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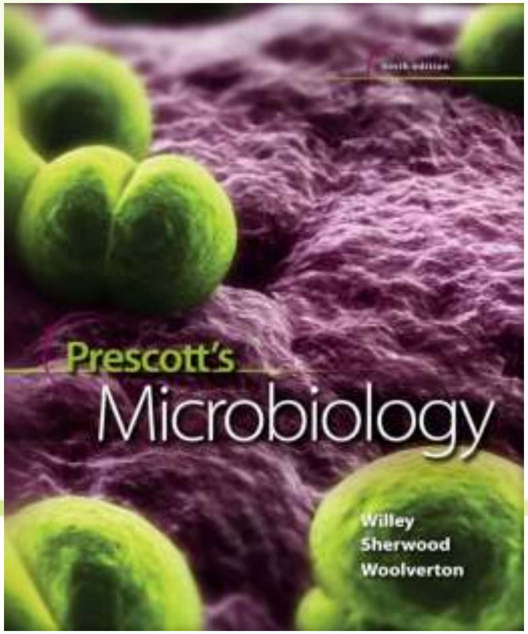


Microbial Growth

Reproductive Strategies

- The reproductive strategies of eukaryotic microbes
  - asexual and sexual, haploid or diploid
- *Bacteria* and *Archaea*
  - **haploid only, asexual - binary fission, budding, filamentous**
  - all must replicate and segregate the genome prior to division

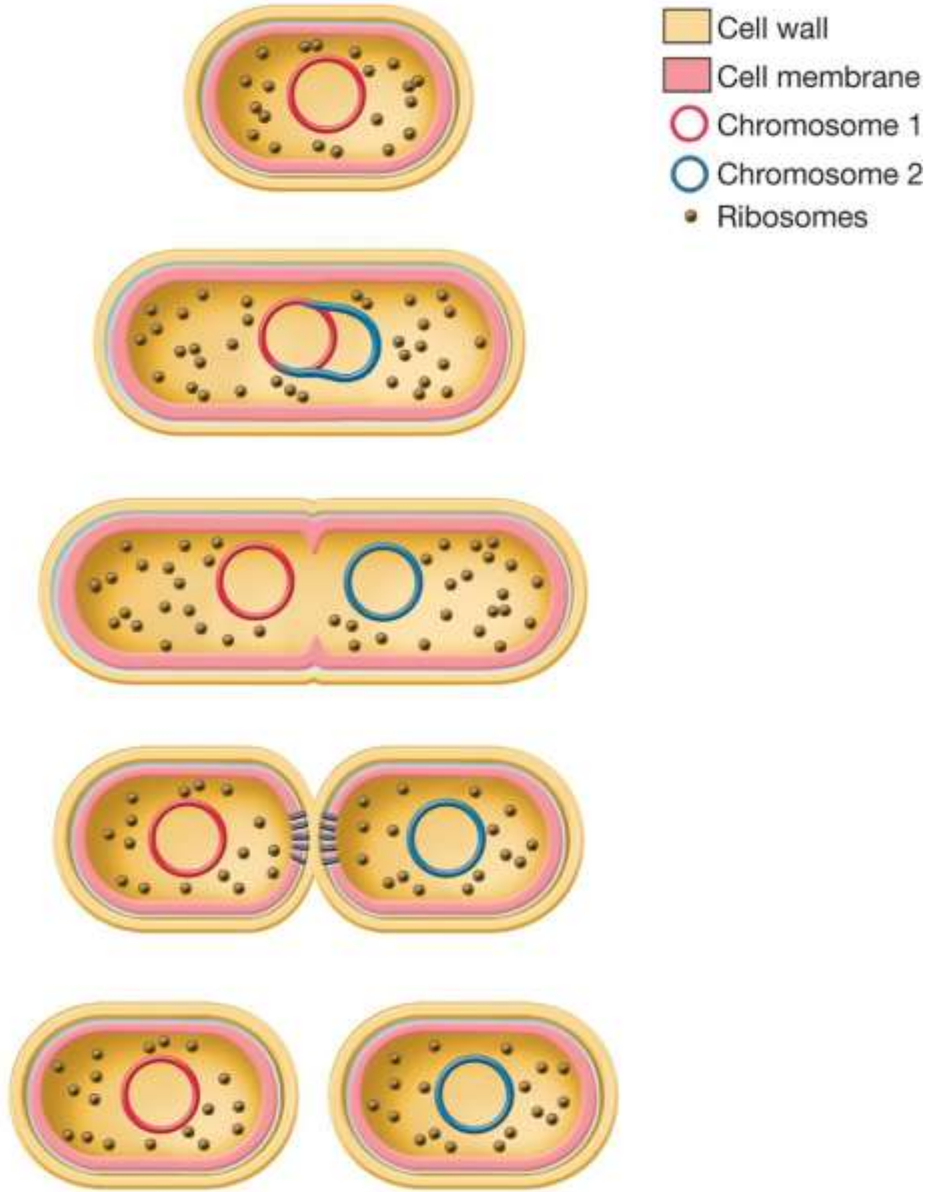
(a) A young cell at early phase of cycle

(b) A parent cell prepares for division by enlarging its cell wall, cell membrane, and overall volume. DNA replication then starts.

(c) The septum begins to grow inward as the chromosomes move toward opposite ends of the cell. Other cytoplasmic components are distributed to the two developing cells.

(d) The septum is synthesized completely through the cell center, creating two separate cell chambers.

(e) At this point, the daughter cells are divided. Some species separate completely as shown here, while others remain attached, forming chains, doublets, or other cellular arrangements.

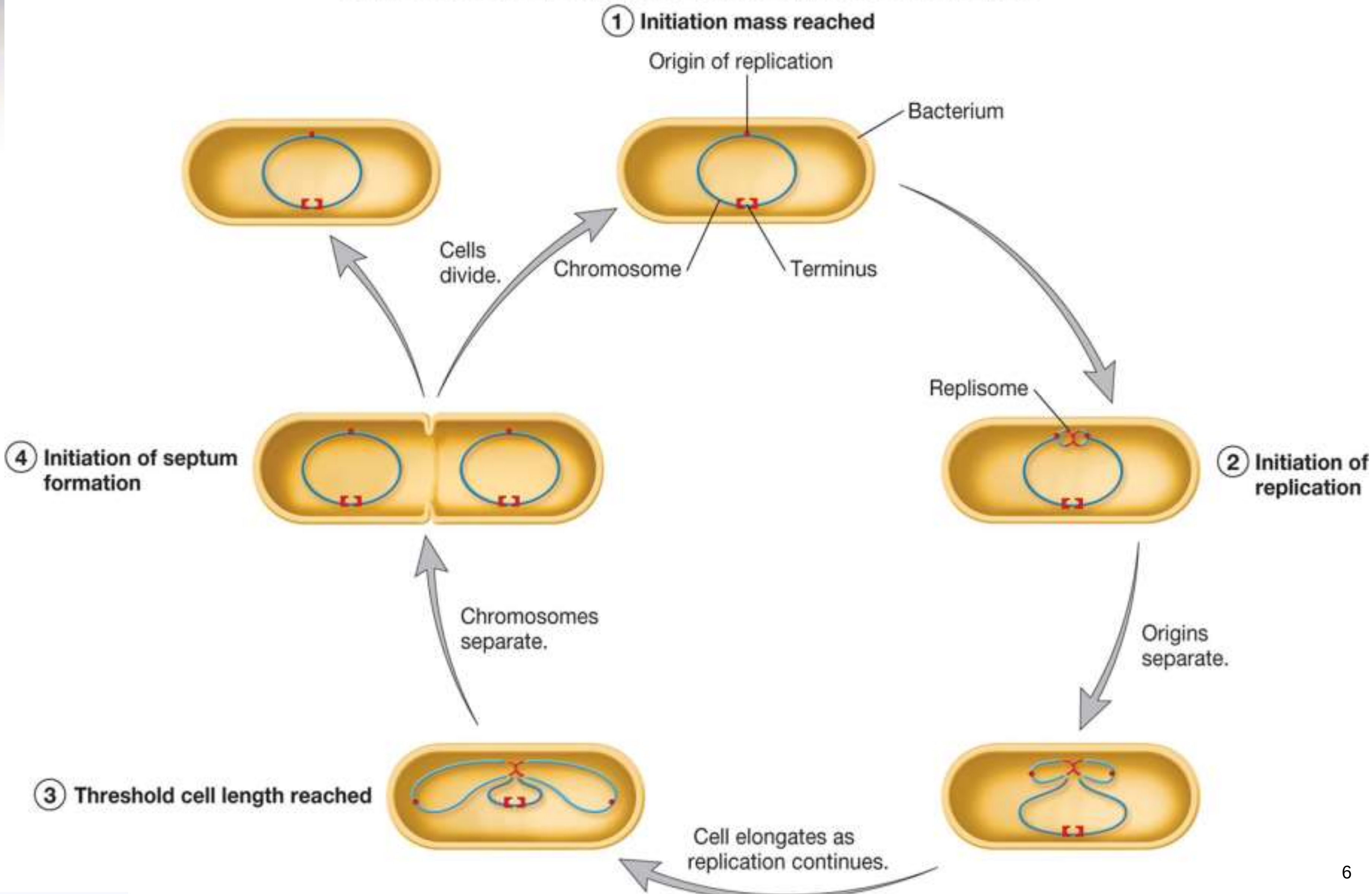


Bacterial Cell Cycle

- Cell cycle is sequence of events from formation of new cell through the next cell division
  - most bacteria divide by binary fission
- Two pathways function during cycle
  - **DNA replication and partition**
  - **cytokinesis**

Chromosome Replication and Partitioning - 1

- Most bacterial chromosomes are circular
- **Single origin of replication** – site at which replication begins
- **Terminus** – site at which replication is terminated, located opposite of the origin
- **Replisome** – group of proteins needed for DNA synthesis
- DNA replication **proceeds in both directions from the origin**
- Origins move to opposite ends of the cell


1 Initiation mass reached
Origin of replication
Bacterium
Chromosome
Terminus
Cells divide.
Replisome
2 Initiation of replication
Origins separate.
Cell elongates as replication continues.
3 Threshold cell length reached
Chromosomes separate.
4 Initiation of septum formation

Chromosome Partitioning

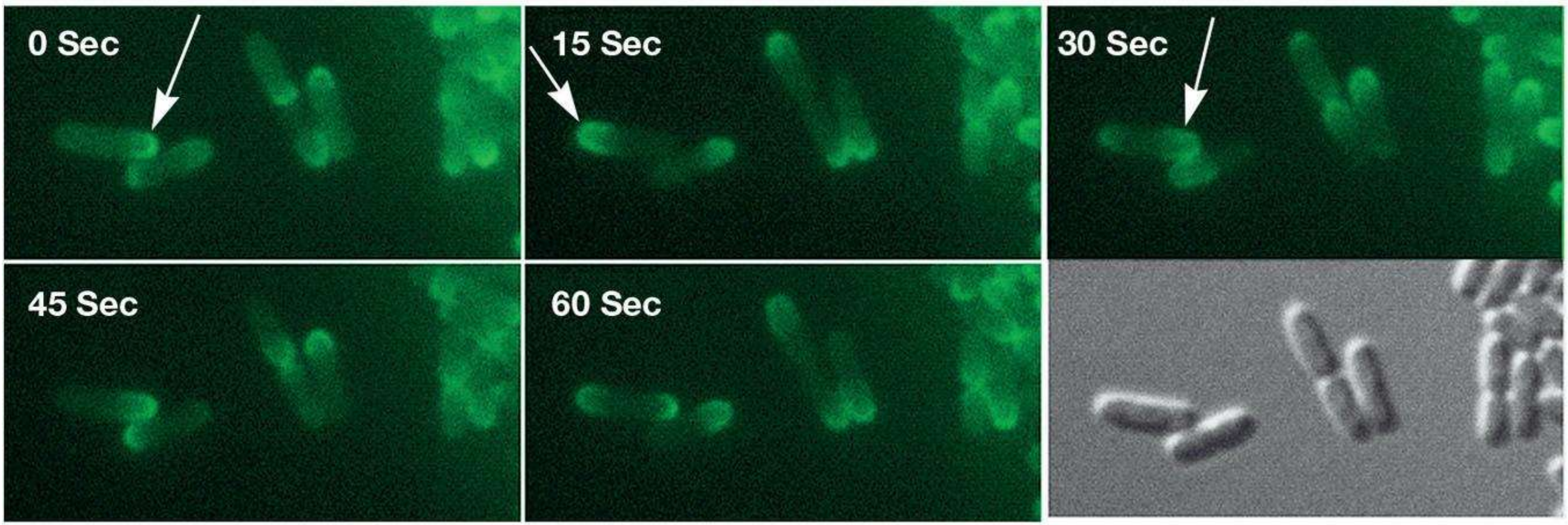
- **Replisome pushes, or condensation of, daughter chromosomes to opposite ends**
- **MreB (*mur*ein cluster *B*)** – an actin homolog, plays role in determination of cell shape as spiral inside cell periphery, and chromosome segregation
  - new origins associate with MreB tracks
  - if MreB is mutated, chromosomes do not segregate

Cytokinesis - Septation

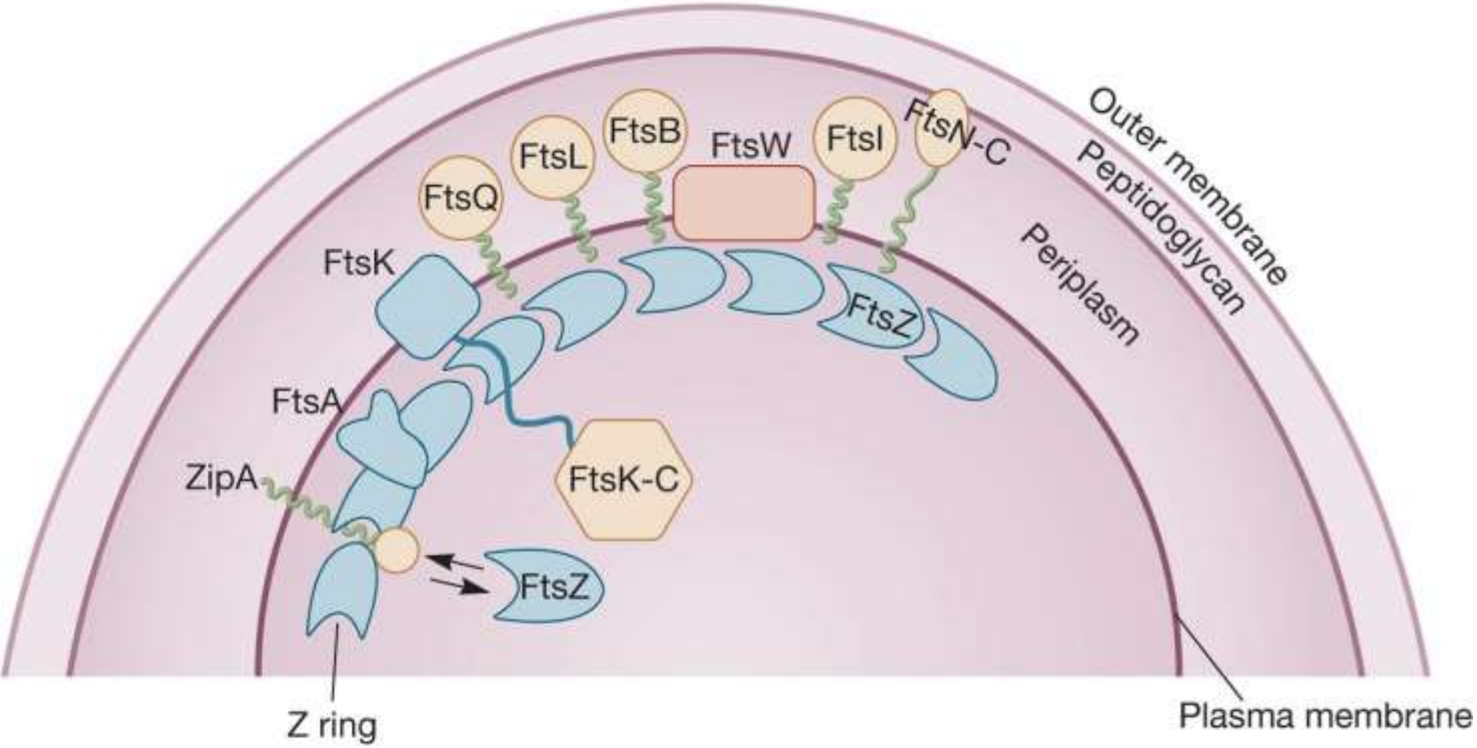
- **Septation** – formation of cross walls between daughter cells
- **Several steps**
  – **selection of site for septum formation**
  – **assembly of Z ring**
  – **linkage of Z ring to plasma membrane (cell wall)**
  – **assembly of cell wall synthesizing machinery**
  – **constriction of cell and septum formation**

Z Ring Formation - Role in Septation

- **Protein FtsZ**
  - tubulin homologue, found in most bacteria and archaea
  - polymerization forms Z ring, filaments of meshwork
- **MinCDE** system in *E. coli* limits the Z ring to the center of the cell
  - MinC, MinD, MinE oscillate from one side of cell to other
  - link Z ring to cell membrane
  - Z ring constricts and cell wall synthesis of septal wall



Reprinted with permission from the American Society for Microbiology (*Microbe* 2008, 3(7): 629–36); 7.15a: Dr. Joachim Reitner



The *E. coli* divisome

Table 7.1 Divisome Proteins and Their Functions

Divisome Protein	Function
FtsA, ZipA	Anchor Z ring to plasma membrane
FtsZ	Forms Z ring
FtsK	Chromosome segregation and separation of chromosome dimers
FtsQLB	May provide a scaffold for assembly of proteins involved in peptidoglycan synthesis
FtsI[1], FtsW	Peptidoglycan synthesis
FtsN	Thought to trigger constriction initiation

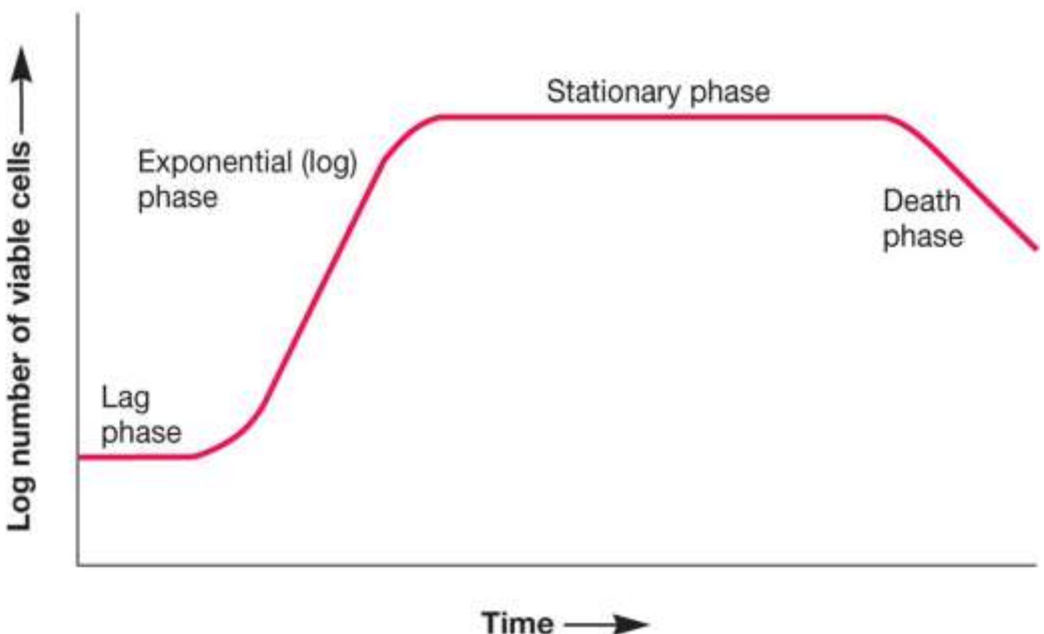
1 FtsI is also known as penicillin-binding protein 3 (PBP3).

Growth

- Increase in cellular constituents that may result in:
  - **increase in cell number**
  - **increase in cell size**
- Growth refers to population growth rather than growth of individual cells

The Growth Curve

- Observed when microorganisms are cultivated in **batch culture**
- Usually plotted as logarithm of cell number versus time
- Has four distinct phases



Lag Phase

- **Cell synthesizing new components**
  - e.g., to replenish spent materials(لتجديد المواد المستهلكة)
  - e.g., to adapt to new medium or other conditions
- **Varies in length**
  - in some cases can be very short or even absent

Exponential Phase

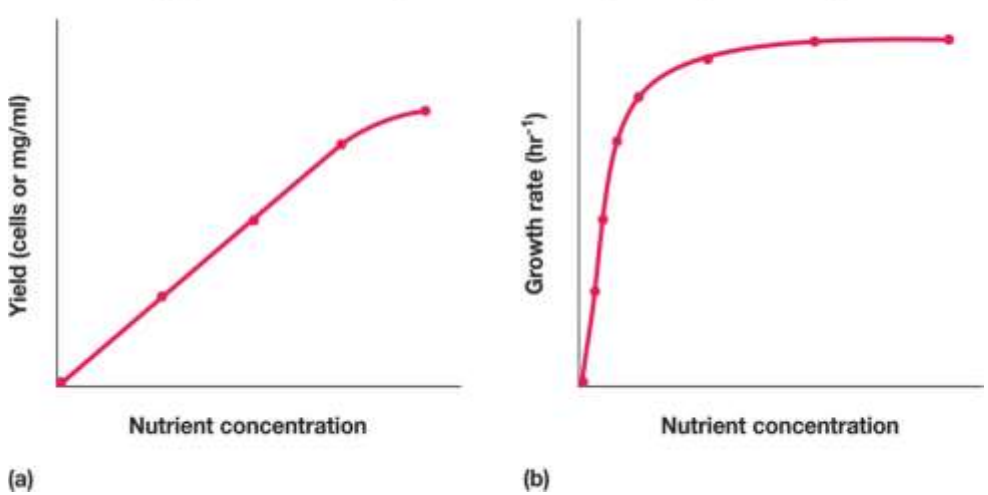
- Also called log phase
- **Rate of growth and division is constant and maximal**
- Population is **most uniform** in terms of chemical and physical properties during this phase

Balanced Growth

- During log phase, cells exhibit balanced growth
  - cellular constituents manufactured at constant rates relative to each other

Unbalanced Growth

- Rates of synthesis vary relative to each other
- Occurs under a variety of conditions
  - **change in nutrient levels**
    - shift-up (poor medium to rich medium)
    - shift-down (rich medium to poor medium)
  - change in environmental conditions



Stationary Phase

- Closed system population growth eventually ceases, total number of viable cells remains constant
  - active cells stop reproducing or reproductive rate is balanced by death rate

Possible Reasons for Stationary Phase

- Nutrient limitation
- Limited oxygen availability
- Toxic waste accumulation
- Critical population density reached

Stationary Phase and Starvation Response

- Entry into stationary phase due to starvation and other stressful conditions activates survival strategy
  - **morphological changes**
    - e.g., endospore formation
  - **decrease in size, protoplast shrinkage, and nucleoid condensation**
  - **RpoS protein assists RNA polymerase in transcribing genes for starvation proteins**

Starvation Responses

- **Production of starvation proteins**
  - increase cross-linking in cell wall
  - Dps protein protects DNA
  - chaperone proteins prevent protein damage
- Cells are called **persister cells**
  - **long-term survival**
  - **increased virulence**

Senescence and Death Phase

- Two alternative hypotheses
  - cells are **Viable But Not Culturable (VBNC)**
    - cells alive, but dormant, capable of new growth when conditions are right
- **Programmed cell death**
  - fraction of the population genetically programmed to die (commit suicide)

The Influence of Environmental Factors on Growth

- Most organisms grow in fairly moderate environmental conditions(ظروف بيئية معتدلة الى حدٍ ما)
- **Extremophiles**
  - grow under harsh conditions that would kill most other organisms

Table 7.2 Microbial Responses to Environmental Factors

Descriptive Term	Definition	Representative Microorganisms
Solute and Water Activity		
Osmotolerant	Able to grow over wide ranges of water activity or osmotic concentration	*Staphylococcus aureus, Saccharomyces rouxii*
Halophile	Requires high levels of sodium chloride, usually above about 0.2 M, to grow	*Halobacterium, Dunaliella, Ectothiorhodospira*
pH		
Acidophile	Growth optimum between pH 0 and 5.5	*Sulfolobus, Picrophilus, Ferroplasma, Acontium*
Neutrophile	Growth optimum between pH 5.5 and 8.0	*Escherichia, Euglena, Paramecium*
Alkaliphile	Growth optimum between pH 8.0 and 11.5	*Bacillus alcalophilus, Natronobacterium*
Temperature		
Psychrophile	Grows at 0°C and has an optimum growth temperature of 15°C or lower	*Bacillus psychrophilus, Chlamydomonas nivalis*
Psychrotroph	Can grow at 0–7°C; has an optimum between 20 and 30°C and a maximum around 35°C	*Listeria monocytogenes, Pseudomonas fluorescens*
Mesophile	Has growth optimum between 20 and 45°C	*Escherichia coli, Trichomonas vaginalis*
Thermophile	Can grow at 55°C or higher; optimum often between 55 and 65°C	*Geobacillus stearothermophilus, Thermus aquaticus, Cyanidium caldarium, Chaetomium thermophile*
Hyperthermophile	Has an optimum between 85 and about 113°C	*Sulfolobus, Pyrococcus, Pyrodictium*
Oxygen Concentration		
Obligate aerobe	Completely dependent on atmospheric O_2 for growth	*Micrococcus luteus*, most protists and fungi
Facultative anaerobe	Does not require O_2 for growth but grows better in its presence	*Escherichia, Enterococcus, Saccharomyces cerevisiae*
Aerotolerant anaerobe	Grows equally well in presence or absence of O_2	*Streptococcus pyogenes*
Obligate anaerobe	Does not tolerate O_2 and dies in its presence	*Clostridium, Bacteroides, Methanobacterium*
Microaerophile	Requires O_2 levels between 2–10% for growth and is damaged by atmospheric O_2 levels (20%)	*Campylobacter, Spirillum volutans, Treponema pallidum*
Pressure		
Piezophile (barophile)	Growth more rapid at high hydrostatic pressures	*Photobacterium profundum, Shewanella benthica*

Solutes and Water Activity

- Changes in osmotic concentrations in the environment may affect microbial cells
  - **hypotonic solution** (lower osmotic concentration)
    - water enters the cell
    - cell swells may burst
  - **hypertonic** (higher osmotic concentration)
    - water leaves the cell
    - membrane shrinks from the cell wall (plasmolysis) may occur

Water Activity (a_w)

- Water activity of a solution is 1/100 the relative humidity of solution
- Also equal to **ratio of solution's vapor pressure (Psoln) to that of pure water (Pwater)**
- Aw = Psoln/ Pwater
  - **low water activity means most water is bound**
- **Osmotolerant microbes can grow over wide ranges of water activity**

Solutes and Water Activity

- water activity (a_w)
  - **amount of water available to organisms**
  - reduced by interaction with solute molecules (osmotic effect)

    higher [solute] ⇒ lower a_w

  - reduced by adsorption to surfaces (matric effect)

Microbes Adapt to Changes in Osmotic Concentrations

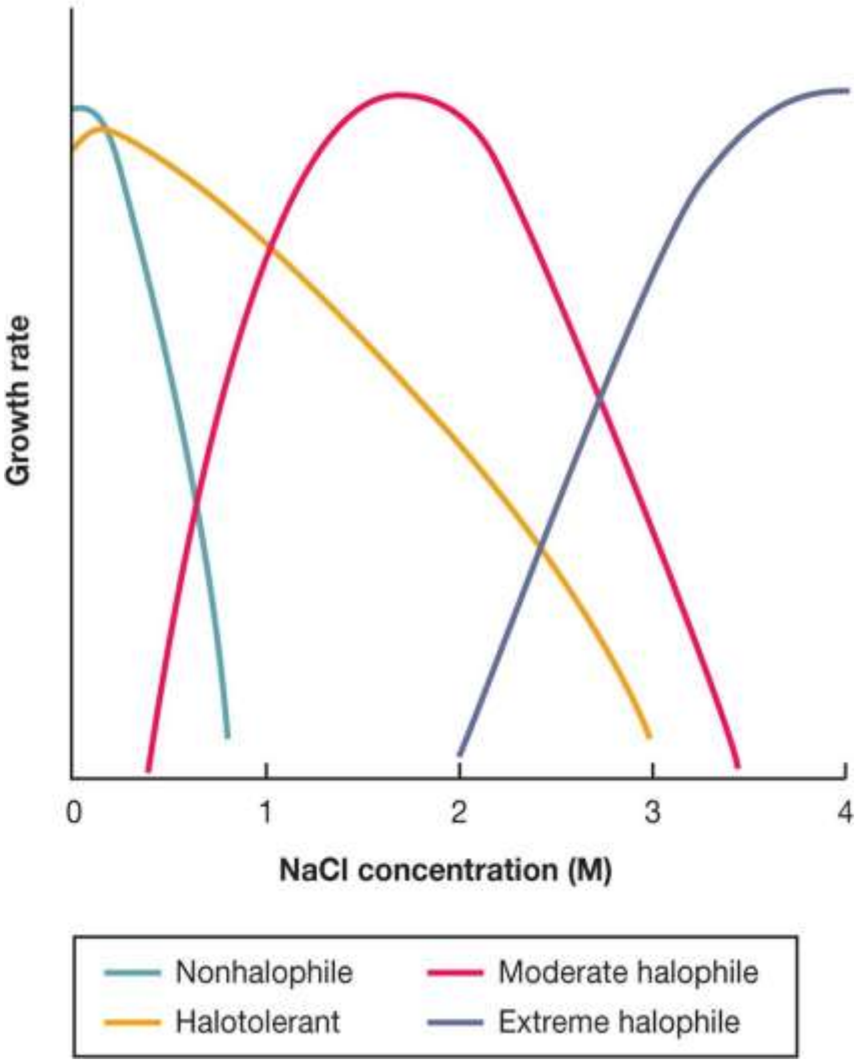
- Reduce osmotic concentration of cytoplasm in hypotonic solutions
  - **mechanosensitive** (MS) channels in plasma membrane allow solutes to leave
- **Increase internal solute concentration with <u>compatible solutes</u> to increase their internal osmotic concentration in hypertonic solutions**
  - solutes compatible with metabolism and growth

Extremely Adapted Microbes

- **Halophiles**
  - grow optimally in the presence of NaCl or other salts at a concentration above about 0.2M
- **Extreme halophiles**
  - require salt concentrations of **2M and 6.2M**
  - extremely high concentrations of potassium
  - cell wall, proteins, and plasma membrane require high salt to maintain stability and activity

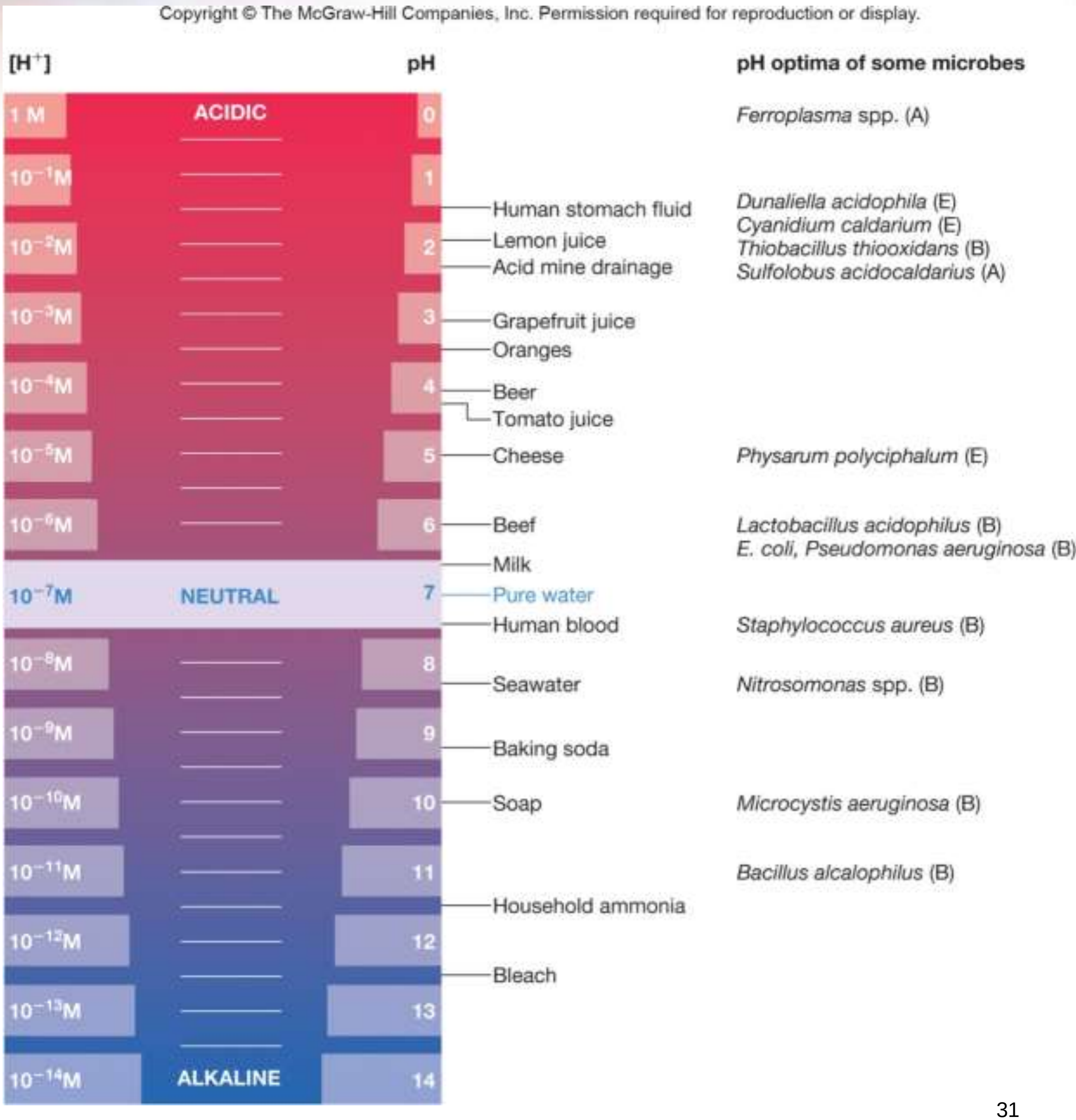
Effects of NaCl on Microbial Growth

- **Halophiles**
  - grow optimally at >0.2 M
- Extreme halophiles
  - require >2 M



pH

- measure of the relative acidity of a solution
- negative logarithm of the hydrogen ion concentration



pH

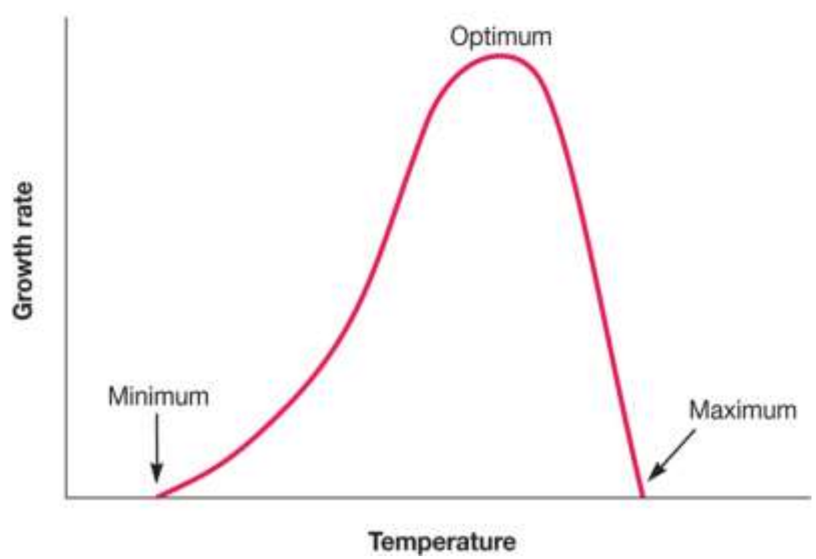
- **Acidophiles**
  - growth optimum between pH 0 and pH 5.5
- **Neutrophiles**
  - growth optimum between pH 5.5 and pH 7
- **Alkaliphiles (alkalophiles)**
  - growth optimum between pH 8.5 and pH 11.5

pH

- Most microbes maintain an internal pH near neutrality
  - the plasma membrane is impermeable(غير نافذ/كتيم) to proton
  - exchange potassium for protons
- **Acidic tolerance response**
  - **pump protons out of the cell**
  - **some synthesize acid and heat shock proteins that protect proteins**
- **Many microorganisms change the pH of their habitat by producing acidic or basic waste products**

Temperature

- Microbes cannot regulate their internal temperature
- Enzymes have optimal temperature at which they function optimally
- High temperatures may inhibit enzyme functioning and be lethal(قاتل)
- Organisms exhibit distinct **cardinal growth** temperatures
  - **minimal**
  - **maximal**
  - **optimal**



Temperature Ranges for Microbial Growth

- psychrophiles – 0º C to 20º C
- psychrotrophs – 0º C to 35º C
- mesophiles – 20º C to 45º C
- thermophiles – 55º C to 85º C
- hyperthermophiles – 85º C to 113º C

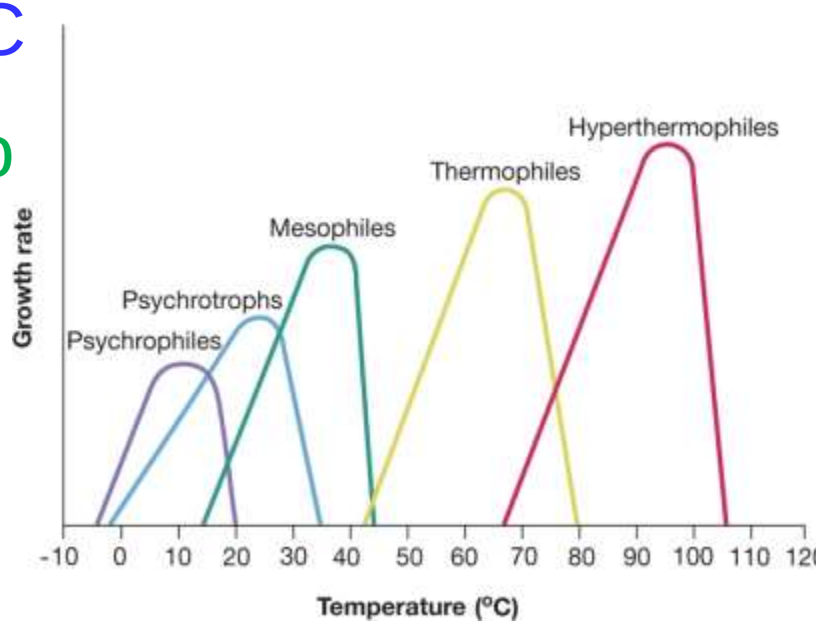


Table 7.3 Temperature Ranges for Microbial Growth

Microorganism	Minimum	Optimum	Maximum
	CARDINAL TEMPERATURES (°C)		
Nonphotosynthetic Bacteria and Archaea			
Bacillus psychrophilus	−10	23–24	28–30
Pseudomonas fluorescens	4	25–30	40
Enterococcus faecalis	0	37	44
Escherichia coli	10	37	45
Neisseria gonorrhoeae	30	35–36	38
Thermoplasma acidophilum	45	59	62
Thermus aquaticus	40	70–72	79
Pyrococcus abyssi	67	96	102
Pyrodictium occultum	82	105	110
Pyrolobus fumarii	90	106	113
Photosynthetic Bacteria			
Anabaena variabilis	ND[1]	35	ND
Synechococcus eximius	70	79	84
Protists			
Chlamydomonas nivalis	−36	0	4
Amoeba proteus	4–6	22	35
Skeletonema costatum	6	16–26	>28
Trichomonas vaginalis	25	32–39	42
Tetrahymena pyriformis	6–7	20–25	33
Cyclidium citrullus	18	43	47
Fungi			
Candida scotti	0	4–15	15
Saccharomyces cerevisiae	1–3	28	40
Mucor pusillus	21–23	45–50	50–58

1 ND, not determined.

Adaptations of Thermophiles

- Protein structure stabilized by a variety of means
  - e.g., more H bonds
  - e.g., more proline
  - e.g., chaperones
- Histone-like proteins stabilize DNA
- Membrane stabilized by variety of means
  - e.g., **more saturated, more branched and higher molecular weight lipids**
  - e.g., **ether linkages(روابط) (archaeal membranes)**

Oxygen Concentration

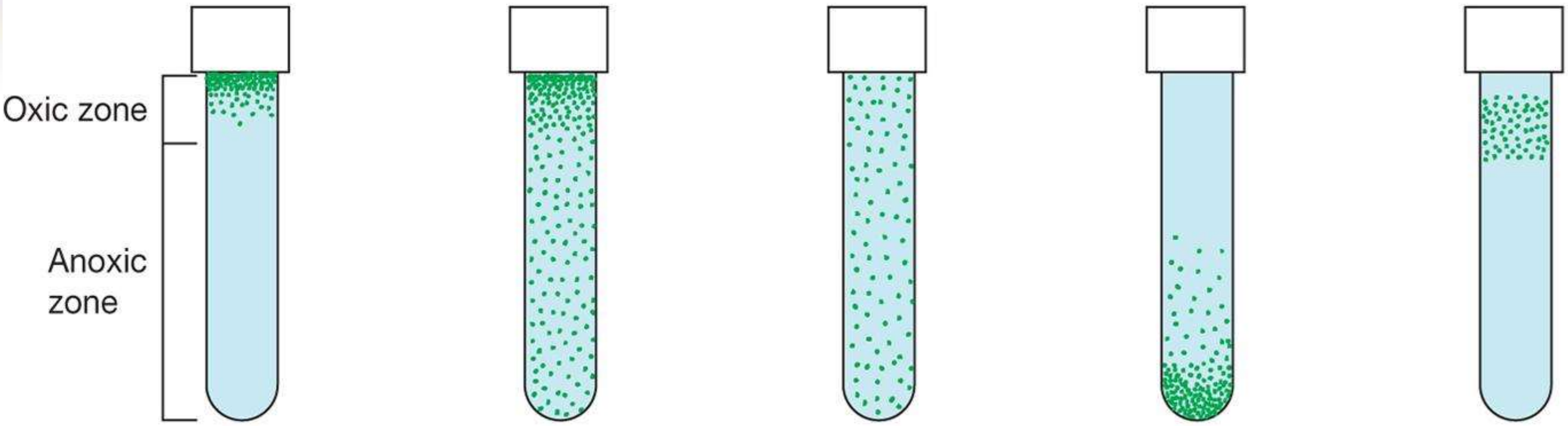
- growth in oxygen correlates with(يرتبط مع) microbes energy conserving metabolic processes and the electron transport chain (ETC) and nature of terminal electron acceptor

Oxygen and Bacterial Growth

- **Aerobe**
  - – grows in presence of atmospheric oxygen (O_2) which is 20% O_2
- **Obligate aerobe** – requires O_2
- Anaerobe
  - – grows in the absence of O_2
- Obligate anaerobe
  - – usually killed in presence of O_2

Oxygen and Bacterial Growth

- **Microaerophiles**
  - requires 2–10% O_2
- **Facultative anaerobes**
  - do not require O_2 but grow better in its presence
- **Aerotolerant anaerobes**
  - grow with or without O_2



Obligate aerobe	Facultative anaerobe	Aerotolerant anaerobe	Strict anaerobe	Microaerophile

Enzyme content

+ SOD + Catalase	+ SOD + Catalase	+ SOD – Catalase	– SOD – Catalase	+ SOD +/– Catalase (low levels)

Basis of Different Oxygen Sensitivities

- Oxygen easily reduced to toxic reactive oxygen species (ROS)
  - superoxide radical
  - hydrogen peroxide
  - hydroxyl radical
- **Aerobes produce protective enzymes**
  - **superoxide dismutase (SOD)**
  - **catalase**
  - **peroxidase**

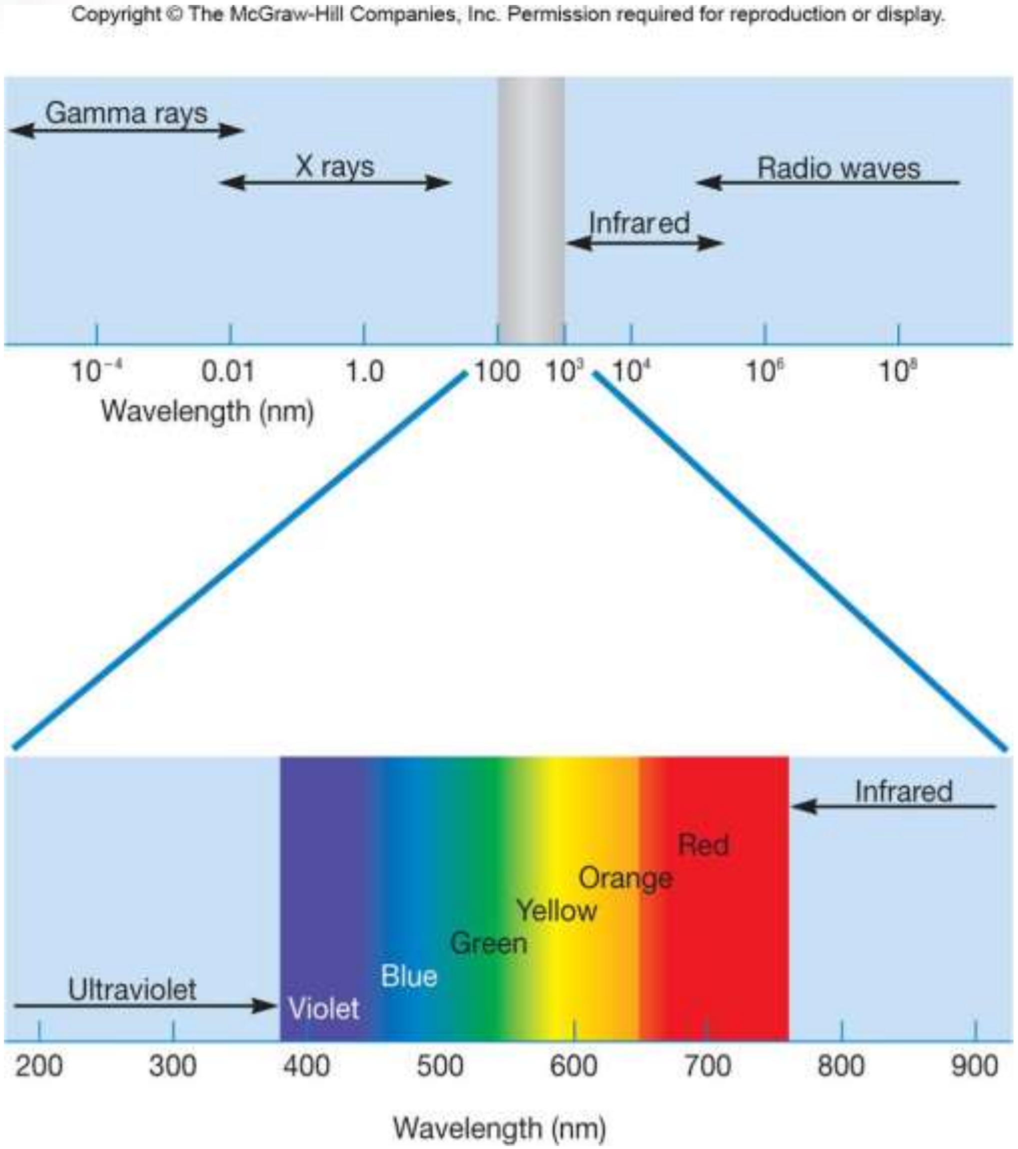
Strict Anaerobic Microbes

- All **strict anaerobic microorganisms lack or have very low quantities of**
  - **superoxide dismutase**
  - **catalase**
- These microbes cannot tolerate O_2
- Anaerobes must be grown without O_2
  - **work station with incubator**
  - **gaspak anaerobic system**

Pressure

- Microbes that live on land and water surface live at 1 atmosphere (atm)
- Some *Bacteria* and *Archaea* live **in deep sea with very high hydrostatic pressures**
- **Barotolerant**
  - adversely affected by increased pressure, but not as severely as nontolerant organisms
- **Barophilic** (peizophilic) organisms
  - **require or grow more rapidly in the presence of increased pressure**
  - **change membrane fatty acids to adapt to high pressures**

- The Electromagnetic Spectrum



Radiation Damage

- Ionizing radiation
  - x-rays and gamma rays
  - **mutations → death (sterilization(تعقيم))**
  - Disrupts(تعطيل) chemical structure of many molecules, including DNA
    - **damage may be repaired by DNA repair mechanisms if small dose**
  - *Deinococcus radiodurans*
    - extremely resistant to DNA damage

Radiation Damage…

- Ultraviolet (UV) radiation
  - wavelength most effectively absorbed by DNA is 260 nm
  - **mutations → death**
  - causes formation of **thymine dimers** in DNA
  - requires **direct exposure** on microbial surface
  - DNA damage can be repaired by several repair mechanisms

Radiation Damage…

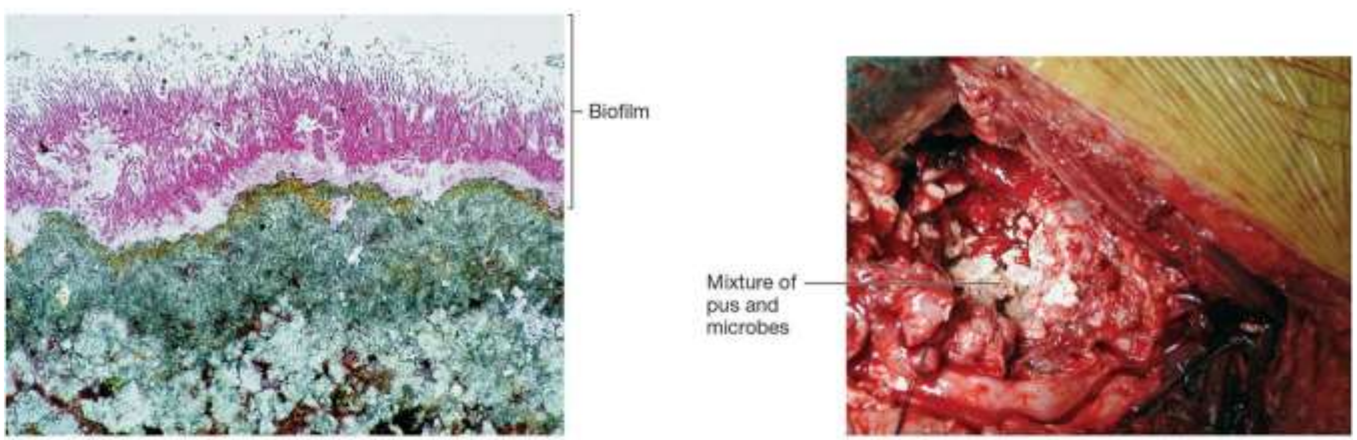
- **Visible light**
  - **at high intensities generates singlet oxygen ($^1O_2$)**
    - powerful oxidizing agent
  - **carotenoid pigments**
    - protect many light-exposed microorganisms from photooxidation

Microbial Growth in Natural Environments

- Microbial environments are complex, constantly changing, often contain low nutrient concentrations (oligotrophic environment) and may expose a microorganism to overlapping gradients of nutrients and environmental factors

Biofilms

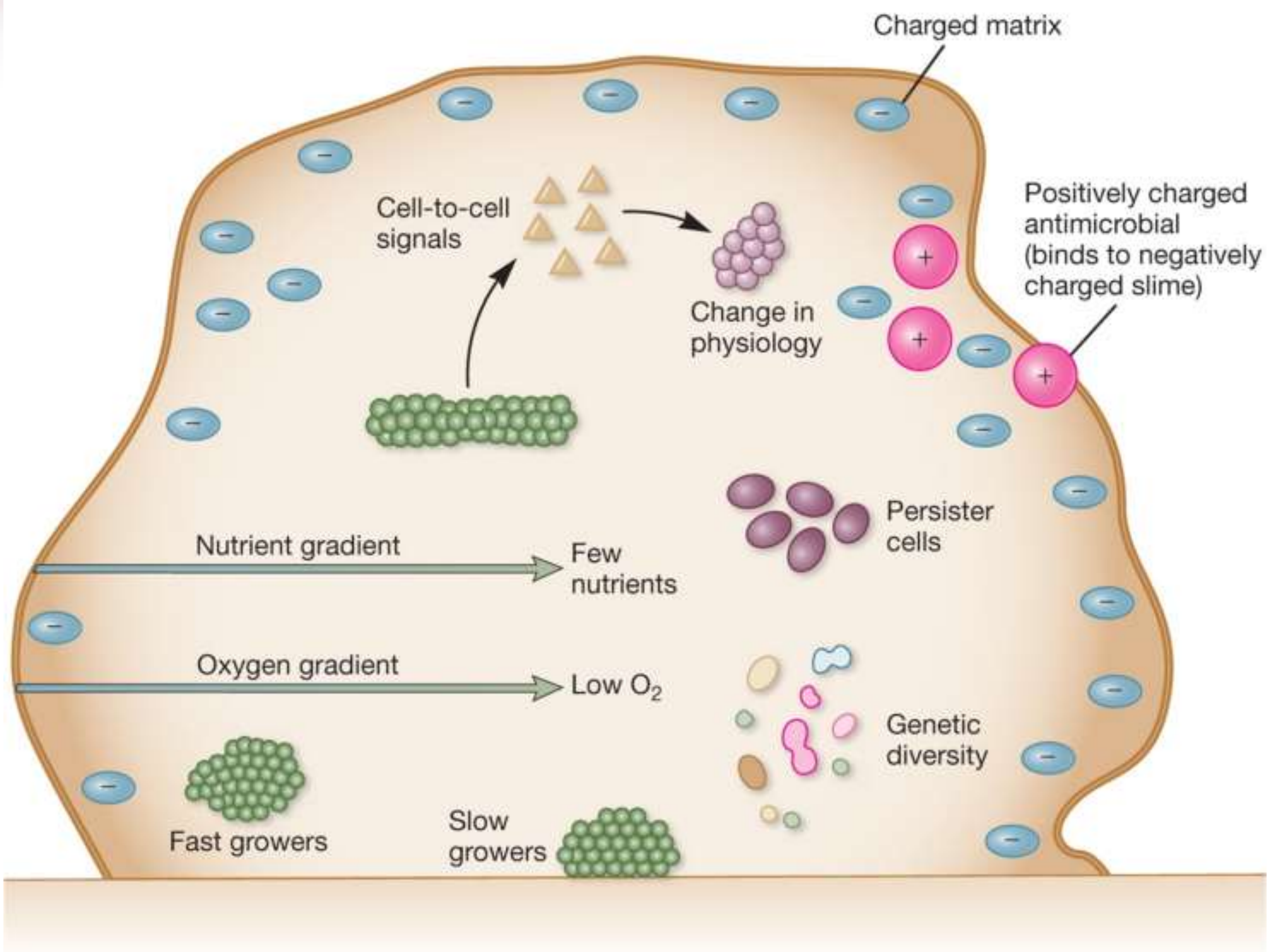
- Most microbes grow attached to surfaces (**sessile**) rather than free floating (planktonic)
- These attached microbes are members of complex, slime enclosed communities called a **biofilm**
- Biofilms are ubiquitous in nature in water
- Can be formed on any conditioned surface



(a) Biofilm on surface of a stromatolite

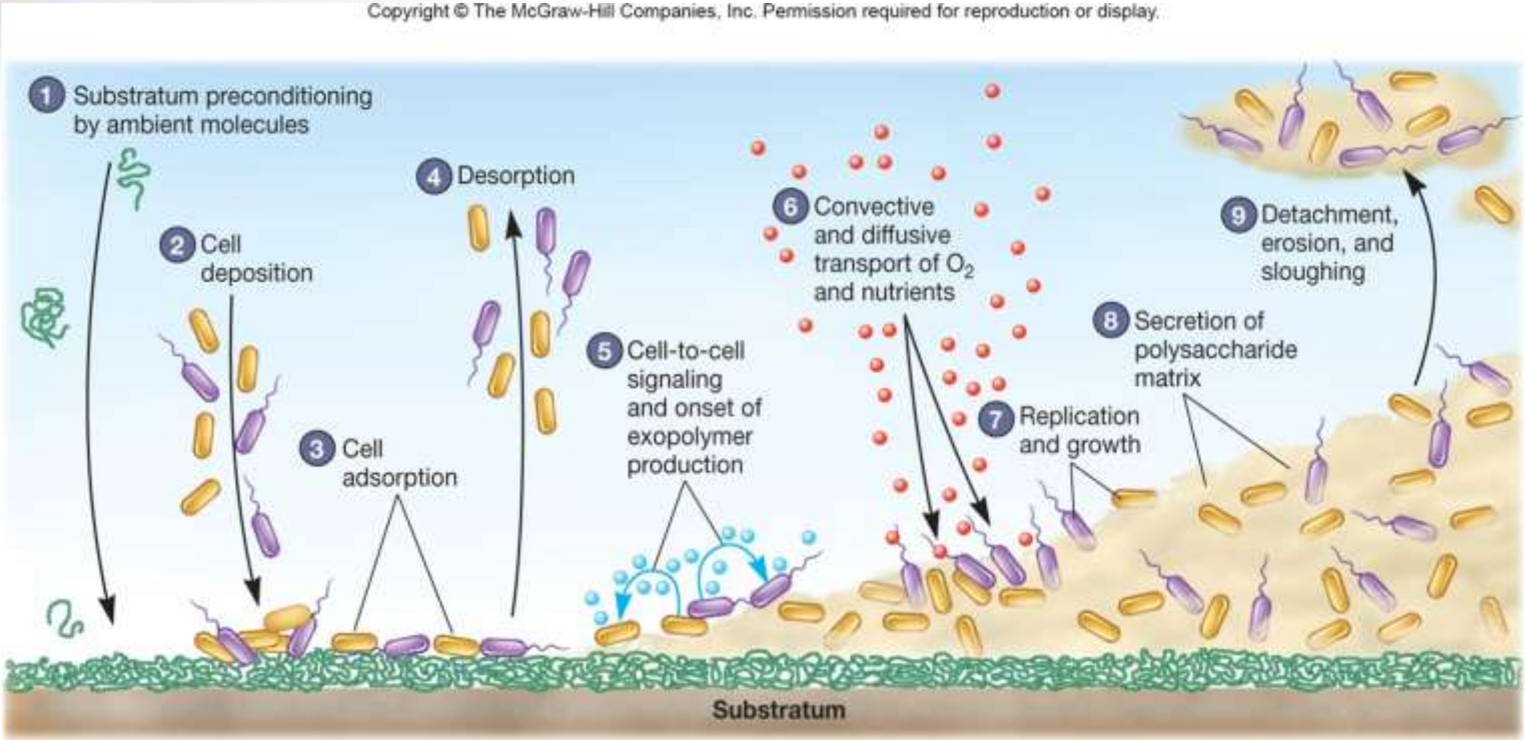
(b) Infected tissue after hip replacement

a: Dr. Joachim Reitner; b: Courtesy Nicholas G. Sotereanos, M.D.



Charged matrix
Cell-to-cell signals
Change in physiology
Positively charged antimicrobial (binds to negatively charged slime)
Persister cells
Nutrient gradient
Few nutrients
Oxygen gradient
Low O_2
Genetic diversity
Fast growers
Slow growers

Biofilm Formation



- Microbes reversibly attach to conditioned surface and release **polysaccharides, proteins, and DNA** to form the extracellular polymeric substance (EPS)
- Additional polymers are produced as microbes reproduce and biofilm matures

Biofilms

- a mature biofilm is a complex, **dynamic community of microorganisms**
- **heterogeneity** is differences in metabolic activity and locations of microbes
- interactions occur among the attached organisms
  - exchanges take place metabolically, DNA uptake and communication

Biofilm Microorganisms

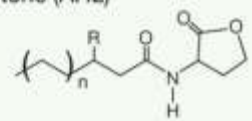
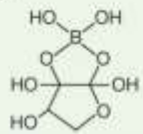
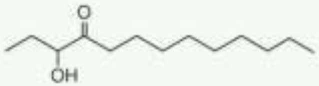
- The **EPS** and change in attached organisms' physiology protects microbes from harmful agents
  - – UV light, antibiotics, antimicrobials
- **When formed on medical devices, such as implants, often lead to illness**
- **Sloughing off(تخلي عن/نزع) of organisms can result in contamination of water phase above the biofilm such as in a drinking water system**

Cell to Cell Communication Within the Microbial Populations

- Bacterial cells in biofilms communicate in a density-dependent manner called **quorum sensing**
- Produce small proteins that increase in concentration as microbes replicate and convert a microbe to a **competent state**
  - DNA uptake occurs, bacteriocins are released

Quorum Sensing

- **Acylhomoserine lactone (AHL)** is an autoinducer molecule produced by many gram-negative organisms
  - diffuses across plasma membrane
  - once inside the cell, induces expression of target genes regulating a variety of functions

Signal and Structure	Representative Organism	Function Regulated
N-acylhomoserine lactone (AHL) 	*Vibrio fischeri* *Agrobacterium tumefaciens* *Erwinia carotovora* *Pseudomonas aeruginosa* *Burkholderia cepacia*	Bioluminescence Plasmid transfer Virulence and antibiotic production Virulence and biofilm formation Virulence
Furanosylborate (AI-2) 	*Vibrio harveyi*[a]	Bioluminescence
Cyclic thiolactone (AIP-II) 	*Staphylococcus aureus*	Virulence
Hydroxy-palmitic acid methyl ester (PAME) 	*Ralstonia solanacearum*	Virulence
Methyl dodecenoic acid 	*Xanthomonas campestris*	Virulence
Farnesoic acid 	*Candida albicans*	Dimorphic transition and virulence
3-hydroxytridecan-4-one 	*Vibrio cholerae*	Virulence

[a] Other bacteria make a form of AI-2 that lacks boron.

7.8 Continuous culture of microorganisms

1. Distinguish batch culture and continuous culture
2. Differentiate chemostats and turbidostats
3. Discuss the relationship between the dilution rate of a chemostat and population size and growth rate

The Continuous Culture of Microorganisms

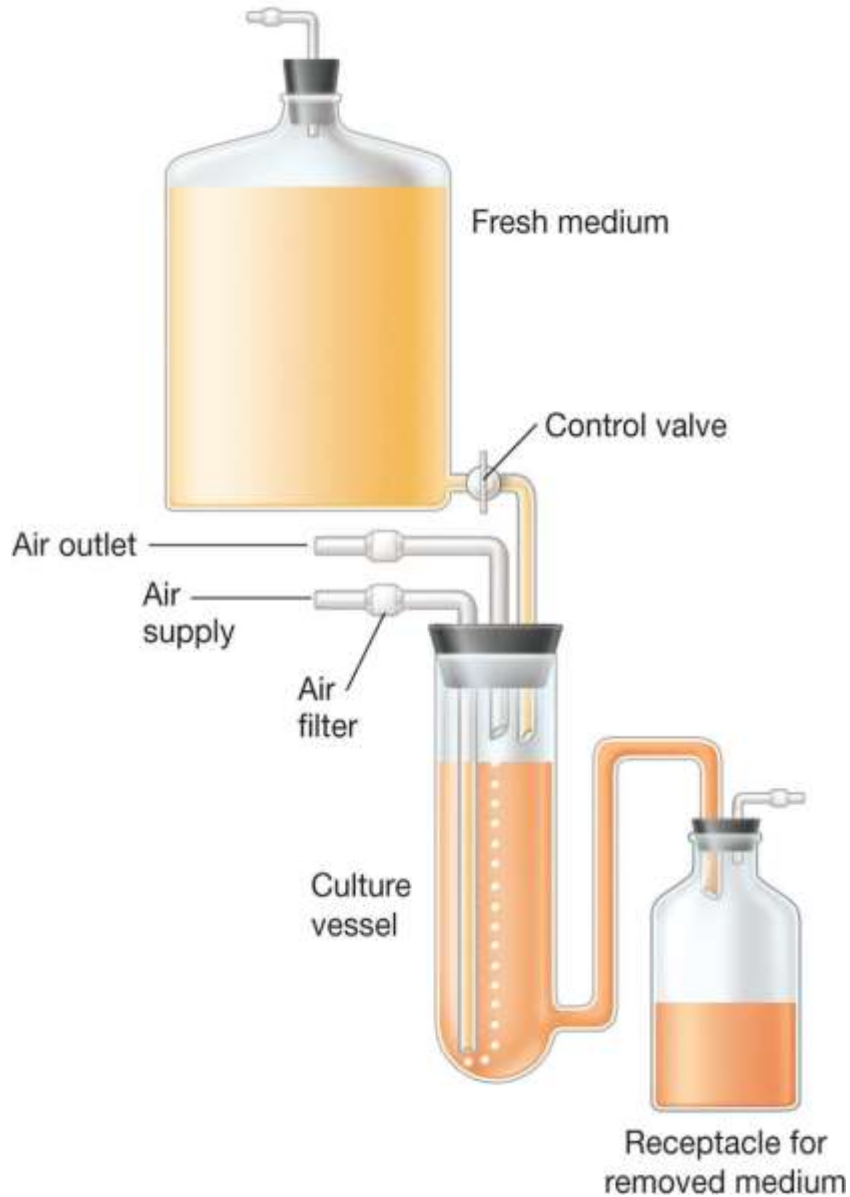
- **Growth in an open system**
  - continual provision of nutrients
  - continual removal of wastes
- **Maintains cells in log phase at a constant biomass concentration for extended periods**
- Achieved using a continuous culture system

Importance of Continuous Culture Methods

- **Constant supply of cells in exponential phase growing at a known rate**
- Study of microbial **growth at very low nutrient concentrations, close to those present in natural environment**
- Study of interactions of microbes under conditions resembling(تشبه) those in aquatic environments
- Food and industrial microbiology

The Chemostat

- Rate of incoming medium = rate of removal of medium from vessel
- An essential nutrient is in limiting quantities



Dilution Rate and Microbial Growth

dilution rate – rate at which medium flows through vessel relative to vessel size

note: cell density maintained at wide range of dilution rates and chemostat operates best at low dilution rate