CHAPTER 3

Bacterial physiology and genetics

Bacterial physiology

Growth

Bacteria, like all living organisms, require nutrients for metabolic purposes and for cell division, and grow best in an environment that satisfies these requirements. Chemically, bacteria are made up of polysaccharide, protein, lipid, nucleic acid and peptidoglycan, all of which must be manufactured for successful growth.

Nutritional requirements

Oxygen and hydrogen

Both oxygen and hydrogen are obtained from water; hence, water is essential for bacterial growth. In addition, the correct oxygen tension is necessary for balanced growth. While the growth of aerobic bacteria is limited by availability of oxygen, anaerobic bacteria may be inhibited by low oxygen tension.

Carbon

Carbon is obtained by bacteria in two main ways:

1. **Autotrophs**, which are free-living, non-parasitic bacteria, use carbon dioxide as the carbon source.
2. **Heterotrophs**, which are parasitic bacteria, utilize complex organic substances such as sugars as their source of carbon dioxide and energy.

Inorganic ions

Nitrogen, sulphur, phosphate, magnesium, potassium and a number of trace elements are required for bacterial growth.

Organic nutrients

Organic nutrients are essential in different amounts, depending on the bacterial species:

- Carbohydrates are used as an energy source and as an initial substrate for biosynthesis of many substances.
- Amino acids are crucial for growth of some bacteria.
- Vitamins, purines and pyrimidines in trace amounts are needed for growth.

Reproduction

Bacteria reproduce by a process called **binary fission**, in which a parent cell divides to form a **progeny** of two cells. This results in a **logarithmic growth rate** – one bacterium will produce 16 bacteria after four generations. The **doubling** or **mean generation time** of bacteria may vary (e.g. 20 min for *Escherichia coli*, 24 h for *Mycobacterium tuberculosis*); the shorter the doubling time, the faster the multiplication rate. Other factors that affect the doubling time include the amount of nutrients, the temperature and the pH of the environment.

Bacterial growth cycle

The growth cycle of a bacterium has four main phases (Fig. 3.1):

1. **Lag phase**: may last for a few minutes or for many hours as bacteria do not divide immediately but undergo a period of adaptation with vigorous metabolic activity.
2. **Log** (logarithmic, exponential) **phase**: rapid cell division occurs, determined by the environmental conditions.
3. **Stationary phase**: this is reached when nutrient depletion or toxic products cause growth to slow until the number of new cells produced balances the number of cells that die. The bacteria have now achieved their **maximal cell density** or **yield**.
4. **Decline** or **death phase**: this is marked by a decline in the number of live bacteria.

Growth regulation

Bacterial growth is essentially regulated by the nutritional environment. However, both intracellular and extracellular regulatory events can modify the growth rate. Intracellular factors include:

- **end product inhibition**: the first enzyme in a metabolic pathway is inhibited by the end product of that pathway
- **catabolite repression**: enzyme synthesis is inhibited by catabolites.

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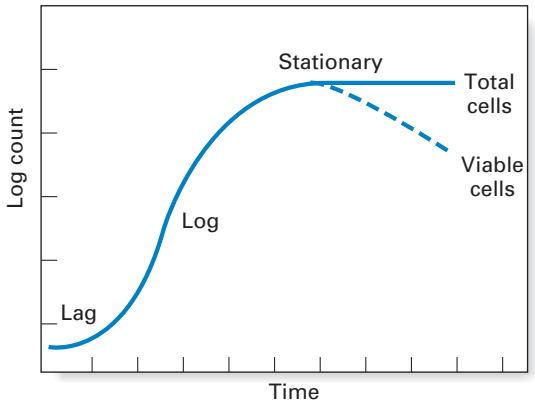

Fig. 3.1 Bacterial growth curve. Lag, lag phase of growth; Log, logarithmic phase of growth.

Extracellular factors that modify bacterial growth are:

- **Temperature**: the optimum is required for efficient activity of many bacterial enzymes, although bacteria can grow in a wide range of temperatures. Accordingly, bacteria can be classified as:
  - **mesophiles**, which grow well between 25 and 40 °C, comprising most medically important bacteria (that grow best at body temperature)
  - **thermophiles**, which grow between 55 and 80 °C (*Thermus aquaticus*, for instance, grows in hot springs and its enzymes such as *Taq* polymerase are therefore heat resistant, a fact exploited by molecular biologists in the polymerase chain reaction (PCR) (see below))
  - **psychrophiles**, which grow at temperatures below 20 °C.
- **pH**: the hydrogen ion concentration of the environment should be around pH 7.2–7.4 (i.e. physiological pH) for optimal bacterial growth. However, some bacteria (for example, lactobacilli) have evolved to exploit ecological niches, such as carious cavities where the pH may be as low as 5.0.

Aerobic and anaerobic growth

A good supply of oxygen enhances the metabolism and growth of most bacteria. The oxygen acts as the hydrogen acceptor in the final steps of energy production and generates two molecules: hydrogen peroxide (H_2O_2) and the free radical superoxide (O_2). Both of these are toxic and need to be destroyed. Two enzymes are used by bacteria to dispose of them: the first is **superoxide dismutase**, which catalyses the reaction:

$$2O_2 + 2H^+ \rightarrow H_2O_2 + O_2$$

and the second is **catalase**, which converts hydrogen peroxide to water and oxygen:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Bacteria can therefore be classified according to their ability to live in an oxygen-replete or an oxygen-free environment (Fig. 3.2, Table 3.1). This has important practical implications, as clinical specimens must be incubated in the laboratory under appropriate gaseous conditions for the pathogenic bacteria to grow. Thus, bacteria can be classified as follows:

- **obligate (strict) aerobes**, which require oxygen to grow because their adenosine triphosphate (ATP)-generating system is dependent on oxygen as the hydrogen acceptor (e.g. *M. tuberculosis*)
- **facultative anaerobes**, which use oxygen to generate energy by respiration if it is present, but can use the fermentation pathway to synthesize ATP in the absence of sufficient oxygen (e.g. oral bacteria such as *mutans* streptococci, *E. coli*)
- **obligate (strict) anaerobes**, which cannot grow in the presence of oxygen because they lack either superoxide dismutase or catalase, or both (e.g. *Porphyromonas gingivalis*)
- **microaerophiles**, that grow best at a low oxygen concentration (e.g. *Campylobacter fetus*).

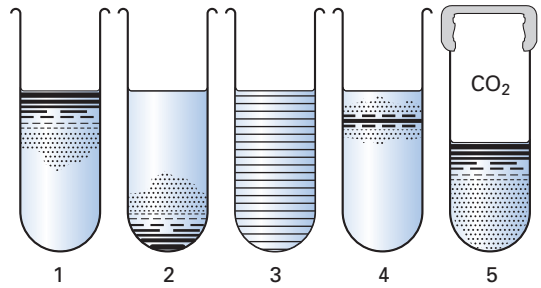

Fig. 3.2 Atmospheric requirements of bacteria, as demonstrated in agar shake cultures. (**1**) Obligate aerobe; (**2**) obligate anaerobe; (**3**) facultative anaerobe; (**4**) microaerophile; (**5**) capnophilic organism (growing in carbon dioxide-enriched atmosphere). (See also Table 3.1.)

Table 3.1 Effect of oxygen on the growth of bacteria

Degree of oxygenation	Term	Example
Oxygen essential for growth	Obligate aerobe	*Pseudomonas aeruginosa*
Grows well under low oxygen concentration (5%)	Microaerophile	*Campylobacter fetus*
Grows in the presence or absence of oxygen	Facultative anaerobe[a]	*Streptococcus milleri*
Only grows in the absence of oxygen	Obligate anaerobe	*Porphyromonas gingivalis*

[a]Facultative anaerobes may be subgrouped as capnophiles or capnophilic organisms if they grow well in the presence of 8–10% carbon dioxide (e.g. *Legionella pneumophila*).

Bacterial genetics

Genetics is the study of inheritance and variation. All inherited characteristics are encoded in DNA, except in RNA viruses.

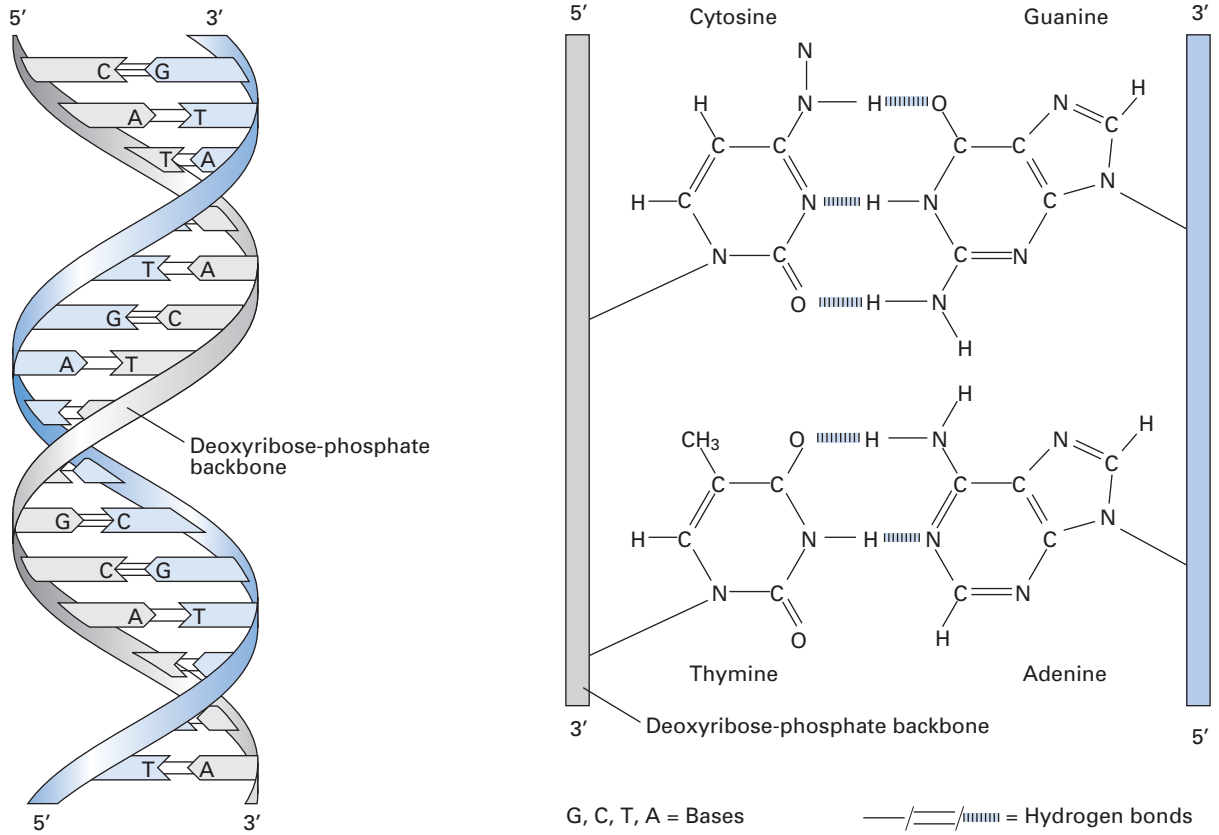

Fig. 3.3 The structure of DNA.

The bacterial chromosome

The bacterial chromosome contains the genetic information that defines all the characteristics of the organism. It is a single, continuous strand of DNA (Fig. 3.3) with a closed, circular structure attached to the cell membrane of the organism. The 'average' bacterial chromosome has a molecular weight of 2×10^9.

Replication

Chromosome replication is an accurate process that ensures that the progeny cells receive identical copies from the mother cell. The replication process is initiated at a specific site on the chromosome (*oriC* site) where the two DNA strands are locally denatured. A complex of proteins binds to this site, opens up the helix and initiates replication. Each strand then serves as a template for a complete round of DNA synthesis, which occurs in both directions (bidirectional) and on both strands, creating a **replication bubble** (Fig. 3.4). The two sites at which the replication occurs are called the **replication forks**. As replication proceeds, the replication forks move around the molecule in opposite directions opening up the DNA strands, synthesizing two new complementary strands until the two replication forks meet at a termination site. Of the four DNA strands now available, each daughter cell receives a parental strand and a newly synthesized strand. This process is called **semiconservative replication**. Such chromosomal replication is synchronous with cell division, so that each cell receives a full complement of DNA from the mother cell.

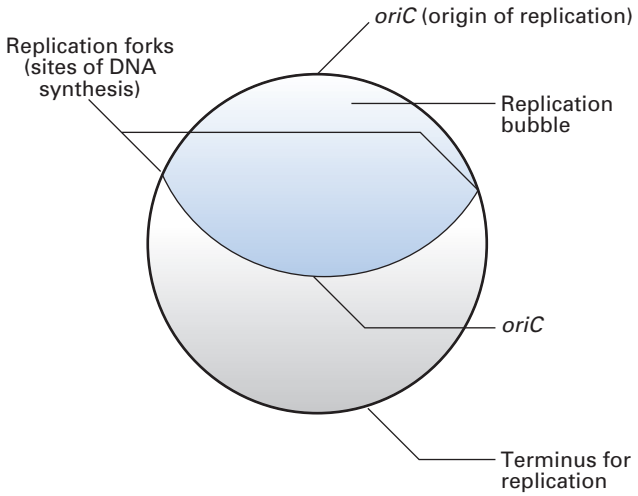

Fig. 3.4 Bidirectional replication of a circular bacterial chromosome.

The main enzyme that mediates DNA replication is **DNA-dependent DNA polymerase**, although a number of others take part in this process. When errors occur during DNA replication, repair mechanisms excise incorrect nucleotide sequences with nucleases, replace them with the correct nucleotides and religate the sequence.

Bacteria have evolved mechanisms to delete foreign nucleotides from their genomes. **Restriction enzymes** are mainly used for this purpose, and they cleave double-stranded DNA

at specific sequences. The DNA fragments produced by restriction enzymes vary in their molecular weight and can be demonstrated in the laboratory by gel electrophoresis. Hence, these restriction enzymes are used in many clinical analytical techniques to cleave DNA and to characterize both bacteria and viruses (see below).

Genes

The genetic code of bacteria is contained in a series of units called **genes**. As the normal bacterial chromosome has only one copy of each gene, bacteria are called **haploid** organisms (as opposed to higher organisms, which contain two copies of the gene and hence are **diploid**).

A gene is a chain of **purine** and **pyrimidine** nucleotides. The genetic information is coded in triple nucleotide groups or **codons**. Each codon or triplet nucleotide codes for a specific amino acid or a regulatory sequence, e.g. start and stop codons. In this way, the structural genes determine the sequence of amino acids that form the protein, which is the gene product.

The genetic material of a typical bacterium (e.g. *E. coli*) comprises a single circular DNA with a molecular weight of about 2×10^9 and composed of approximately 5×10^6 base pairs, which in turn can code for about 2000 proteins.

Genetic variation in bacteria

Genetic variation can occur as a result of mutation or gene transfer.

Mutation

A mutation is a change in the base sequence of DNA, as a consequence of which different amino acids are incorporated into a protein, resulting in an altered phenotype. Mutations result from three types of molecular change, as follows.

Base substitution

This occurs during DNA replication when one base is inserted in place of another. When the base substitution results in a codon that instructs a different amino acid to be inserted, the mutation is called a missense mutation; when the base substitution generates a termination codon that stops protein synthesis prematurely, the mutation is called a nonsense mutation. The latter always destroys protein function.

Frame shift mutation

A frame shift mutation occurs when one or more base pairs are added or deleted, which shifts the reading frame on the ribosome and results in the incorporation of the wrong amino acids 'downstream' from the mutation and in the production of an inactive protein.

Insertion

The insertion of additional pieces of DNA (e.g. transposons) or an additional base can cause profound changes in the reading frames of the DNA and in adjacent genes (Fig. 3.5).

Mutations can be induced by chemicals, radiation or viruses.

Gene transfer

The transfer of genetic information can occur by:

- conjugation
- transduction
- transformation
- transposition.

Clinically, the most important consequence of DNA transfer is that antibiotic-resistant genes are spread from one bacterium to another.

Conjugation

This is the mating of two bacteria, during which DNA is transferred from the donor to the recipient cell (Fig. 3.6A). The mating process is controlled by an **F (fertility) plasmid**, which carries the genes for the proteins required for mating, including the protein pilin, which forms the sex pilus (conjugation tube). During mating, the pilus of the donor (male) bacterium carrying the F factor (F+) attaches to a receptor on the surface of the recipient (female) bacterium. The latter is devoid of an F plasmid (F–). The cells are then brought into direct contact with each other by 'reeling in' of the sex pilus. Then the F factor DNA is cleaved enzymatically, and one strand is transferred across the bridge into the female cell. The process is completed by synthesis of the complementary strand to form a double-stranded F plasmid in both the donor and recipient cells. The recipient now becomes an F+ male cell that has the ability to transmit the plasmid further. The new DNA can integrate into the recipient's DNA and become a stable component of its genetic material. Complete transfer of the bacterial DNA takes about 100 min.

Transduction

Transduction is a process of DNA transfer by means of a bacterial virus – a **bacteriophage (phage)**. During the replication of the phage, a piece of bacterial DNA is incorporated, accidentally, into the phage particle and is carried into the recipient cell at the time of infection (Fig. 3.6B). There are two types of transduction:

1. Generalized transduction occurs when the phage carries a segment from any part of the bacterial chromosome. This may occur when the bacterial DNA is fragmented after phage infection, and pieces of bacterial DNA the same size as the phage DNA are incorporated into the latter.
2. Specialized transduction occurs when the phage DNA that has been already integrated into the bacterial DNA is excised and carries with it an adjacent part of the bacterial DNA. Phage genes can cause changes in the phenotype of the host bacterium; for example, toxin production in *Corynebacterium diphtheriae* is controlled by a phage gene. This property is lost as soon as the phage DNA is lost in succeeding reproductive cycles.

Plasmid DNA can also be transferred to another bacterium by transduction. However, the donated plasmid can function independently without recombining with bacterial DNA. The ability to produce an enzyme that destroys penicillin (β-lactamase) is mediated by plasmids that are transferred between staphylococci by transduction.

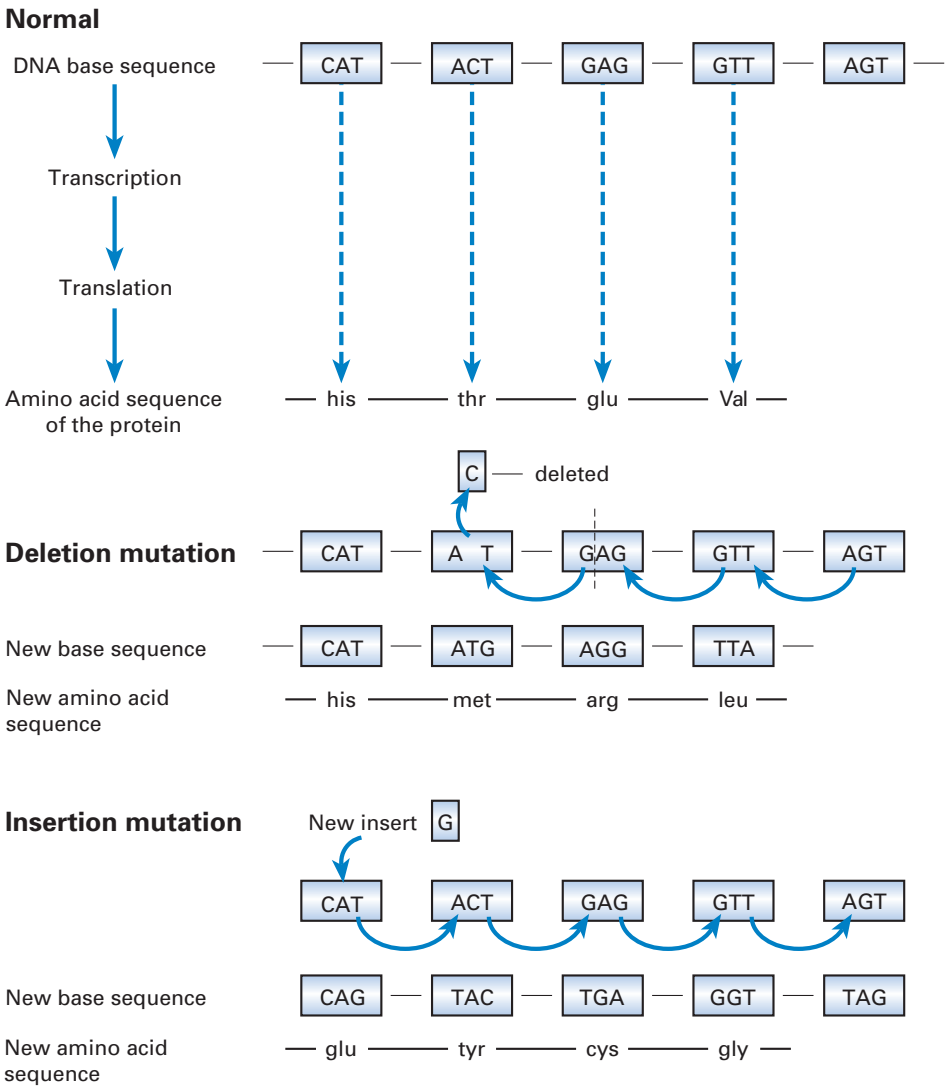

Fig. 3.5 Events that entail mutation: the effect of the deletion and insertion of a single base on the amino acid sequence (and the quality of the protein thus produced) is shown.

Transformation

This is the transfer of exogenous bacterial DNA from one cell to another. It occurs in nature when dying bacteria release their DNA, which is then taken up by recipient cells and recombined with the recipient cell DNA. This process appears to play an insignificant role in disease (Fig. 3.6C).

Transposition

This occurs when transposable elements (transposons; see below) move from one DNA site to another within the genome of the same organism (e.g. *E. coli*). The simplest transposable elements, called 'insertion sequences', are less than 2 kilobases in length and encode enzymes (*transposase*) required for 'jumping' from one site to another (Fig. 3.6D).

Recombination

When the DNA is transferred from the donor to the recipient cell by one of the above mechanisms, it is integrated into the host genome by a process called recombination. There are two types of recombination:

1. **Homologous recombination**, in which two pieces of DNA that have extensive homologous regions pair up and exchange pieces by the processes of breakage and reunion.
2. **Non-homologous recombination**, in which little homology is necessary for recombination to occur. A number of different enzymes (e.g. endonucleases, ligases) are involved in the recombination process.

Plasmids

Plasmids are extrachromosomal, double-stranded circular DNA molecules within the size range 1–200 MDa. They are capable of replicating independently of the bacterial chromosome (i.e. they are replicons). Plasmids occur in both Gram-positive and Gram-negative bacteria, and several different plasmids can often coexist in one cell.