

Chapter 5: Microbial growth characteristics

5.1. Introduction

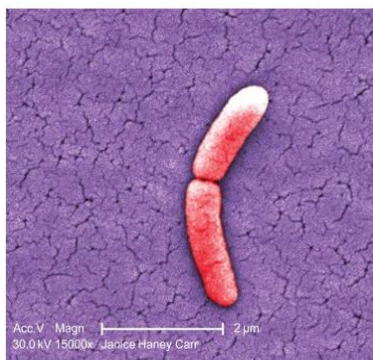
Microbial growth refers to the increase in cell numbers or mass when microorganisms are exposed to favorable environments such as food. Understanding microbial growth is essential because it is directly linked to: food spoilage, foodborne diseases and food bioprocessing (fermentation). Knowledge of growth characteristics helps in isolating unknown microbial strains, studying their properties, and designing methods to control, stimulate, or destroy microorganisms in food.

5.2. Microbial reproduction or growth

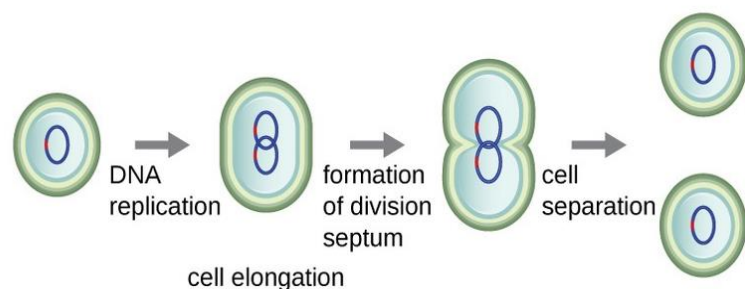
5.2.1. Binary fission

The most common mechanism of cell replication in bacteria is a process called binary fission, which is depicted in Figure 1. Before dividing, the cell grows and increases its number of cellular components. Next, the replication of DNA starts at a location on the circular chromosome called the origin of replication, where the chromosome is attached to the inner cell membrane. Replication continues in opposite directions along the chromosome until the terminus is reached.

In bacteria, a form of sexual recombination can occur that involves transfer of genetic materials from a donor to a recipient cell (e.g., conjugation). However, this is quite different from sexual reproduction, which is facilitated through the union of two cells (gametes) of opposite mating type.



(a)



(b)

Figure 1. (a) The electron micrograph depicts two cells of *Salmonella typhimurium* after a binary fission event. (b) Binary fission in bacteria starts with the replication of DNA as the cell elongates. A division septum forms in the center of the cell. Two daughter cells of similar size form and separate, each receiving a copy of the original chromosome. (credit a: modification of work by Centers for Disease Control and Prevention)

Table 1 : Cell arrangements after division.

Arrangement	Description	Example
Chains	Cells divide in one plane and remain attached	<i>Streptococcus, Bacillus</i>
Clusters	Cells divide in multiple planes and stay together	<i>Staphylococcus</i>
Tetrads	Four-cell arrangements	<i>Pediococcus</i>

Yeasts and molds can also reproduce asexually. A yeast cell produces a bud that initially is much smaller in size and remains attached to the surface of the original cell. As it grows in size, it also can produce a bud, giving an appearance of a chain of buds growing on the surface of the original yeast cell. A yeast cell can have several buds forming on its surface. As the buds mature, they separate from the original cell. Molds can grow in size by cell division or elongation at the tip of a hypha. They can also form large numbers of asexual spores on specialized structure. Both yeasts and molds can also reproduce sexually. Viruses do not reproduce by themselves. Instead, they attach on the surface and inject their nucleic acid inside specific host cells, which then replicate the viral nucleic acid and produce viral particles. The viral particles are released into the environment following lysis of the host cells. Viruses associated with foodborne diseases do not increase in numbers by replication in food. However, if the specific host bacterial cells and the bacteriophages are present in a food, replication can occur.

5.2.2. Generation time (G)

In prokaryotes (Bacteria and Archaea), the generation time is also called the doubling time and is defined as the time it takes for the population to double through one round of binary fission. Bacterial doubling times vary enormously. Whereas *Escherichia coli* can double in as little as 20 minutes under optimal growth conditions in the laboratory, bacteria of the same species may need several days to double in especially harsh environments. Most pathogens grow rapidly, like *E. coli*, but there are exceptions. For example, *Mycobacterium tuberculosis*, the causative agent of tuberculosis, has a generation time of between 15 and 20 hours. On the other hand, *M. leprae*, which causes Hansen's disease (leprosy), grows much more slowly, with a doubling time of 14 days.

✓ *Calculating number of cells*

It is possible to predict the number of cells in a population when they divide by binary fission at a constant rate. As an example, consider what happens if a single cell divides every 30 minutes for 24 hours. The diagram in Figure 3 shows the increase in cell numbers for the first three generations.

Number of generations (n)	Number of cells	Each division adds two new cells
0	1	
1	2	
2	4	
3	8	

Figure 3. The parental cell divides and gives rise to two daughter cells. Each of the daughter cells, in turn, divides, giving a total of four cells in the second generation and eight cells in the third generation. Each division doubles the number of cells.

The number of cells increases exponentially and can be expressed as 2^n , where n is the number of generations. If cells divide every 30 minutes, after 24 hours, 48 divisions would have taken place. If we apply the formula 2^n , where n is equal to 48, the single cell would give rise to 2^{48} or 281,474,976,710,656 cells at 48 generations (24 hours). When dealing with such huge numbers, it is more practical to use scientific notation. Therefore, we express the number of cells as 2.8×10^{14} cells.

In our example, we used one cell as the initial number of cells. For any number of starting cells, the formula is adapted as follows:

$$N_t = N_0 \times 2^n$$

Where:

N_t = Number of bacteria at time t

N_0 = Initial number of bacteria at time 0

n = Number of generations (the number of times the population doubled)

From this, the number of generations (n) can be solved for:

$$n = \frac{\log(N_t) - \log(N_0)}{\log(2)} = 3.3 \times \log\left(\frac{N_t}{N_0}\right)$$

Finally, the generation time (G) is the total time (t) divided by the number of generations (n):

$$G = \frac{t}{n} = \frac{t}{3.3 \times \log(N_t/N_0)}$$

5.2.3. The growth curve

Microorganisms grown in closed culture (also known as a batch culture), in which no nutrients are added and most waste is not removed, follow a reproducible growth pattern referred to as the growth curve. An example of a batch culture in nature is a pond in which a small number of cells grow in a closed environment. The culture density is defined as the number of cells per unit volume. In a closed environment, the culture density is also a measure of the number of cells in the population. Infections of the body do not always follow the growth curve, but correlations can exist depending upon the site and type of infection. When the number of live cells is plotted against time, distinct phases can be observed in the curve.

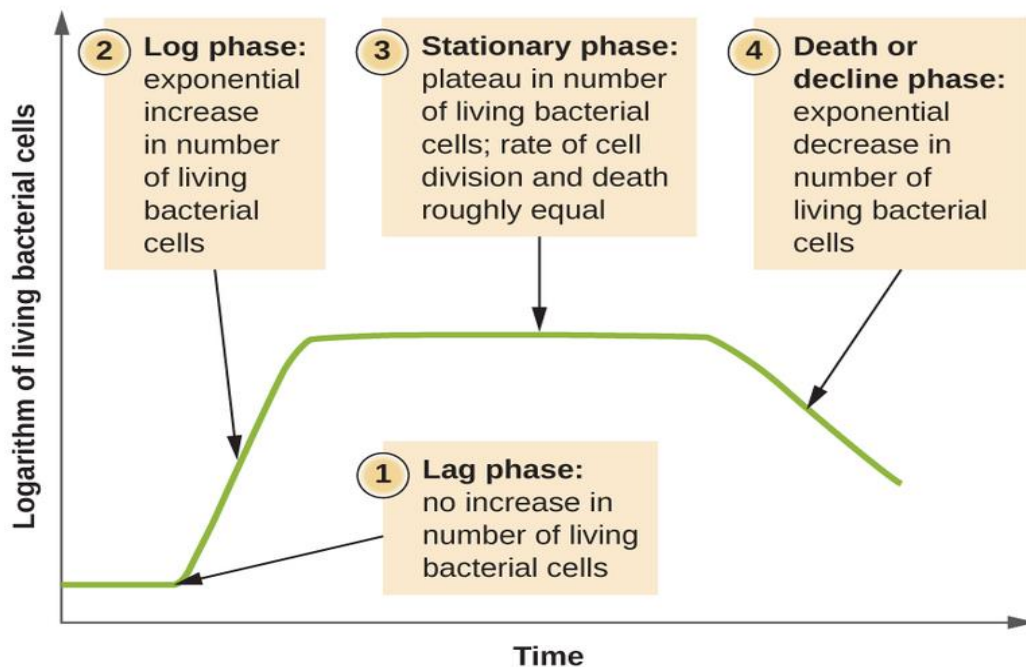


Figure 4. The growth curve of a bacterial culture is represented by the logarithm of the number of live cells plotted as a function of time. The graph can be divided into four phases according to the slope, each of which matches events in the cell. The four phases are lag, log, stationary, and death.

- ✓ **Lag phase:** Inoculated cells adjust to the new medium. Cell numbers do not increase, but cells enlarge, become metabolically active, synthesize proteins, and repair transfer damage. Duration depends on species/genetics, medium composition, and inoculum size.
- ✓ **Log (exponential) phase:** Cells divide actively by binary fission, so numbers increase exponentially. Growth rate and metabolic activity are constant; generation time is genetically determined under given conditions. Cells are preferred for research/industry and are most susceptible to disinfectants and antibiotics targeting protein, DNA, and cell-wall synthesis.
- ✓ **Stationary phase:** Growth slows and then plateaus because waste accumulates, nutrients are depleted, and oxygen becomes limiting. New cell formation equals cell

death, so live population density is constant at carrying capacity. Cells adopt survival metabolism, become less susceptible to antibiotics targeting synthesis, may sporulate, produce secondary metabolites/antibiotics, and express virulence factors (e.g., quorum sensing in *S. aureus*).

- ✓ **Death (decline) phase:** Toxic waste accumulation and nutrient exhaustion cause deaths to exceed divisions, leading to exponential decline. Many cells lyse and release nutrients; survivors may maintain viability or form endospores. Persister cells have slow metabolism and are medically important in chronic infections such as tuberculosis.

5.2.4. Specific growth rate μ

The specific growth rate μ (often expressed in h^{-1} or min^{-1}) is the rate at which a bacterial population grows per unit of biomass or per cell during the exponential growth phase.

✓ **Basic formula**

During exponential growth: $N_t = N_0 e^{\mu t}$

where: N_t = number of bacteria at time t

N_0 = initial number of bacteria

μ = specific growth rate

t = time

Taking the natural logarithm: $\ln(N_t) = \ln(N_0) + \mu t$

Therefore:

$$\mu = \frac{\ln(N_t) - \ln(N_0)}{t}$$

or, between two time points t_1 and t_2 :

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1}$$

This is the slope of the line obtained by plotting $\ln(N)$ versus time.

Relationship with generation time G :

$$\mu = \frac{\ln(2)}{G} \approx \frac{0.693}{G}$$

5.3. Nature of microbial growth in food

5.3.1. Mixed population

Food normally contains a mixed population of bacteria, yeasts, and molds. Growth characteristics differ from pure cultures.

- Species/strains best adapted to the food environment (intrinsic and extrinsic) will outgrow others and become predominant
- This can occur even if they are initially present in low numbers
- Example: Refrigerated storage selects for psychrotrophic (cold-tolerant) microorganisms over mesophiles
- If two species grow optimally but one has a shorter generation time, the faster-growing one becomes predominant
- Bacteria often spoil foods faster than yeasts and molds because they generally have shorter generation times

5.3.2. Sequence of growth

Different species can become predominant in sequence during storage.

Mechanism:

1. Initially, one or two types grow optimally
2. Their growth changes the environment (e.g., consumes oxygen, produces metabolites)
3. The changed environment becomes favorable for a different type
4. This shift can occur several times during storage

Example: Ground meat in a bag: Aerobes grow first and consume oxygen → environment becomes anaerobic → anaerobes/facultative anaerobes grow

5.3.3. Growth in succession (Diauxic growth)

Growth in stages separated by a short lag phase when a microorganism metabolizes two or more nutrients, one preferred over the other.

Mechanism:

1. First growth phase: utilizes the preferred nutrient
2. Short lag phase: adaptation
3. Second growth phase: utilizes the less preferred nutrient

Example: Certain lactic acid bacteria and Gram-negative bacteria in fresh meat: first: utilize limiting carbohydrates. Then: utilize nonprotein nitrogenous (NPN) substances (e.g., amino acids). Each stage shows exponential and stationary phases with a lag phase in between.

5.3.4. Symbiotic growth

Mutual benefit during growth between two or more microbial types.

Mechanism:

- One type produces metabolic products needed by the second type
- The second type produces nutrients that stimulate the first type

Example : Yogurt production

- *Streptococcus thermophilus* hydrolyzes milk proteins → produces amino acids → stimulates *Lactobacillus delbrueckii* subsp. *bulgaricus*
- *Lactobacillus* produces formate → stimulates *Streptococcus* growth
- Both are necessary for a desirable product

5.3.5. Synergistic growth

A type of symbiotic growth where each microorganism can grow independently, but together they show greatly increased growth rate and by-product formation; more than the additive amounts produced separately.

Example : Yogurt

- *Str. thermophilus* alone: ~8–10 ppm acetaldehyde
- *Lab. delbrueckii* subsp. *bulgaricus* alone: ~8–10 ppm acetaldehyde
- Together: 30 ppm or more acetaldehyde (much higher than additive)
- Acetaldehyde is the desirable flavor component of yogurt

Application: Two separate strains/species can be used to induce synergistic growth in fermented food production.

5.3.6. Antagonistic growth

Two or more microorganisms adversely affect each other's growth; one may interfere with or kill the other.

Mechanisms:

- Production of antimicrobial compounds (e.g., bacteriocins)
- Production of wall-degrading enzymes

Examples:

- Some Gram-positive bacteria produce bacteriocins that kill other Gram-positive bacteria
- Some yeasts produce wall-degrading enzymes that reduce mold growth