)$	40
60 (1 hour)	10	3	$2^3 (= 2 \times 2 \times 2)$	80
80	10	4	$2^4 (= 2 \times 2 \times 2 \times 2)$	160
100	10	5	$2^5 (= 2 \times 2 \times 2 \times 2 \times 2)$	320
120 (2 hours)	10	6	$2^6$	640
140	10	7	$2^7$	1,280
160	10	8	$2^8$	2,560
180 (3 hours)	10	9	$2^9$	5,120
200	10	10	$2^{10}$	10,240
220	10	11	$2^{11}$	20,480
240 (4 hours)	10	12	$2^{12}$	40,960

## 4.2. PROKARYOTIC GROWTH IN NATURE

- ✗ Microorganisms historically studied in laboratory
- ✗ But dynamic, complex conditions in nature have profound effect on microbial growth, behavior
  - + Cells sense changes, adjust to surroundings
  - + Synthesize compounds useful for growth
  - + Can live singly
    - ✗ Most live in polysaccharide-encased communities
    - ✗ Termed biofilms
    - ✗ Cause slipperiness of rocks in stream bed, slimy “gunk” in sink drains, scum in toilet bowls, dental plaque

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# BIOFILMS

## ✕ Formation of biofilm



**Planktonic bacteria move to the surface and adhere.**

**Bacteria multiply and produce extracellular polymeric substances (EPS).**

**Other bacteria may attach to the EPS and grow.**

**Cells communicate and create channels in the EPS that allow nutrients and waste products to pass.**

**Some cells detach and then move to other surfaces to create additional biofilms.**



# BIOFILMS

- ✗ Biofilms have characteristic architectures
  - + Channels through which nutrients and wastes pass
  - + Cells communicate by synthesizing chemical signals
- ✗ Biofilms have important implications
  - + Dental plaque leads to tooth decay, gum disease
  - + Most infections (e.g., ear infections, cystic fibrosis)
  - + Industrial concerns: accumulations in pipes, drains
  - + Biofilm structure shields microbes growing within
    - ✗ May be hundreds of times more resistant
  - + Biofilms can also be helpful
    - ✗ Bioremediation, wastewater treatment

# INTERACTIONS OF MIXED MICROBIAL COMMUNITIES

- ✗ Prokaryotes regularly grow in close association
  - + Many different species
  - + Interactions can be cooperative
    - ✗ Can foster growth of species otherwise unable to survive
      - ★ Strict anaerobes can grow in mouth if others consume  $O_2$
      - ★ Metabolic waste of one can serve as nutrient for other
  - + Interactions often competitive
    - ✗ Some synthesize toxic compounds to inhibit competitors



## 4.3. OBTAINING A PURE CULTURE

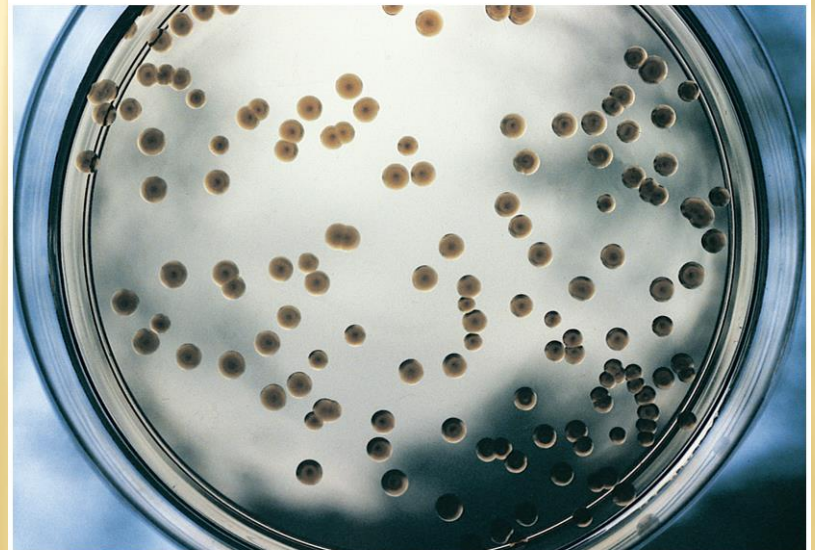
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- ✗ Pure culture defined as population of cells derived from a single cell
  - + Allows study of single species
- ✗ Pure culture obtained using aseptic technique
  - + Minimizes potential contamination
- ✗ Cells grown on culture medium
  - + Contains nutrients dissolved in water
  - + Can be broth (liquid) or solid gel

# GROWING MICROORGANISMS ON A SOLID MEDIUM

- ✗ Need culture medium, container, aseptic conditions, method to separate individual cells
  - + With correct conditions, single cell will multiply
  - + Form visible colony (~1 million cells easily visible)
  - + Agar used to solidify
    - ✗ Not destroyed by high temperatures
    - ✗ Liquifies above 95°C
    - ✗ Solidifies below 45°C
    - ✗ Few microbes can degrade
  - + Growth in Petri dish
    - ✗ Excludes contaminants
    - ✗ Agar plate

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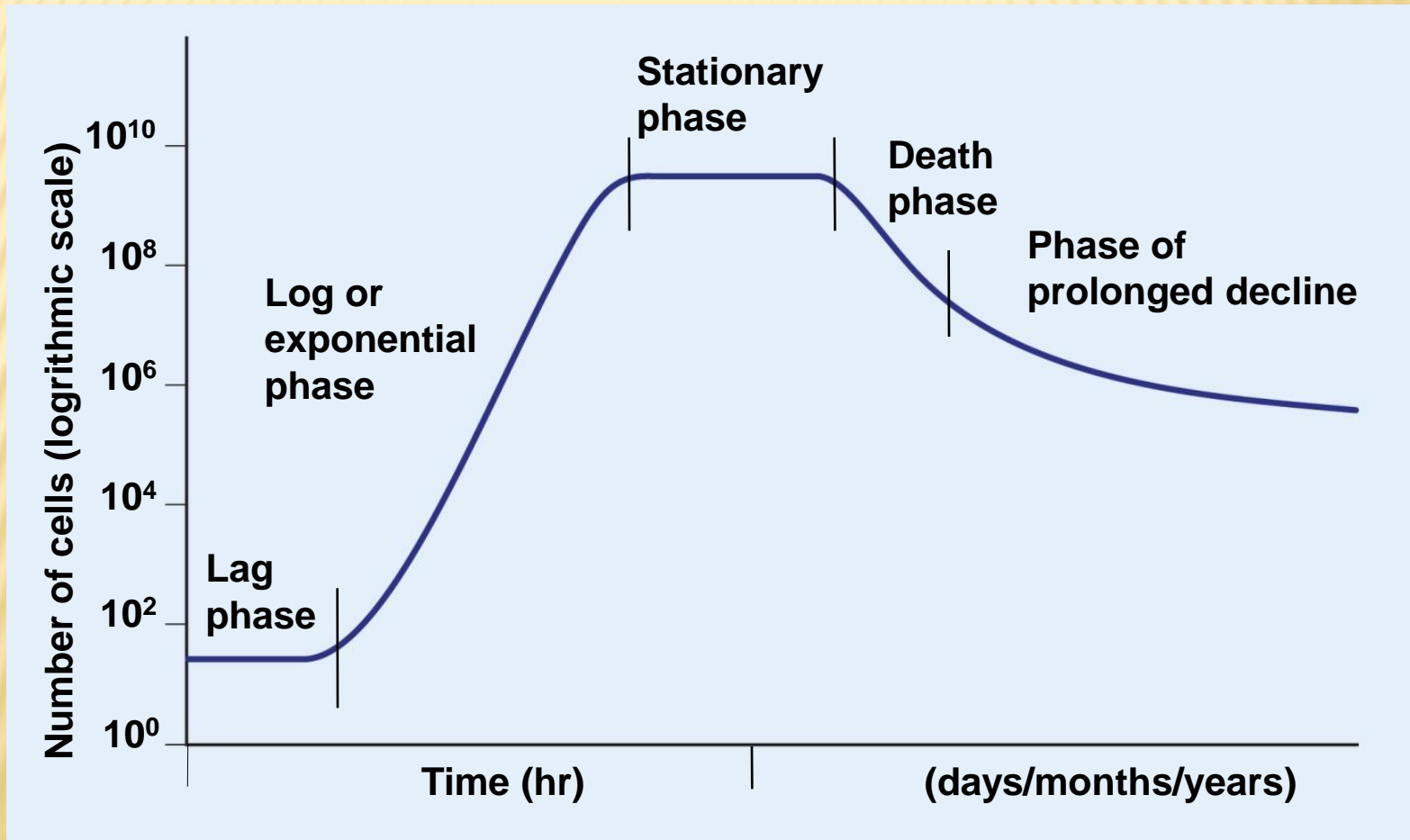
## 4.4. PROKARYOTIC GROWTH IN LABORATORY CONDITIONS

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- ✗ Prokaryotes grown on agar plates or in tubes or flasks of broth
  - + Closed systems
    - ✗ Nutrients not renewed; wastes not removed
  - + Termed batch cultures
    - ✗ Yields characteristic growth curve
  - + Open system required to maintain continuous growth
    - ✗ Termed continuous culture
    - ✗ Nutrients added, wastes removed continuously

# THE GROWTH CURVE

- ✗ Growth curve characterized by five stages





# THE GROWTH CURVE

## ✗ Lag phase

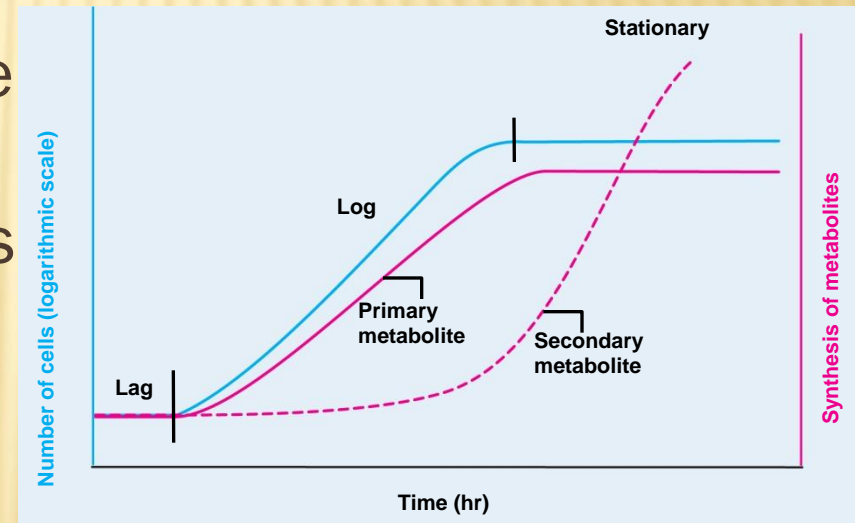
- + Number of cells does not increase
- + Begin synthesizing enzymes required for growth
- + Delay depends on conditions

## ✗ Exponential (log) phase

- + Cells divide at constant rate
- + Generation time measured
- + Most sensitive to antibiotics
- + Production of primary metabolites

✗ Important commercially

- + Secondary metabolite production occurs as nutrients are depleted and wastes accumulate



# THE GROWTH CURVE

---

## ✕ Stationary phase

- + Nutrient levels too low to sustain growth
- + Total numbers remain constant
  - ✕ Some die, release contents; others grow

## ✕ Death phase

- + Total number of viable cells decrease
  - ✕ Cells die at constant rate
  - ✕ Exponential, but usually much slower than cell growth

## ✕ Phase of prolonged decline

- + Some fraction may survive
- + Adapted to tolerate worsened conditions



# COLONY GROWTH

- ✗ Colonies and liquid cultures share similarities
- ✗ Important differences based on location
  - + Position of single cell determines its environment
  - + Edge of colony has  $O_2$ , nutrients
  - + Center of colony has depleted  $O_2$ , nutrients
    - ✗ Accumulation of potentially toxic wastes including acids
  - + Colony may range from exponential growth at edges, death phase in center

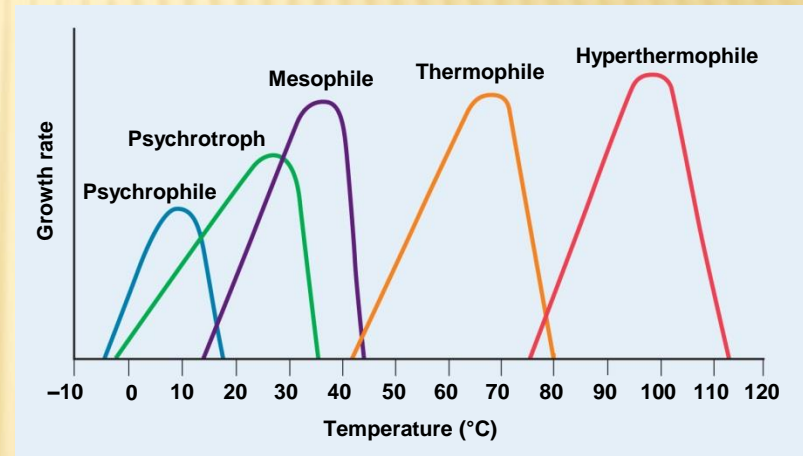
## 4.5. ENVIRONMENTAL FACTORS THAT INFLUENCE MICROBIAL GROWTH

---

- ✗ Prokaryotes inhabit nearly all environments
  - + Some live in comfortable habitats favored by humans
  - + Some live in harsh environments
    - ✗ Termed extremophiles; most are *Archaea*
- ✗ Major conditions that influence growth
  - + Temperature
  - + Atmosphere
  - + pH
  - + Water availability

# TEMPERATURE REQUIREMENTS

- ✗ Each species has well-defined temperature range
  - + Optimum growth usually close to upper end of range
  - + Psychrophile:  $-5^{\circ}$  to  $15^{\circ}\text{C}$ 
    - ✗ Found in Arctic and Antarctic regions
  - + Psychrotroph:  $20^{\circ}$  to  $30^{\circ}\text{C}$ 
    - ✗ Important in food spoilage
  - + Mesophile:  $25^{\circ}$  to  $45^{\circ}\text{C}$ 
    - ✗ Pathogens  $35^{\circ}$  to  $40^{\circ}\text{C}$
  - + Thermophiles:  $45^{\circ}$  to  $70^{\circ}\text{C}$ 
    - ✗ Common in hot springs
  - + Hyperthermophiles:  $70^{\circ}$  to  $110^{\circ}\text{C}$ 
    - ✗ Usually members of *Archaea*
    - ✗ Found in hydrothermal vents





# TEMPERATURE REQUIREMENTS

- ✖ Proteins of thermophiles resist denaturing
  - + Thermostability comes from amino acid sequence
    - ✖ Number and position of bonds, which determine structure
- ✖ Temperature and food preservation
  - + Refrigeration ( $\sim 4^{\circ}\text{C}$ ) slows spoilage by limiting growth of otherwise fast-growing mesophiles
    - ✖ Psychrophiles, psychrotrophs can still grow, but slowly
  - + Freezing preserves food; not effective at killing microbes
- ✖ Temperature and disease
  - + Temperature of different parts of human body differs
    - ✖ Some microbes cause disease in certain parts
    - ✖ E.g., Hansen's disease (leprosy) involves coolest regions (ears, hands, feet, fingers) due to preference of *M. leprae*


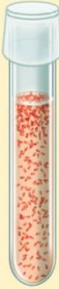



# OXYGEN REQUIREMENTS

- ✗ Boil nutrient agar to drive off  $O_2$ ; cool to just above solidifying temperature; inoculate; gently swirl
- + Growth demonstrates organism's  $O_2$  requirements

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**TABLE 4.3**

**Oxygen ( $O_2$ ) Requirements of Prokaryotes**

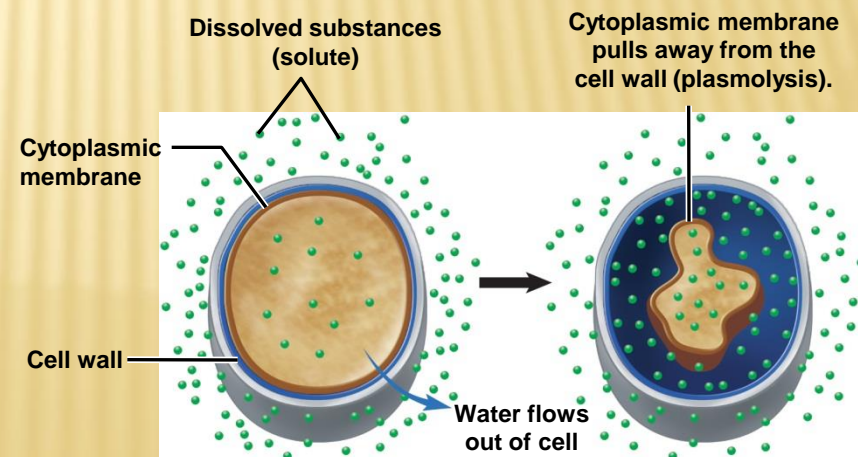
	Obligate aerobe	Facultative anaerobe	Obligate anaerobe	Microaerophile	Aerotolerant anaerobe
					
<b>Growth characteristics</b>	Grows only when $O_2$ is available.	Grows best when $O_2$ is available, but also grows without it.	Cannot grow when $O_2$ is present.	Grows only if small amounts of $O_2$ are available.	Grows equally well with or without $O_2$ .
<b>Use of <math>O_2</math> in energy-harvesting processes</b>	Requires $O_2$ for respiration.	Uses $O_2$ for respiration, if available.	Does not use $O_2$ .	Requires $O_2$ for respiration.	Does not use $O_2$ .
<b>Typical mechanisms to protect against reactive oxygen species</b>	Produces superoxide dismutase and catalase.	Produces superoxide dismutase and catalase.	Does not produce superoxide dismutase or catalase.	Produces some superoxide dismutase and catalase.	Produces superoxide dismutase but not catalase.

- ✗ Bacteria survive a range of pH; have optimum
  - + Most maintain constant internal pH, typically near neutral
    - ✗ Pump out protons if in acidic environment
    - ✗ Bring in protons if in alkaline environment
  - + Most microbes are neutrophiles
    - ✗ Range of pH 5 to 8; optimum near pH 7
    - ✗ Food can be preserved by increasing acidity
    - ✗ *H. pylori* grows in stomach; produces urease to split urea into CO<sub>2</sub> and ammonia to decrease acidity of surroundings
  - + Acidophiles grow optimally at pH below 5.5
    - ✗ *Picrophilus oshimae* has optimum pH of less than 1!
  - + Alkaliphiles grow optimally at pH above 8.5



# WATER AVAILABILITY

- ✗ All microorganisms require water for growth
  - + Dissolved salts, sugars make water unavailable to cell
    - ✗ If solute concentration is higher outside of cell, water diffuses out (osmosis)
    - ✗ Salt, sugar used to preserve food
  - + Some microbes withstand or even require high salt
  - + Halotolerant: withstand up to 10% (e.g., *Staphylococcus*)
  - + Halophiles: require high salt concentrations
    - ✗ Marine bacteria ~3%
    - ✗ Extreme halophiles  $\geq 9\%$  (Dead Sea, Utah's salt flats)



## 4.6. NUTRITIONAL FACTORS THAT INFLUENCE MICROBIAL GROWTH

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- ✗ Prokaryotes have remarkable metabolic diversity
- ✗ Require nutrients to synthesize cell components
  - + Lipid membranes, cell walls, proteins, nucleic acids
  - + Made from subunits: phospholipids, sugars, amino acids, nucleotides
    - ✗ Subunits composed of chemical elements including carbon and nitrogen
- ✗ Key considerations:
  - + Required elements
  - + Growth factors
  - + Energy sources
  - + Nutritional diversity



## 4.6. NUTRITIONAL FACTORS THAT INFLUENCE MICROBIAL GROWTH

### ✖ Required elements

- + Major elements make up cell components
- + Carbon source distinguishes different groups
  - ✖ Heterotrophs use organic carbon
  - ✖ Autotrophs use inorganic carbon as  $\text{CO}_2$  (carbon fixation)
- + Nitrogen required for amino acids, nucleic acids
  - ✖ Many use ammonia (some convert nitrate to ammonia)
  - ✖ Nitrogen fixation important
- + Iron, phosphorous often limiting
- + Trace elements usually available (cobalt, zinc, copper, molybdenum, manganese)

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**TABLE 4.4**

**Representative Functions  
of the Major Elements**

Chemical	Function
Carbon, oxygen, and hydrogen	Component of amino acids, lipids, nucleic acids, and sugars
Nitrogen	Component of amino acids and nucleic acids
Sulfur	Component of some amino acids
Phosphorus	Component of nucleic acids, membrane lipids, and ATP
Potassium, magnesium, and calcium	Required for the functioning of certain enzymes; additional functions as well
Iron	Part of certain enzymes



## 4.6. NUTRITIONAL FACTORS THAT INFLUENCE MICROBIAL GROWTH

---

- ✗ Energy sources

  - + Sunlight, chemical compound

- ✗ **Photo**trophs obtain energy from sunlight

  - + Plants, algae, photosynthetic bacteria

- ✗ **Chem**otrophs extract energy from chemicals

  - + Mammalian cells, fungi, many types of prokaryotes

  - + Sugars, amino acids, fatty acids common sources

  - + Some prokaryotes use inorganic chemicals such as hydrogen sulfide, hydrogen gas

## 4.6. NUTRITIONAL FACTORS THAT INFLUENCE MICROBIAL GROWTH

### × Nutritional diversity

- + **Photo**auto**tro**phs: energy from sunlight; carbon from  $\text{CO}_2$
- + **Photo**heterotrophs: energy from sunlight; carbon from organic compounds
- + **Chemo**litho**auto**tro**phs** (also termed chemoautotrophs, chemolithotrophs): energy from inorganic compounds; carbon from  $\text{CO}_2$
- + **Chemo**organoheterotrophs (also termed chemoheterotrophs, chemoorganotrophs): energy and carbon from organic compounds

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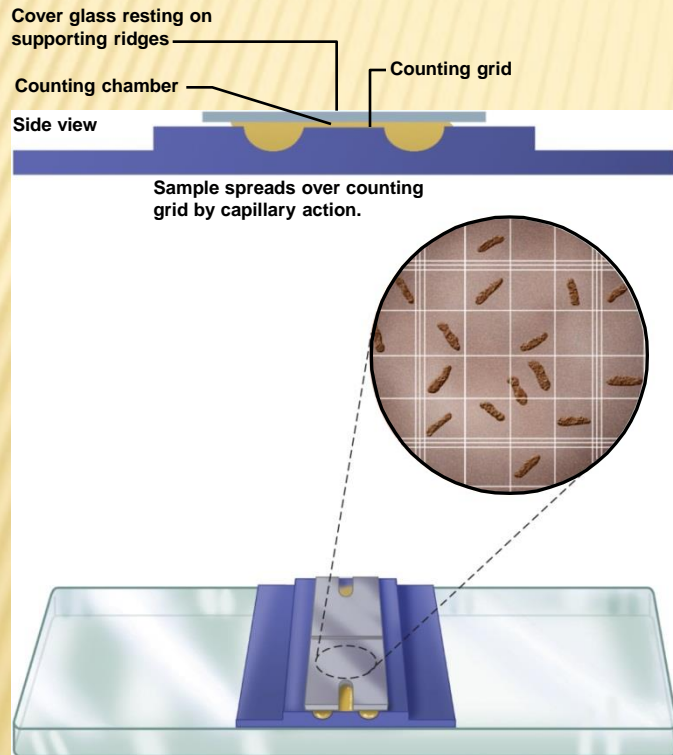
**TABLE 4.5**

**Energy and Carbon Sources Used by Different Groups of Prokaryotes**

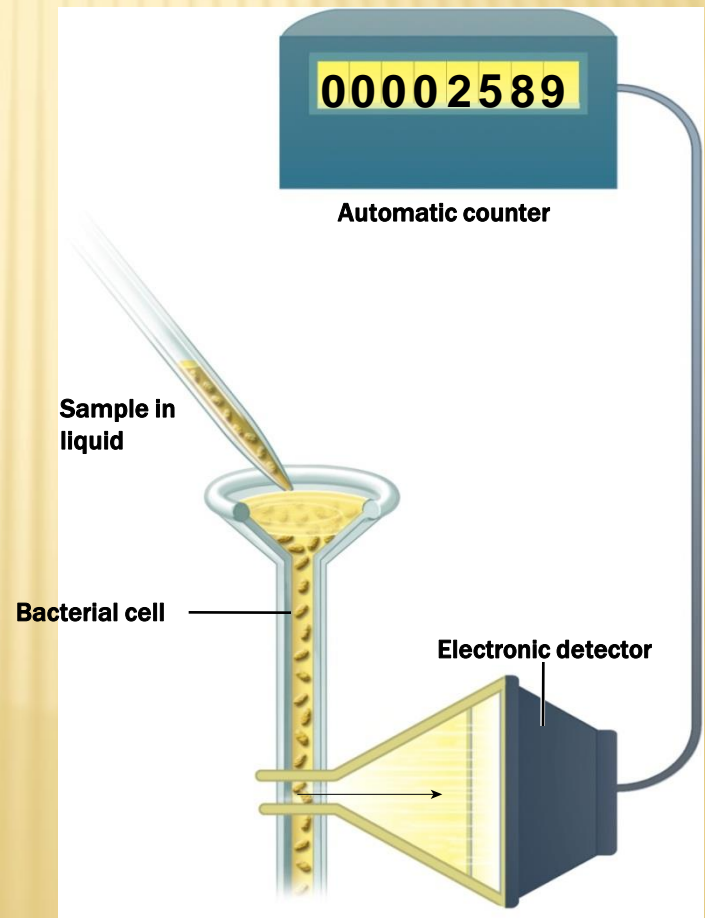
Type	Energy Source	Carbon Source
Photoautotroph	Sunlight	$\text{CO}_2$
Photoheterotroph	Sunlight	Organic compounds
Chemolithoautotroph	Inorganic chemicals ( $\text{H}_2$ , $\text{NH}_3$ , $\text{NO}_2^-$ , $\text{Fe}^{2+}$ , $\text{H}_2\text{S}$ )	$\text{CO}_2$
Chemoorganoheterotroph	Organic compounds (sugars, amino acids, etc.)	Organic compounds

# 4.8. METHODS TO DETECT AND MEASURE MICROBIAL GROWTH

- ✗ Direct cell counts: total numbers (living plus dead)
  - ✗ Direct microscope count
  - ✗ Cell-counting instruments (Coulter counter, flow cytometer)



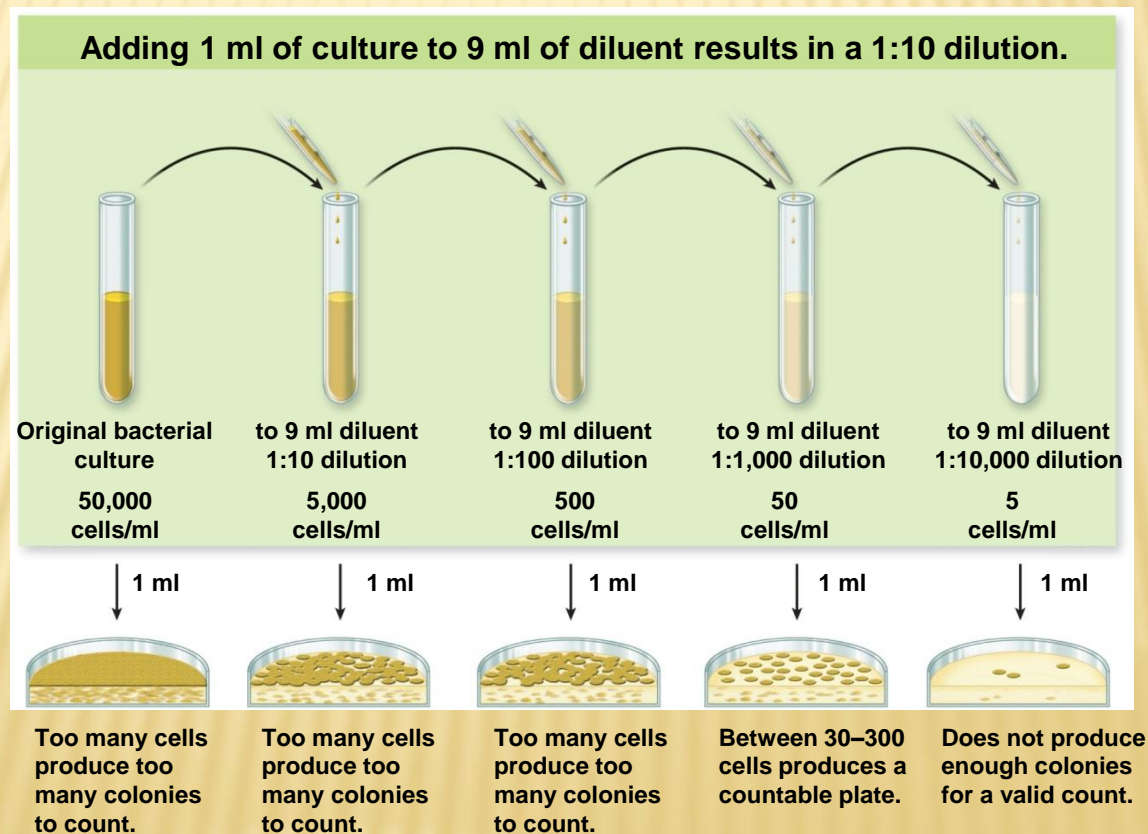
Using a microscope, the cells in several large squares like the one shown are counted and the results averaged. To determine the number of cells per ml, that number must be multiplied by 1/volume (in ml) held in the square. For example, if the square holds  $1/1,250,000$  ml, then the number of cells must be multiplied by  $1.25 \times 10^6$  ml.





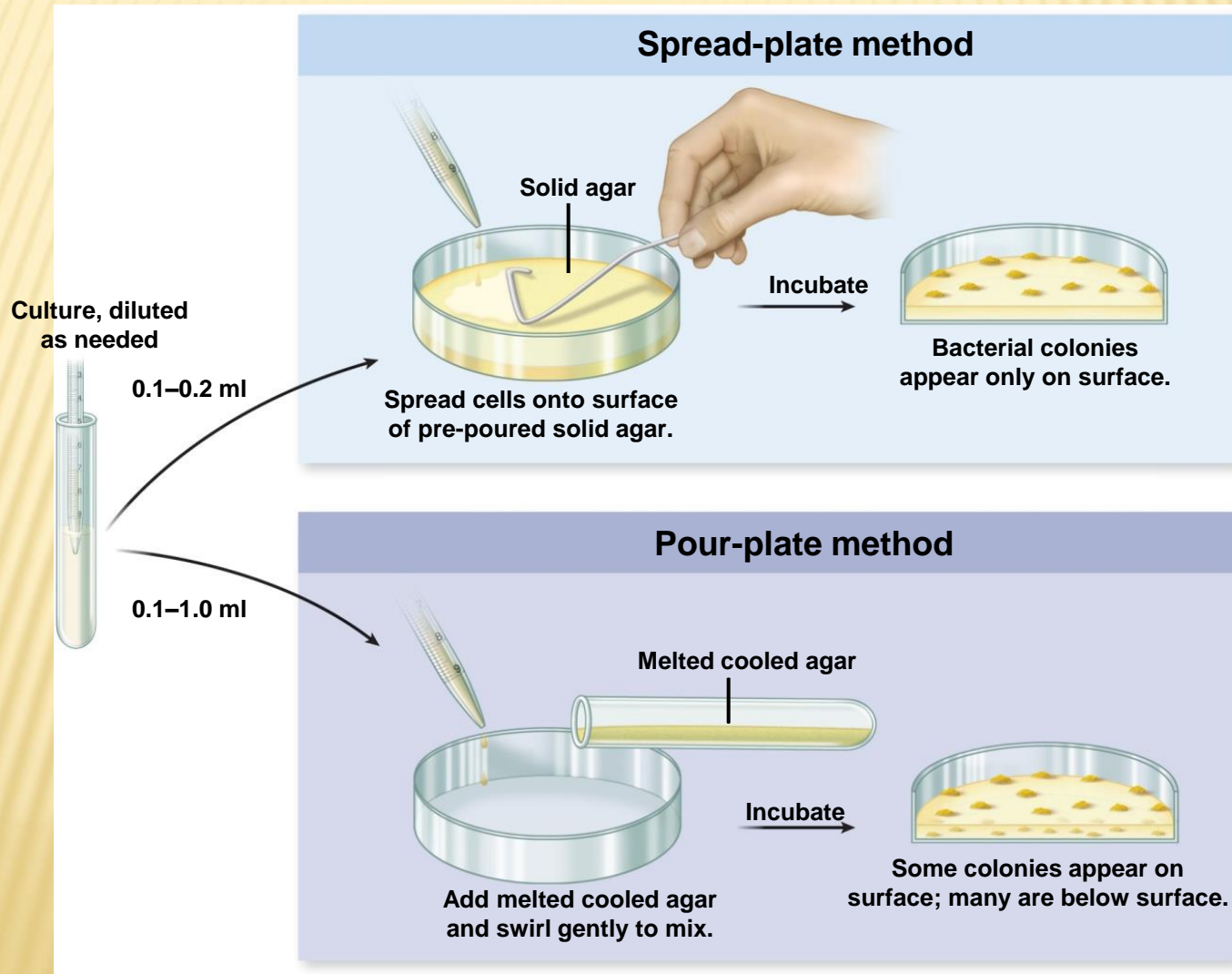
## 4.8. METHODS TO DETECT AND MEASURE MICROBIAL GROWTH

- ✗ Viable cell counts: cells capable of multiplying
  - + Can use selective, differential media for particular species
  - + Plate counts: single cell gives rise to colony
    - ✗ Plate out dilution series: 30–300 colonies ideal



## 4.8. METHODS TO DETECT AND MEASURE MICROBIAL GROWTH

+ Plate counts determine colony-forming units (CFUs)

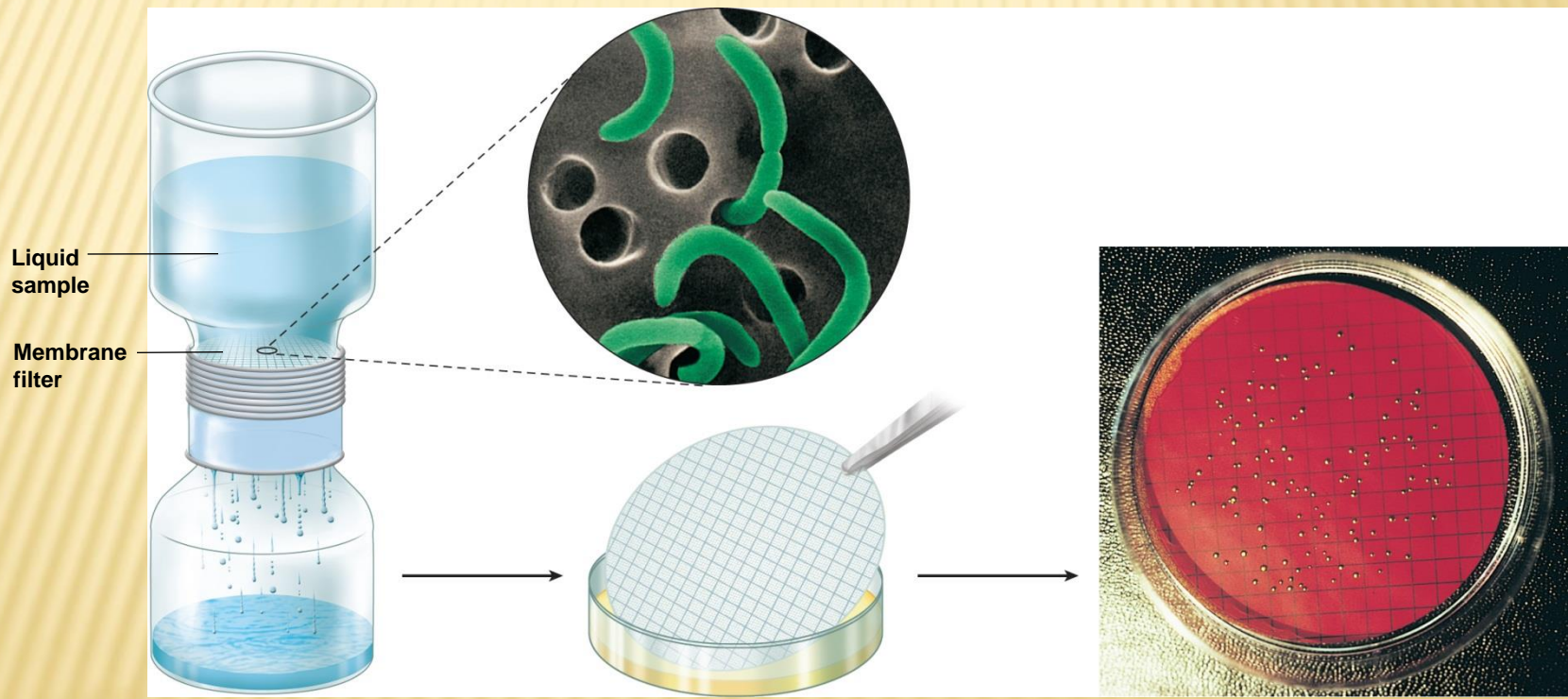




## 4.8. METHODS TO DETECT AND MEASURE MICROBIAL GROWTH

### + Membrane filtration

- × Concentrates microbes by filtration
- × Filter is incubated on appropriate agar medium



A known volume of liquid is passed through a sterile membrane filter; the filter retains bacterial cells.

The membrane filter is placed on an appropriate agar medium and incubated.

The number of colonies that grow on the filter indicates the number of bacterial cells in the volume filtered.



# 4.8. METHODS TO DETECT AND MEASURE MICROBIAL GROWTH

## ✖ Measuring biomass

- + Turbidity is proportional to concentration of cells
- + Measured with spectrophotometer

