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1.1 CYTOLOGICAL BASIS OF MICROORGANISMS

The cell is the fundamental unit of all living organisms. Various subunit structures and chemical substances found on and inside the cell make complex cellular functions possible. Microbes can be divided into three major groups according to their morphological structure, degree of differentiation, and chemical composition: eukaryote, prokaryote, and acellular microorganisms (Figure 1.1(A)–(D)).

1.2 MICROBIAL MORPHOLOGY

Microorganisms, also known as microbes, are tiny organisms that are only visible under an optical microscope or an electron microscope. They are small in size and simple in structure. Microbes reproduce quickly, can tolerate a wide range of environmental conditions, are widely distributed, highly variable, and tend to congregate.

1.2.1 Microbial Size

As many types of microbes exist, they vary widely in size. Generally, the units used to measure microbes are μm and nm. Most cocci are 1 μm in diameter. Bacilli can be further divided into coccobacilli, brevibacteria, and long bacilli, and measure approximately 1–10 μm in length and 0.3–1 μm in width. Spirochetes measure approximately 6–20 μm in length and 0.1–0.2 μm in width. Fungi are several times larger than bacteria. Most viruses are smaller than 150 nm and are only visible under the electron microscope. The same microbes can change in size depending on their environment or age (Figure 1.2).

1.2.2 Microbial Morphology

Different types of microbes have different, but characteristic, shapes. Under suitable conditions, the shape and size of microbes are relatively stable. It is important to know the morphological structure of microbes, as it provides us with a better understanding of microbial physiology, pathogenic mechanisms, antigenic features, and allows us to identify them by species. In addition, knowledge of microbial morphology can be helpful in diagnosing disease and in preventing microbial infections.

1. Bacteria are complex and highly variable microbes. They come in four basic shapes: spherical (cocci), rod-shaped (bacilli), arc-shaped (vibrio), and spiral (spirochete) (Figure 1.3(A)).

2. Fungi are divided into unicellular and multicellular according to the number of cells that make up the organism. Unicellular fungi, such as Saccharomyces and other yeast-like fungi, are usually round or oval. Multicellular fungi have hyphae and spores. The hyphae and spores of different fungi are shaped differently (Figure 1.3(B) and (C)).

3. Many viruses are spherical or almost spherical, some are rod-shaped (often seen in plant viruses), filamentous (e.g., freshly isolated influenza virus), bullet-shaped (e.g., rabies virus), brick-shaped (e.g., poxvirus), and tadpole-shaped (e.g., bacteriophage) (Figure 1.3(D)).

1.3 MICROBIAL CELL STRUCTURE

Although different microbes possess different cellular structures, there are certain commonalities within groups of microbes.

1.3.1 Basic Bacterial Structures

The architecture of bacterial cells consists of basic and special structures. Basic structures include the cell wall, cell membrane, cytoplasm, nuclear material, ribosome, plasmid, etc. Special structures, which are only found in some bacteria, include the flagellum, pilus, capsule, spore, etc. (Figure 1.4).
1.3.1.1 Cell Wall

The cell wall is the outermost structure of the bacterial cell and is located outside the cell membrane. It is transparent, tough, and flexible. The average thickness ranges from 15 to 30 nm. It mainly consists of peptidoglycan, also called murein, glycopeptide, or mucopeptide. Bacteria are classified into gram-positive and gram-negative based on the appearance of the cells after Gram stain. The peptidoglycan of gram-positive bacteria is composed of a glycan backbone, tetrapeptide side chains, and a pentapeptide cross-linking bridge (Figure 1.5(A)). The peptidoglycan of gram-negative bacteria is composed of a glycan backbone and a tetrapeptide side chain (Figure 1.5(B)).

Gram-positive and gram-negative bacteria have unique structures other than peptidoglycan in their cell walls (Figure 1.5(C) and (D)). Other substances, such as compound polysaccharide, surface protein, proteins M and G of *Streptococcus*, protein A of *Staphylococcus aureus*, etc. are found on the outer layer of the cell wall of some gram-positive bacteria.

1.3.1.2 Cell Wall-Deficient Bacteria (Bacterial L Form)

Cell wall-deficient bacteria are strains of bacteria that lack cell walls. The peptidoglycan that makes up the cell wall can be destroyed or inhibited by physical, chemical, or biological factors. When gram-positive bacteria lack a cell wall, the cytoplasm is surrounded by the cell membrane, and the entire structure is known as a protoplast. When gram-negative bacteria do not have a cell wall, the cytoplasm is protected by the outer membrane, and the entire structure is called a spheroplast. Bacteria
1.3 Microbial cell Structure

that have lost their cell wall are still capable of growing and dividing as cell wall-deficient bacteria. Examples of these were first isolated in 1935 by Emmy Klieneberger-Nobel, who named them “L-forms” after the Lister Institute in London where she was working at the time. L-form bacteria give rise to a variety of cell morphologies and sizes and can be spherical, rod-shaped, filiform, etc. The rate of growth and division of L-form bacteria is slow. They also form distinctive bacterial colonies when plated on agar. Some L-form strains have a tendency to revert to the normal phenotype when the conditions that were used to produce the cell wall deficiency are reduced. L-form bacteria are difficult to stain or stain unevenly. In a Gram stain test, L-form bacteria always show up as gram-negative, due to the lack of a cell wall.

1.3.1.3 Cell Membrane

The cell membrane is a selectively permeable biological membrane found inside the cell wall and surrounding the cytoplasm. It is made of a lipid bilayer. The cell membrane is compact and flexible, and measures approximately 7.5 nm in thickness. It accounts for 10–30% of the bacterial cell dry weight. The structure of the bacterial cell membrane resembles that of eukaryotic cell membranes, except it is deficient in cholesterol. The lipid bilayer is embedded with carrier proteins and zymoprotein, which possess specific functions.

The cell membrane of some bacteria can form invaginations into the cytoplasm called mesosomes.

1.3.1.4 Cytoplasm

The cytoplasm is the gel-like substance enclosed within the cell membrane, which is made up of water, proteins, lipids, nucleic acids, inorganic salts, etc. Most metabolic activities take place within the cytoplasm, and subcellular structures, such as ribosomes, plasmids, and cytoplasmic granules, are located in the cytoplasm.

Ribosomes are found in cytoplasm. They are approximately 15–20 nm in diameter and are composed of a small (30S) and a large (50S) subunit. The association between subunits requires the presence of Mg²⁺. Ribosomes are made up of 30% ribosomal proteins and 70% ribosomal RNA.

Plasmids are small, circular, double-stranded DNA molecules and are extrachromosomal genetic material. They can replicate independently of chromosomal DNA and transmit genes encoding drug resistance, bacteriocins, toxins, and more from one bacterium to another via conjugation and transduction.

Cytoplasmic granules is a general term referring to many types of cytoplasmic inclusion granules. They are an intracytoplasmic (inside the cytoplasm of a cell) form of storing nutrients and energy and include molecules such as polysaccharides, lipids, phosphates, etc. They are not essential or permanent structures in cells. Cytoplasmic granules are also known as metachromatic granules because they may stain into different colors than other bacterial cell structures.

1.3.1.5 Nuclear Material

The bacterial nuclear material is also called the nucleoid. It is a piece of double-stranded DNA devoid of nuclear membrane, nucleolus, or histones and is the bacterial equivalent of chromatin. The function of the nucleoid is similar to that of the nucleus in eukaryotic cells and encodes genes necessary for activities and traits such as growth, metabolism, reproduction, heredity, mutation, etc.

1.3.2 Special Bacterial Structures

1.3.2.1 Capsule

The capsule is a layer of slime that lies outside the bacterial cell wall. It is secreted by bacteria and diffuses into the surrounding medium. Based on its appearance when examined by light microscope, the bacterial capsule is classified into two types: microcapsule, which is less than 0.2 μm in thickness and escapes optical detection; and capsule or large capsule, which is over 0.2 μm in thickness, binds tightly to the cell wall, and presents an obvious boundary under optical microscope. The capsule shows up as negatively stained when ordinary
1. ORAL MICROBES

**FIGURE 1.3** (A) Basic shape of bacteria. (B) Fungal spores. (C) Fungal hyphae. (D) Morphology and structure of viruses: 1. Poxvirus, 2. Paramyxovirus, 3. Orthomyxovirus, 4. Coronavirus, 5. Togaviridae, 6. Adenovirus, 7. Bullet-shaped virus, 8. Herpes virus, 9. T2 bacteriophage, 10. Reovirus, 11. Papovavirus, 12. Picornavirus, 13. Picodnavirus, 14. Tobacco mosaic virus.

**FIGURE 1.4** Schematic representation of bacterial cell structure.
staining techniques are used. It appears as a clear halo around the bacterium when stained samples are examined by light microscope. Using special staining, the capsule can be stained differently from the bacterial cell (Figure 1.6). Most bacterial capsules are composed of polysaccharides, but a few capsules are made of polypeptides.

Capsular polysaccharides are highly hydrated molecules in which water accounts for more than 95% of the composition. They bind to phospholipids or lipid A on the cell surface through covalent bonds. The capsule is considered as an important virulence factor because it protects bacteria from engulfment by eukaryotic immune cells, desiccation, and helps bacteria adhere to surfaces.

1.3.2.2 Flagellum

The flagellum is a lash-like appendage that protrudes from the cell body and usually measures 5–20 μm in length and 10–30 nm in diameter. It is the locomotive organelle of motile bacteria such as *Selenomonas* and *Wolinella succinogenes*. The flagellum is composed of three parts: basal body, hook, and filament (Figure 1.7(A)). Different bacteria can have anywhere from one or two flagella to hundreds of flagella (Figure 1.7(B)). Flagella can only be observed directly by electronic microscope or by light microscope after special staining (Figure 1.7(C)). The flagellum is involved in the pathogenesis of some diseases and is antigenic (for example, antigen H). Examples of flagellate bacteria include *Vibrio cholerae*. 

**FIGURE 1.5** (A) Schematic representation of *Staphylococcus aureus* cell wall peptidoglycan. M: N-Acetylmuramic acid, G: N-acetylglucosamine, O: β-1,4 glycosidic bond, a: L-alanine, b: d-aspartic acid, c: l-lysine, d: d-aspartic acid, x: glycine. (B) Schematic representation of *Escherichia coli* cell wall peptidoglycan. M: N-Acetylmuramic acid, G: N-acetylglucosamine, Ala: alanine, Glu: glutamic acid, DAP: diaminopimelic acid. (C) Schematic of gram-positive bacteria cell wall. Gram-positive bacteria have a thick (20–80 nm) cell wall composed of 15–50 layers of peptidoglycan, many teichoic acids, and some teichuronic acid. Teichoic acids are unique to gram-positive bacterial cell walls and constitute a class of important antigens related to the serotype classification of certain bacterial species. Teichoic acid accounts for about 50% of the dry weight of the cell wall. It is a polymer consisting of ribitol or glycerin residues that are bound by phosphodiester bonds into a long chain, which is then anchored in peptidoglycan. Teichoic acids are classified into cell wall teichoic acids and membrane teichoic acids (also known as lipoteichoic acid, LTA) according to the cellular structure to which they are anchored. (D) Schematic representation of the cell wall of gram-negative bacteria. Gram-negative bacteria have a comparatively thin cell wall, approximately 10–15 nm in thickness. It is made up of one to two layers of peptidoglycan and other complex structures. On the outside of the peptidoglycan is the outer membrane, which is the main component of the gram-negative bacteria cell wall and accounts for approximately 80% of the dry weight of the cell wall. The outer membrane is composed of three layers: lipoprotein, lipid bilayer, and lipopolysaccharide (LPS) ordered from the interior to the exterior: OMP: outer membrane protein, PP: porin, BP: nutrient binding protein, CP: carrier protein, M: N-acetylmuramic acid, G: N-acetylglucosamine.
1. ORAL MICROBES

1. ORAL MICROBES

and Campylobacter jejuni, which use multiple flagella to propel themselves through the mucus lining of the small intestine to reach the epithelium and produce toxin.

Flagella can be classified as monotrichous, amphitrichous, lophotrichous, and peritrichous according to their number and location.

1.3.2.3 Pilus

The pilus is a hair-like structure associated with bacterial adhesion and related to bacterial colonization and infection. Pili are primarily composed of oligomeric pilin proteins, which arrange helically to form a cylinder. New pilin protein molecules insert into the base of the pilus. Pili are antigenic, and genes encoding pili can be located in the bacterial chromosome or in plasmids. Pili are not locomotive structures. They are classified into ordinary

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**FIGURE 1.6** Capsule of S. pneumoniae (Murs staining method). Bacterial cells are stained red and the capsule around the cell appears as blue transparent circles.

**FIGURE 1.7** (A) Schematic of E. coli flagellum. Basal body: Located at the base of the flagellum. The basal body, embedded in the cell wall and cell membrane, is the output device. It acts as an engine to provide energy for locomotion. The nearby switch determines the direction of rotation. Hook: This structure points directly away from the cell and has a sharp bend (about 90°) from which filaments protrude. Filament: This filiform structure protrudes from the bacterial cell. It is a hollow tube made of the protein flagellin. Its acts like a ship’s or plane’s propeller to move the bacterial cell. (B) Examples of bacterial flagellar arrangement. (C) Periplasmic flagella (flagella staining). The bacterial cell is stained red and the flagella are stained light red around the bacterial cell.
1.3.1.2.4 Spore

The spore is a small round or oval body that forms in bacteria due to cytoplasmic dehydration under unfavorable conditions (Figure 1.8(A)). It is surrounded by multiple membrane layers and has low permeability. Only gram-positive bacteria can form spores, including species such as *Bacillus subtilis*, *Clostridium tetani* (Figure 1.8(B)), etc. The spore contains a complete karyoplasm and enzymatic system and can maintain all the essential activities for the bacteria to remain alive.

![Diagram of bacterial spore](A)

The multiple membrane layers of the spore are, from the exterior to the interior, as follows: spore coating, spore shell, outer membrane, cortex, cell wall of spore, and inner membrane, which surrounds the nucleus of the spore.

Spores are difficult to stain due to their thick cell wall. Special staining is required to stain the spore and distinguish it from the bacterial cell (Figure 1.8(B)).

The size, morphology, and location of the spore differ between bacterial species and can be used to help identify bacteria (Figure 1.8(C)). For example, the *C. tetani* spore is round and larger than the transverse diameter of the bacterial cell, forming a drumstick-like structure, as the spore is located at the tip of the bacterial cell (Figure 1.8(B)).

1.3.3 Basic Structure of Virus

Viruses are a kind of acellular microbe consisting mainly of nucleic acid and proteins. Some viruses are composed of a small amount of lipids and polysaccharides. The basic structure of viruses is made up by the

![Spore stain of C. tetani](B)

**FIGURE 1.8** (A) Schematic representation of the bacterial spore. (B) Spore stain of *C. tetani* (fuchsin-methylene blue stain). (C) Size, morphology, and location of bacterial spores.
1. ORAL MICROBES

viral core, viral capsid, as well as a membrane envelope in some viruses (Figure 1.9). The size, morphology, and structure of viruses play important roles in viral taxonomy and in diagnosing viral infections.

1. Viral core: namely the nucleic acid component, which makes up the genome of the virus. The viral core provides genetic information that determines pathogenicity, antigenicity, proliferation, heredity, variation, etc. The chemical components of the viral core are DNA or RNA, based on whether the virus is classified as a DNA virus or an RNA virus. Nucleic acid can be single or double stranded. The relative molecular mass of the viral core is 2–160 × 10^6.

2. Viral capsid: it is a protein shell that surrounds and protects the nucleic acid of the virus. The viral capsid is sometimes associated with the viral nucleic acid, and this structure is known as the nucleocapsid. In virions without an envelope, the nucleocapsid makes up the entirety of the virus. The viral capsid is composed of repeated protein subunits known as capsomers, which are made of one or more proteins known as the chemical subunit or structural subunit.

3. Envelope: this is the one or two layers of membrane that surround the capsid of some viruses, which is a structure unique to the class of viruses known as enveloped viruses. The envelope is formed during the maturation process when certain viruses bud out from the cell membrane. Therefore, the envelope can be composed of the host cell membrane and/or the nuclear membrane. The surface of some viral envelopes carries protein protrusions called peplomers or spikes.

1.4 MICROBIAL PHYSIOLOGY

Bacterial cells are synthesis machines that multiply themselves. The growth and division of bacteria include approximately 2000 different types of biochemical reactions that mediate energy conversion or enzymatic biosynthesis.

1.4.1 Binary Fission Reproduction

Binary fission is a process by which many prokaryotes reproduce from a single cell into two new cells (Figure 1.10(A)). Bacteria reproduce asexually by binary fission. Cocci can divide from different planes to form different arrangements. Bacilli divide along their horizontal axis; however, some bacterial species such as Mycobacterium tuberculosis occasionally split by branching.

During cell division, the cell volume increases and a diaphragm is generated where the cell division is to take place. Then, a single cell will divide into two cells (Figure 1.10(B) and (C)). Under suitable conditions, the majority of bacteria divide quickly, about 20–30 min for one division. However, the growth of some bacterial species can be relatively slow. For example, M. tuberculosis takes 18–20 h to complete one round of division.

1.4.2 Bacterial Growth

Mastering the fundamentals of bacterial growth allows the researcher to change culture conditions artificially, adjust the bacterial phases of growth and reproduction, and use beneficial bacteria more efficiently. Bacterial growth involves inoculating a certain number of bacteria into a suitable liquid medium and checking the number of viable cells at different time intervals. With the collected information, it is possible to generate a growth curve using culture time as the horizontal axis and the logarithmic number of viable cells in the culture as the vertical axis (Figure 1.11). Growth curves can generally be divided into four major sections.

1. Lag phase: During this process, the bacteria are adapting to their new environment. The volume of bacterial cells increases and their metabolism is active, but cell division is slow and reproduction is minimal.

2. Logarithmic phase: In this period, bacteria grow rapidly and divide and reproduce at a constant speed. The number of bacteria increases exponentially and the number of viable cells increases logarithmically.

3. Stationary phase: The bacterial growth rate gradually decreases, and the number of dead bacteria increases. The number of newly produced bacteria is approximately equal to the number of dying bacteria, and the number of viable cells remains relatively stable.

4. Decline phase: Bacterial growth rate slows and stops, and the number of dead bacteria is higher than that
1.5 Microbial Genetics

Like the other living organisms, microorganisms have heredity and variable characteristics. Heredity keeps microbial genetic traits relatively stable to ensure the reproduction of the species, while variations produce changes in the microorganisms that are useful for...

Figure 1.10 (A) Binary fission in bacilli. (B) Synthesis of gram-positive bacterial cell wall. During cell division, the volume of the cell increases and a new cell wall is formed. New cell wall materials are added to the preexisting cell wall to maintain structural integrity. (C) Division of gram-positive bacteria (SEM). Cell division in *Streptococcus gordonii*, showing a clear division.

Figure 1.11 The growth curve of *E. coli*.

Of viable cells. Cells become polymorphic, showing morphologies such as cell deformation, swelling, or autolysis.
microbial survival and evolution and ultimately lead to the generation of new species.

1.5.1 Heredity

Heredity is the similarity in biological traits between offspring and its parent. Cells can be considered as a chemical plant in which information can be stored and transformed into a useful product. Enzymes are the molecular machines that catalyze specific chemical reactions. The information is stored DNA, which exists in the cell as two long molecular coiled chains (DNA double helix). The intracellular genetic information is replicated, transcribed, and translated by enzymes, which then leads to protein synthesis (Figure 1.12).

1.5.2 Variation

Variation refers to the differences between offspring and its parent under certain conditions, including variations in morphology and structure, virulence, drug resistance, and so on. The variability of microorganisms is divided into genetic variation and nongenetic variation. The former is due to changes in bacterial gene structure. The new characteristics can be stably transmitted to future generations, which is why this type of variation is called genotype variation, as the change is mostly irreversible (Figure 1.13). The latter is caused by the influence of certain environmental conditions that do not change the genetic structure of bacteria. The change is not transmitted to the offspring and is therefore called a phenotypic variation. Genetic variation is rarely influenced by external environmental factors. Therefore, genetic variation tends to occur in individual bacterial cells, while phenotypic variation tends to occur in a bacterial flora due to the effect of environmental factors. These variations can revert with the removal of the stimulating environmental factors.

1.5.3 Genetic Material of Bacteria

Nucleic acids are the basis of organismal heredity. Two types of nucleic acid exist: DNA and RNA. DNA is the genetic material in prokaryotic and eukaryotic organisms, while the genetic material in viruses is DNA and RNA. The genetic material found in microorganisms includes chromosomes, plasmids, bacteriophages, and transposable elements.

Bacteriophages are viruses that infect bacteria, fungi, actinomycetes, mycoplasmas, and spirochetes. They inject their genetic material into the infected host cell and can induce bacterial cell lysis under certain conditions. Bacteriophages, known as phages, can only reproduce in specific host strains and have high specificity for...
their host. The specificity is related to phage cell binding molecules and the structure and complementarity of the host bacterial strain’s surface receptor molecules.

Since phages are small, they can only be observed under electron microscope. They can be divided into three basic morphologies: tadpole-shaped, spherical, and rod-shaped. Most phages are tadpole-shaped and consist of a head and a tail (Figure 1.14A).

The relationship between the phage life cycle and its host bacteria is shown in Figure 1.14(B). Phages that infect bacteria can produce two outcomes. The first, observed in virulent phage, involves phage multiplication resulting in the production of many progeny phages, bacterial cell lysis, and cell death. This cycle is known as the lytic cycle. The second outcome is lysogeny, observed in temperate phages. It involves the integration of phage nucleic acid with the bacterial chromosome, resulting in the formation of a prophage. The phage genetic material is reproduced when the bacterial cell divides.

1.5.4 Mechanism of Microbial Variation

Genetic variation in microorganisms has its basis in mutations caused by changes in their genetic sequence. These changes are stable and heritable. Mutations can generally be classified as gene mutations and chromosomal aberrations. The spontaneity and randomness of microbial mutations can be tested by using fluctuation test or replica plating (Figure 1.15A and B).

1.5.5 Gene Transfer and Recombination

Gene transfer is a process by which exogenous genetic material from a donor cell is transferred to the receptor cell. However, simply the process of transferring genetic material is not enough, as the recipient cell must be able to accommodate exogenous genes. Integration between the transferred gene and the DNA of the recipient cell is a process known as recombination, whereby the recipient cell acquires certain characteristics of the donor strain. Gene transfer and recombination in bacteria can take place through processes such as transformation, conjugation, transduction, cell fusion, and lysogenic conversion.

1.5.5.1 Transformation

Transformation takes place when donor bacteria DNA is cleaved and free DNA fragments are directly taken up by receptors. As a result, recipient cells acquire certain genetic traits from the donor cell. This phenomenon was confirmed in *Streptococcus pneumoniae*, *Staphylococci*, and *Haemophilus influenzae*. Griffith first showed that bacterial transformation takes place by infecting mice with *S. pneumoniae* in 1928. An outline of the experiment is shown in Figure 1.16.

1.5.5.2 Conjugation

Conjugation is the method by which bacteria physically connect with one another through their pilus to transfer genetic material (mainly plasmid DNA). Plasmid transfer from the donor to the recipient cell results in the recipient cell acquiring some of the genetic traits of the donor cell. Plasmids that can be transferred through conjugation are called conjugative plasmids, which include the F, R, Col, and virulence plasmids. The F plasmid encodes the pilus, controls pilus formation and whether or not the pilus enables conjugation (Figure 1.17A).

Plasmids that cannot be transferred between bacteria through a pilus are called nonconjugative plasmids.

**FIGURE 1.14** (A) The structural model of a phage. The phage head is icosahedral and is approximately 80×100 nm in size. It consists of the capsid protein that surrounds the internal nucleic acid. The tail is a tubular structure composed of protein, including the tail whiskers, tail collar, tail tube, tail sheath, tail fibers, and tail baseplate. (B) Lysogenic and lytic cycles of lysogenic phage.
FIGURE 1.15 (A) Fluctuation test: The fluctuation test shows that mutations preexist in a population of bacteria in the absence of selection. This was tested by Luria and Delbrück using naturally existing phage-resistant mutations in bacterial populations. A given concentration (10^3/ml) of Escherichia coli sensitive to specific phages was inoculated in two equal volumes (10 ml) of broth medium. One inoculum was concentrated in a large test tube and the other was evenly distributed into 50 small test tubes. After 24–36 h incubation under the same conditions, the bacterial cultures from the large and small tubes were plated onto phage-containing plates, and the number of colonies was measured. The results typically showed that of 50 phage-coated plates inoculated with bacteria from the large test tube, the fluctuation in the colony number was small (3–7). On the other hand, when the 50 small tubes were plated onto 50 plates, the number of the colonies tended to fluctuate significantly, from zero colonies to several hundreds. (B) Replica plating: Replica plating (Lederberg et al., 1952) involves plating antibiotic-susceptible strains on agar plates in the absence of antibiotics until scattered single colonies grow. Using a block covered with sterile velvet, gently press the velvet onto the surface of the agar plates so that bacterial colonies are imprinted onto the sticky velvet surface. Then, press the velvet surface onto an agar plate containing antibiotics. After an appropriate culture time, bacteria susceptible to the antibiotics are completely suppressed, but drug-resistant colonies will be visible on the plate. We can find colonies corresponding to drug-resistant colonies on the original plates lacking antibiotics. The drug-resistant colonies can then be transplanted to culture broth containing the appropriate antibiotics to observe bacterial growth. Although the bacterial colonies on the original agar plate have never been in contact with antibiotics, they are nonetheless resistant to antibiotic drugs.

FIGURE 1.16 *Streptococcus pneumoniae* transformation in mice. S. pneumoniae with a polysaccharide capsule belong to the virulent type III strain. These colonies are smooth (S) in appearance. S. pneumoniae without the polysaccharide capsule belong to the avirulent type II strain and appear as rough (R) type colonies. In the classic Griffith experiment, type II-R bacteria and type III-S bacteria were injected into mice. Mice that received the type II-R bacteria survived, and those that received the type III-S bacteria died. The type III-S bacteria were isolated from the blood of the dead mice and were heated until they no longer active. These dead type III-S bacteria were injected into mice, and the mice survived. However, when dead type III-S bacteria and live type II-R bacteria were both injected into the same mice, the mice died, and type III-S bacteria were isolated from their blood. This experiment showed that the live type II-R type bacteria were able to obtain genetic material from dead type III-S bacteria that transformed them from an avirulent strain to a virulent one. It also suggests that the genetic material encoded the capsule virulence factor from type III-S bacteria.
1.5 Microbial Genetics

The nonconjugative plasmid ColE1 is relatively low in molecular mass and does not encode the necessary gene required for it to be transferred from one cell to another. However, if there is another conjugative plasmid in the host cell, ColE1 can tag along and be transferred from one cell to another. For example, the F plasmid that encodes the genes necessary for pilus formation can help ColE1 transfer from one cell to another (Figure 1.17(B)).

Conjugation is widespread in gram-negative bacteria and can be observed in almost all members of the Enterobacteriaceae. Some gram-positive bacteria have been reported to conjugate (e.g., Streptococcus, Bacillus subtilis), and the phenomenon has also been observed in Streptomyces.

1.5.5.3 Transduction

Transduction uses a temperate phage as the vehicle by which DNA from a donor cell is transferred into a recipient cell. Transduction can transfer larger fragments of DNA than transformation. According to the genes involved in transduction, the process can be divided into generalized transduction and restricted transduction (Figure 1.18(A) and (B)).

Generalized transduction can transfer plasmids. The transduction of plasmid R in S. aureus is a very important clinical feature.

In generalized transduction, the packaged DNA can be from any part of the donor strain chromosome. When phages exit the lysogenic phase, the prophage will excise the packaged DNA. In some cases, the prophage will remain integrated into the host chromosome, and the packaged DNA will be transduced into a new cell. This process is called stable gene transfer. In other cases, the packaged DNA will be released from the prophage and will be transduced into a new cell. This process is called abortive transduction.

FIGURE 1.17 (A) The transfer and replication of the F plasmid during conjugation. Bacteria possessing the F plasmid and F-pili are the male equivalent strain (F⁺), while bacteria lacking the F plasmid and F-pili are the female equivalent strain (F⁻). When the F⁺ and F⁻ strains are present in the same environment, the F-pilus from the F⁺ bacterial cell conjugates to the F⁻ surface receptor. The F-pilus gradually shortens so that the two cells are pulled close to each other and a channel is formed. Plasmid DNA from the F⁺ bacteria breaks at the origin of transfer (oriT) and the 5' end extends through the channel into the F⁻ bacteria. Single-stranded DNA in both bacterial cells replicate by rolling circle replication, and each cell forms a complete F plasmid. Therefore, the donor cell has not lost its F plasmid after the transfer, and the recipient cell becomes F⁺ strain bacteria after receiving the F plasmid. (B) Nonconjugative plasmid (ColE1) induced to transfer by F plasmid. The ColE1 plasmid can be induced to transfer from a donor to a recipient cell. This process requires two genes encoded on the ColE1 plasmid: the specific site bom on ColE1 DNA (also known as the nic locus) and the nuclease encoded by the mobA gene. When both F and ColE1 plasmids are present in the same bacterial cell, the mobA gene is transcribed and its product creates a single strand break at the bom locus to form a gap. As a result, the ColE1 plasmid goes from a supercoiled plasmid to an open loop.

FIGURE 1.18 (A) Generalized mode of transduction. (B) Restricted mode of transduction.
itself from the bacterial chromosome and enter into the lytic phase. In the latest stages of the lytic phase, phage DNA is replicated in large quantities and errors can occur during phage assembly. Approximately one in $10^5$–$10^7$ phages will contain bacterial DNA fragments that have been mistakenly packaged into the phage head, which results in the formation of a transducing phage. Transducing phages can infect another host bacterium and inject the DNA fragment they are carrying into the recipient cell, thereby transferring DNA from one bacterial cell to another.

Restricted transduction, or specific transduction, describes the process in which transduction is restricted to specific genes from the chromosome of the donor strain. If phage $\lambda$ is transferred into *E. coli* K12 while it is in the lysogenic phase, the phage DNA is integrated into a specific site in the *E. coli* chromosome, between the galactose (*gal*) and biotin (*bio*) genes.