3

NUTRITION AND GROWTH OF BACTERIA

3.1 INTRODUCTION

Bacteria are prokaryotic organisms that do not contain chlorophyll. They are unicellular and do not show true branching. They differ from eukaryotes in not having a nuclear membrane, a nucleolus, and cell organelles like mitochondria, golgi apparatus and endoplasmic reticulum. They have a single circular chromosome.

This chapter will deal with the growth and multiplication of bacteria and their requirements for the same. It will deal with the energy requirements and their ability to synthesise essential metabolites.

OBJECTIVES

After reading this lesson, you will be able to:

- discuss salient aspects of nutrition, gaseous requirements, temperature requirements and other physiological requirements for growth
- describe how bacteria grow and multiply
- list the salient features of the bacterial growth curve

3.2 GROWTH OF BACTERIA

3.2.1 Bacterial nutrition

The bacterial cell has the same general chemical pattern as the cells of other organisms. The bacterial cell contains water (80% of total weight), proteins, polysaccharides, lipids, nucleic acids, mucopeptides and low molecular weight compounds.

For growth and nutrition of bacteria, the minimum nutritional requirements are water, a source of carbon, a source of nitrogen and some inorganic salts. Water is the vehicle of entry of all nutrients into the cell and for the elimination of waste products.

Bacteria can be classified nutritionally based on their energy requirements and on their ability to synthesise essential metabolites. Bacteria which derive energy from sunlight are called **phototrophs**. Those that obtain energy from chemical reactions are called **chemotrophs**. Bacteria that can synthesise all their organic compounds are called **autotrophs**. They are able to use atmospheric carbon dioxide and nitrogen. They are capable of independent existence in water and soil. They are of no medical importance. Some bacteria are unable to synthesise their own metabolites. They depend on preformed organic compounds. They are called **heterotrophs**. These bacteria are unable to grow with carbon dioxide as the sole source of carbon. Their nutritional requirements vary widely. Some may require only a single organic substance like glucose. Others may need a large number of different compounds like amino acids, nucleotides, lipids, carbohydrates and coenzymes.

Bacteria require a supply of inorganic salts. They require anions like phosphate and sulphate anions and cations like sodium, potassium, magnesium, iron and calcium. Some ions like cobalt may be required in trace amounts.

Some bacteria require certain organic compounds in minute quantities. These are called **growth factors** or bacterial vitamins. Growth factors are called **essential** when growth does not occur in their absence. **Accessory** growth factors are those which enhance growth without being absolutely necessary for it. In many cases, bacterial vitamins are same as vitamins necessary for nutrition of mammals, for example, B group vitamins – thiamine, riboflavin, pyridoxine, nicotinic acid, folic acid and vitamin B12.

3.2.2 Gaseous Requirements

Depending on the influence of oxygen on growth and survival, bacteria are divided into **aerobes** and **anaerobes**. Aerobic bacteria require oxygen for growth. They may be **obligate aerobes** or **facultative anaerobes**. **Obligate aerobes** grow only in the presence of oxygen, for eg. Cholera bacillus. **Facultative anaerobes** are ordinarily aerobic but can grow in the absence of oxygen, though less abundantly. Most bacteria of medical importance are facultative anaerobes. **Anaerobic bacteria**, such as clostridia grow in the absence of oxygen. **Obligate anaerobes** may even die on exposure to oxygen. **Microaerophilic** bacteria are those that grow best in the presence of low oxygen tension.

In case of aerobes, atmospheric oxygen is the final electron acceptor in the process of respiration (aerobic respiration). In this case, the carbon and energy source may be completely oxidised to carbon dioxide and water. Energy is provided by the production of energy-rich phosphate bonds and the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP). This process is called **oxidative phosphorylation**.

Anaerobic bacteria use compounds like nitrates or sulphates instead of oxygen as final electron acceptors in the process of respiration (anaerobic respiration). A more common process used by these bacteria in anaerobic metabolism is **fermentation**. It is defined as the process by which complex organic compounds, such as glucose, are broken down by the action of enzymes into simpler compounds without the use of oxygen. This process leads to the formation of several organic end products such as organic acids and alcohols, as well as of gas (carbon dioxide and hydrogen). For example, *Escherichia coli* ferments glucose with the production of acid and gas. It also ferments lactose. During the process of fermentation, energy-rich phosphate bonds are produced by the introduction of organic phosphate into intermediate metabolites. This process is known as **substrate-level phosphorylation**. The energy-rich phosphate groups so formed are used for conversion of ADP to ATP.

All bacteria require some amounts of carbon dioxide for growth. This is obtained from the atmosphere or from the cellular metabolism of the bacterial cell. Some bacteria like *Brucella abortus* require much higher levels of carbon dioxide (5-10%) for growth. They are called **capnophilic**.

3.2.3 Temperature Requirements

Bacteria vary in their requirement of temperature for growth. For each species, there is a **"temperature range"**, and growth does not occur above the maximum or below the minimum of this range. The temperature at which growth occurs best is known as the **"optimum temperature"**. In the case of most pathogenic bacteria, the optimum temperature is 37ºC.

Bacteria which grow best at temperatures of 25-40ºC are called **mesophilic**, for example *Escherichia coli*. **Psychrophilic** bacteria are those that grow best at temperatures below 20ºC. They are soil and water saprophytes and may cause spoilage of refrigerated food. Thermophilic bacteria are those which grow best at high temperatures, 55-80ºC. They may cause spoilage of underprocessed canned food. Some thermophiles, for example *Geobacillus stearothermophilus*, form spores that are highly thermoresistant.

3.2.4 Other physiological Requirements

Moisture and drying, hydrogen ion concentration, light, osmotic effect and mechanical and sonic stress may also influence the growth and multiplication of bacteria.

3.2.5 Growth and Multiplication of Bacteria

Bacteria divide by binary fission. When a bacterial cell reaches a certain size, it divides to form two daughter cells. Nuclear division is followed by cell division.

The interval of time between two cell divisions, or the time required for a bacterium to give rise to two daughter cells under optimum conditions, is called the generation time or the population doubling time. In *Escherichia coli* and many other medically important bacteria, the generation time is about 20 minutes. Some bacteria are slow-growing. The generation time in tubercle bacilli is about 20 hours. In lepra bacilli, it is as long as about 20 days.

When bacteria are grown in a vessel of liquid medium, multiplication is arrested after a few cell divisions due to depletion of nutrients or accumulation of toxic products. This is a **batch culture**. By the use of special devices for replenishing nutrients and removing bacterial cells (chemostat or turbidostat), it is possible to maintain a **continuous culture** of bacteria for industrial or research purposes. When bacteria multiply in host tissues, the situation may be intermediate between a batch culture and a continuous culture. The source of nutrients may be inexhaustible but the bacteria have to fight the defence mechanisms of the host. Bacteria growing on solid media (for example blood agar, MacConkey agar) form colonies. Each colony represents a cluster of cells derived from a parent cell. In liquid media, growth is diffuse.

Bacterial growth may be considered at two levels: increase in the size of the bacterial cell and increase in the number of cells. Growth in numbers can be studied by bacterial counts. Two types of bacterial counts can be made: total count and viable count. The total count gives the total number of cells in the sample, irrespective of whether they are living or not. It can be done by various methods, for example direct counting under the microscope using counting chambers. The viable count measures the number of living cells, that is, cells capable of multiplication. Viable counts are obtained by dilution or plating methods. In the dilution method, the suspension, whose cell count is to be determined, is serially diluted. The dilutions are made to the point beyond which unit quantities do not yield growth when inoculated into suitable liquid media. Each dilution is inoculated into the respective tubes containing liquid media. The viable count is statistically evaluated from the number of tubes showing growth. This method is not accurate but is used for the estimation of "presumptive coliform count" in drinking water. The presumptive coliform count is a method of estimating the level of pollution of drinking water. In the plating method, appropriate dilutions are inoculated on solid media, either on the surface of plates or as pour plates. The number of colonies that develop after incubation gives an estimate of the viable count. The method commonly employed is that described by Miles and Misra (1938) in which serial dilutions are dropped on the surface of dried plates and colony counts obtained.

3.2.6 Bacterial growth curve

When a bacterium is seeded into a suitable liquid medium and incubated, its growth follows a definite course. If bacterial counts are made at intervals after inoculation and plotted in relation to time, a growth curve is obtained. The curve shows the following phases:

Lag phase: Immediately following the seeding of a culture medium, there is no appreciable increase in number, though there may be an increase in the size of the cells. This initial period is the time required for adaptation to the new environment. The necessary enzymes and metabolic intermediates are built up in adequate quantities for multiplication to proceed. The maximum cell size is obtained towards the end of lag phase. The duration of the lag phase varies with the species, size of the inoculum, nature of the culture medium and environmental factors such as temperature.

Log (logarithmic) or exponential phase: Following the lag phase, the cells start dividing and their numbers increase exponentially or by geometric progression with time. If the logarithm of the viable count is plotted against time, a straight line will be obtained. In this phase, cells are smaller and stain uniformly.

Stationary phase: After a varying period of exponential growth, cell division stops due to depletion of nutrients and accumulation of toxic products. The number of new cells formed is just enough to replace the number of cells that die. Equilibrium exists between the dying cells and the newly formed cells. So, the viable count remains stationary. In this phase, cells are frequently gram variable and show irregular staining. Sporulation occurs at this stage.

Phase of decline: This is the phase when the population decreases due to cell death. Besides nutritional exhaustion and toxic accumulation, cel death may also be caused by autolytic enzymes.

INTEXT QUESTIONS 3.1

1. Bacteria that can synthesise all their organic compounds are called:

   A. Phototrophs　　B. Chemotrophs

   C. Autotrophs　　D. heterotrophs

2. Organisms that are ordinarily aerobic but can grow in the absence of oxygen are called:

   A. Aerobes　　B. Facultative anaerobes

   C. Anaerobes　　D. Obligate anaerobes

3. The process by which complex organic compounds, such as glucose, are broken down by the action of enzymes into simpler compounds without the use of oxygen is called:

   A. Fermentation B. Substrate-level phosphorylation

   C. Oxidative phosphorylation D. Photosynthesis

4. Organisms which are mesophilic grow at:

   A. Temperature of 0-20ºC B. Temperature of 50-60ºC.

   C. Temperature of 25-40ºC D. Concentration of 5-10% CO_2

5. In the bacterial growth curve, the phase in which there is an exponential increase in the number of cells is:

   A. Lag phase B. Log phase

   C. Stationary phase D. Phase of decline

WHAT YOU HAVE LEARNT

- Bacteria are unicellular organisms (prokaryotes) which lack nuclear membrane, nucleolus and other cell organelles like mitochondria, endoplasmic reticulum and golgi apparatus.
- Bacterial cell contains water (80% of total weight), proteins, polysaccharides, lipids, nucleic acids, mucopeptides and low molecular weight compounds.
- For growth and multiplication of bacteria, the mimimal nutritional requirements are water, a source of carbon, a source of nitrogen and some inorganic salts.
- **Phototrophs** are bacteria that derive energy from sunlight while **chemotrophs** derive theirs from chemical reactions.
- **Autotrophs** are bacteria that synthesise all their organic compounds while heterotrophs are unable to do so.
- **Heterotrophs** depend on preformed organic compounds.
- **Aerobic** bacteria require oxygen for growth and may be obligate aerobes or facultative anaerobes.
- **Obligate aerobes** grow only in the presence of oxygen.
- **Facultative anaerobes** grow in the presence or absence of oxygen.
- **Anaerobic** bacteria grow in the absence of oxygen.
- **Obligate anaerobes** may even die on exposure to oxygen.
- **Microaerophilic** bacteria grow best in the presence of low oxygen tension.

- Depending on requirements of temperature for growth, bacteria can be classified as **mesophilic** (25-40ºC), **psychrophilic** (below 20ºC) and **thermophilic** (55-80ºC).
- Moisture and drying, hydrogen ion concentration, light, osmotic effect and mechanical and sonic stress may also influence the growth and multiplication of bacteria.
- Bacteria divide by binary fission.
- When the bacterial cell reaches a certain size, it divides to form two daughter cells.
- Nuclear division is followed by cell division.
- The time interval between two cell divisions is the **generation time** or the population doubling time. It may vary from 20 minutes (coliform bacilli) to 20 hours (tubercle bacilli) to 20 days (lepra bacilli).
- The **bacterial growth curve** consists of a lag phase, a log phase, a stationary phase and a decline phase. This is seen in a liquid medium.
- In the **lag phase**, the bacteria adapt to the environment. There is no appreciable increase in cell number.
- In the **log phase**, there is exponential increase in the number of bacterial cells.
- In the **stationary phase**, there is no increase or decrease in the number of bacterial cells.
- In the **decline phase**, there is a decrease in the bacterial population due to cell death.

TERMINAL QUESTIONS

1. Describe the classification of bacteria based on their nutritional requirements.
2. Explain the temperature requirements of bacteria with suitable examples.
3. Describe the gaseous requirements of bacteria.
4. Define fermentation and give an example of fermentation.
4. Describe the bacterial growth curve with suitable diagrams.

ANSWERS TO INTEXT QUESTIONS

1. C 2. B 3. A 4. C 5. B