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### Part 1: General microbiology

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Welcome to the fifth edition of *Essential Microbiology for Dentistry*!

It is now 22 years since the first edition of this tome was published in 1996, and since then, the science of microbiomics and infectious diseases has advanced in leaps and bounds. The two major reasons for these transformational changes have been the exploding new technology that delivers novel tools for the identification and reclassification of organisms, and the emergence of new organisms, especially the viruses that change the landscape of dental and medical practice. For instance, next generation sequencing (NGS) technology has revolutionized the field of microbial taxonomy and identification of, in particular, the uncultivable organisms, leading to a radical rethink on the quantity and quality of the flora that inhabit our body, including the oral cavity. In this, the fifth edition of this book, I have attempted to incorporate the new data as much as possible while maintaining its popular concise, yet comprehensive outlook.

The fact that you are now reading the fifth edition of the book is testimony to its popularity, with more than 40,000 copies sold in all five continents; Chinese, Polish and Korean translations as well as Middle East Editions (Al-Farabi Version) of the book are now in print, although the e-print of the book appears to be outstripping the hard copy sales. For this, I am deeply grateful to the microbiology teachers in dental schools/colleges, as well as the undergraduates and the postgraduates who are avid fans worldwide.

In compiling this completely revised fifth edition, I have retained the popular features of the last few editions. One major feature of this edition is an expanded section on infection control (Part 6), which I co-edited with Dr Caroline Pankhurst, of University of London, UK. Other novel additional features are sections on NGS technology; the oral microbiome and the microbiota; endodontic infections; implant-related infections; plaque biofilms and the systemic disease axis and the current guidelines on antimicrobial prophylaxis.

Of course, a tome of this nature cannot be produced without the help of many friends and colleagues. The legacy authors of the Immunology Section (Part 2) were Dr Brian Jones and Professor Liwei Lu, from the University of Hong Kong, while Professor Glen C Ulett of Griffith University, Australia expanded and embellished these chapters as well as other sections of the tome. To them, my extreme gratitude.

Once again, I am indebted to the following colleagues worldwide, who graciously permitted the reproduction of their work: Professor H Jenkinson, University of Bristol, UK (Fig. 3.9); Dr Bernard Low, Malaysia (Fig. 5.1); Professor Willie van Heerden, University of Pretoria, South Africa (Figs 18.4 and 19.1); Dr Maribasappa Karched of Kuwait University (Fig. 31.2); Dr Leanor Haley, CDC, Atlanta, USA (Fig. 22.5); Dr Annette Motte, Free University of Berlin, Germany (Fig. 31.8); and Professor Saso Ivanowski, Griffith University, Australia (Fig. 33.8). Figures 38.1 and 38.5 are reproduced from UK Health Technical Memorandum No. 01-05, 2009, with permission from Crown Copyright.

As always, the publishing team at Elsevier led by Martin Mellor, Alison Taylor and Helen Leng has pushed me to beat the deadlines despite my myriad duties. Their professionalism and patience has my admiration and gratitude. Last but not least, Hemamali, Dilani and Asanka have lost some quality family time due to this tome, and I am eternally grateful to them for their tolerance and understanding.

Above all, YOU, the reader, are my most important friend and critic! The many features of this edition are due to your feedback over two decades, and I truly hope that the current edition is the finest product thus far. Nevertheless, no book is perfect—so please keep on sending your comments, either good or bad, to me at lakshman@hku.hk.

Lakshman Samaranayake
Hong Kong
August 2017
The latest edition of *Essential Microbiology for Dentistry* comes with over 300 online MCQs that aim to reinforce the student’s knowledge as well as provide exam practice for both undergraduate training and the post-graduate exams set by the UK Royal Colleges and other similar international bodies.

Reflecting and mirroring the structure of the main textbook, each online learning module presents a mini-series of multiple choice questions covering a range of topics which vary from microbial structure and taxonomy, to physiology and genetics. Different classes of microbes are sequentially explored as well as the host immune response, and the role of effective chemotherapy. Downstream modules related to the importance of systemic disease, principles of infection control and infection control procedures finally complete the picture. The number of questions in each module serve to reflect the importance of the topic to dentistry, either in terms of common, and direct, relevance or by the potential seriousness of its impact on any individual patient.

Designed to perfectly complement the fifth edition of *Essential Microbiology for Dentistry*, readers are encouraged to work through each module at their own pace to achieve an overall percentage score at the end of each exercise. This way, they can see their grades at a glance across the range of topics to show instantly their areas of strength and those that require a revision. When ready, readers can reset the program and repeat the process with the aim of resitting each module to raise their overall score.

Prepared by Professor Lakshman Samaranayake, the questions are designed to provide revision and exam practice in a relaxed, non-pressurised environment with the overall aim of improving real exam grades.

To access this helpful online self-assessment tool, please log in at [https://evolve.elsevier.com/Samaranayake/essential/](https://evolve.elsevier.com/Samaranayake/essential/)
Microbiology (Greek: \textit{mikros} small; \textit{bios} life), so called because it primarily deals with organisms too small for the naked eye to see, encompasses the study of organisms that cause disease, the host response to infection and ways in which such infection may be prevented. For our purposes the subject can be broadly classified into \textbf{general}, \textbf{medical} and \textbf{oral microbiology}.

Dental students need both a basic understanding of general and medical microbiology, and a detailed knowledge of clinical oral microbiology in order to diagnose oral microbial infections, which are intimately related to the overall treatment plan for their patients. Moreover, the two major oral disorders—\textbf{caries} and \textbf{periodontal disease}—that the dental practitioner is frequently called upon to treat are due to changes in the oral bacterial ecosystem and the constituent oral microbiome, and a grasp of these disease processes is essential for their appropriate management.

The impact of these infections on the health and welfare of the community is simply astonishing. For instance, caries has gained the dubious distinction of being the most prevalent disease, affecting almost half (44\%) of the world population in 2010. It has also been estimated, for instance, that caries and periodontal disease are the most costly diseases that the majority of the population has to contend with during their lifetime, and the number of working hours lost due to these infections and the related cost of dental treatment worldwide amount to billions of dollars per annum (e.g., more than 81 billion dollars in the USA in 2006). This is not surprising as it is generally accepted that periodontal disease is the most common affliction of humankind.

The advent of the human immunodeficiency virus (HIV) infection in the early 1980s and the subsequent concerns on cross infection via contaminated blood and instruments have resulted in an increased regimentation of \textbf{infection control} practices in dentistry. Furthermore, many patients are acutely concerned about possible infection transmission in clinical settings because of the intense, and sometimes unwarranted, publicity given to these matters by the media. The dental practitioner should therefore be conversant with all aspects of infection control in the clinical environment, not only to implement infection control measures but also to advise the dental team (dental surgery assistants, dental hygienists and other ancillary personnel) and to allay patients’ unfounded fears. For all these and many other reasons, which the student will discover in the text, the discipline of microbiology is intimately woven into the fabric of dentistry and composes a crucial component of the dental curriculum.

It should also be realized that new microbial diseases emerge incessantly (due to reasons given in the following section), and the book you are now reading is a primer for understanding and managing such future scenarios, especially in the context of infection control.

\section*{A note on emerging and re-emerging infections}

Infectious agents have been adversaries of humans for millennia. Diseases such as plague have wiped out civilizations in ancient times, while humans in turn have won the battle against microbes in more recent times (e.g., eradication of smallpox). Such new diseases are given the terms \textbf{emerging infections} or \textbf{re-emerging infections} (Fig. 1.1), and they are broadly categorized as:

- \textbf{new infections}: caused by agents such as new influenza virus strains emerging periodically to cause epidemics, and the mosquito-borne Zika virus infection.
- \textbf{‘old’ infections}: known disease entities where the aetiological agents have been recently identified through advances in technology (e.g., \textit{Helicobacter pylori} causing gastric ulcer disease).
- \textbf{re-emergent infections}: diseases that have returned with a vengeance due to genetic and structural transformations and attendant increased virulence of the organism (e.g., drug-resistant \textit{Mycobacterium tuberculosis} with its ‘new bag of tricks’).
Why study microbiology?

The reasons for their emergence are manifold and include:

- **societal events**: economic impoverishment (especially in the developing world), war and civil conflicts, as well as mass population migration.
- **health care**: new medical devices, organ/tissue transplantation, immunosuppression, antibiotic abuse and contaminated blood and blood products.
- **human behaviour**: increasing number of sexual partners, injectable drug abuse.
- **environmental changes**: deforestation, drought, floods and global warming.
- **microbial adaptation**: emergence of new species from the wild (e.g., HIV), changes in virulence and toxin production and development of drug resistance.

About this book

This text is divided into six parts in order to highlight the different features of microbiology related to dentistry, but it should be noted that such division is artificial and is merely an attempt to simplify the learning process.

The first few chapters in **Part 1** essentially describe general microbiological features of bacteria and viruses and how they cause human infections (i.e., *pathogenesis*). **Diagnostic microbiology**, by which clinical microbiologists ascertain the nature of agents causing various infections, is described in Chapter 6. The laboratory aspect of this fascinating subject is analogous to the work of a crime detection bureau! When a specimen (e.g., pus, urine) from a patient with an infectious disease is sent to the laboratory for identification of the offending agent, the clinical microbiologist utilizes many methods and techniques, as well as a fair amount of thought and contemplation, to identify the pathogen’s lurking in the clinical sample. In many situations the pathogen may be dead, in which case other, indirect clues via molecular techniques need to be pursued to incriminate the suspect pathogen. Once an offending pathogen is identified, antimicrobial chemotherapy is the mainstay of treatment; a description of chemotherapeutic agents and how they are chosen in the laboratory is given in Chapter 7.

The host responds to infection by mounting an immune response. A highly abbreviated account of **basic immunology** is given in **Part 2**; supplemental reading is essential to augment this material, and the reader is referred to the lists of recommended texts for this purpose. Immunological nomenclature is complex and often difficult: a glossary of terms and abbreviations is therefore provided as an appendix.

Although there are thousands of offending pathogens, only some are of direct relevance to dental practice and to the comprehension of the **mechanisms of disease**; these are described in **Part 3**. Arguably this section may appear to be the most daunting part of the book because of the complex nomenclature of microbes; hence only the salient bacterial genera—some of which are more closely related to dental practice (e.g., streptococci) than others (e.g., legionellae)—are outlined. Similarly, the chapters on viruses and fungi are relatively brief, with thumbnail sketches of only the most relevant organisms.

The major **infections of each organ system** are discussed in **Part 4**, with emphasis on those that are most relevant to dentistry. The student is strongly advised to cross-refer to

Fig. 1.1 Global prevalence of some emerging and re-emerging diseases. *E. coli*, *Escherichia coli*; HIV, human immunodeficiency virus; SARS, severe acute respiratory syndrome; *S. aureus*, *Staphylococcus aureus*; vCJD, variant Creutzfeldt–Jakob disease.
organisms and their characteristics (described in Part 3) when studying this section, as the microbial attributes and the diseases they cause form a single continuum.

**Part 5** specifically outlines the **microbial interactions in the craniofacial region**, in both health and disease. This section should be particularly useful for the later years of the dental curriculum, to reinforce the studies in conservative dentistry, periodontics, oral and maxillofacial surgery and oral medicine.

Last but not least, the subject of **cross infection and its control** in dentistry is encapsulated in **Part 6**. It provides a comprehensive summary of the routine infection control regimens that must be implemented in every dental practice. The relevance of this information in routine clinical practice cannot be overemphasized, and a thorough understanding of this material should pay rich dividends in years to come.

As the student will discover, the comprehensive nature of this text has made almost all the materials significant. Thus the reader will be intellectually challenged to learn a new concept or terminology in almost every sentence or phrase. In addition, an attempt has been made to summarize the information as key facts, to serve as an **aide-mémoire**, at the end of each chapter. It is important, however, that the subject matter is augmented with additional reading, and it is to this end that the list of recommended texts is given. The self-assessment quiz at the end of each chapter, which may not cover all aspects of the preceding narrative, should help the reader to assess knowledge assimilation in key areas.

Finally, in most chapters the text is arranged under the following important features of microbiology, which the student must understand in order to deal with infectious diseases:

- **Epidemiology**: spread, distribution and prevalence of infection in the community.
- **Pathogenesis**: the means by which microbes cause disease in humans, an understanding of which is critical for the successful diagnosis and management of infections.
- **Diagnosis**: detection of an infection; this depends on the collection of the correct specimen in the most appropriate manner, and subsequent interpretation of the laboratory results.
- **Treatment**: antibacterial, antifungal or antiviral therapy combined with supportive therapy leads to resolution of most infections.
- **Prevention** (prophylaxis): immunization is the most useful mode of preventing diseases such as tetanus and hepatitis B; however, increasing public awareness of diseases and their modes of spread significantly helps to curb the spread of infections in the community (e.g., HIV infection).

### Further reading


The aim of this section is to present (1) the structural features of microbes and how they cause disease, and (2) a perspective of diagnostic laboratory methods to explain the relationship between the scientific basis of microbiology and its practical application in patient care. Finally, a comprehensive overview of antimicrobial chemotherapy in dentistry is provided, which should be supplemented with additional reading due to its critical relevance to dental care.
Classification of all living beings including microbes has been attempted by many over centuries (Table 2.1). Traditionally, though they were all classified into two kingdoms, plants and animals, the classification was arbitrary and based on morphological and growth characteristics. With the development of novel techniques, the latter classification was expanded to include five kingdoms: monera, protista, plantae, fungi and animalia. However, the current understanding based on their genetic relatedness is that all forms of life fall into three domains: Archaea, Bacteria and Eucarya. The main differences among Archaea, Bacteria and Eucarya are listed in Table 2.2. Note that taken together, Archaea and Bacteria are also known as prokaryotes (see later text).

Viruses are not included in this classification as they are unique, acellular, metabolically inert organisms and therefore replicate only within living cells. Other differences between viruses and cellular organisms include:

- **Structure.** Cells possess a nucleus or, in the case of bacteria, a nucleoid with DNA. This is surrounded by the cytoplasm where energy is generated and proteins are synthesized. In viruses, the inner core of genetic material is either DNA or RNA, but they have no cytoplasm and hence depend on the host for their energy and proteins (i.e., they are metabolically inert).

- **Reproduction.** Bacteria reproduce by binary fission (a parent cell divides into two similar cells), but viruses disassemble, produce copies of their nucleic acid and proteins, and then reassemble to produce another generation of viruses. As viruses are metabolically inert, they must replicate within host cells. Bacteria, however, can replicate extracellularly (except rickettsiae and chlamydiae, which are bacteria that also require living cells for growth).

### Eukaryotes and prokaryotes

As mentioned earlier, another modification of classifying cellular organisms is to divide them into prokaryotes (i.e., Archaea and Bacteria) and eukaryotes (Greek karyon: nucleus). Fungi, protozoa and humans, for instance, are eukaryotic, whereas bacteria are prokaryotic. In prokaryotes, the bacterial genome, or chromosome, is a single, circular molecule of double-stranded DNA, lacking a nuclear membrane (smaller, single or multiple circular DNA molecules called plasmids may also be present in bacteria), whereas the eukaryotic cell has a true nucleus with multiple chromosomes surrounded by a nuclear membrane.

Bacteria comprise the vast majority of human pathogens, whereas archaea appear rarely to cause human disease and live in extreme environments (e.g., high temperature or salt concentrations). Archaea received little attention traditionally as they cannot be easily cultured in the laboratory. Interestingly, recent studies using novel techniques such as pyrosequencing have uncovered their presence in the oral cavity. Some studies have even shown that certain species of archaea are more frequently found in subgingival plaque in periodontal disease.

## Morphology

### Shape and size

The shape of a bacterium is determined by its rigid cell wall. Bacteria are classified by shape into three basic groups (Fig. 2.1):

1. cocci (spherical)
2. bacilli (rod shaped)
3. spirochaetes (helical).

Some bacteria with variable shapes, appearing as both coccal and bacillary forms, are called pleomorphic (pleo: many; morphic: shaped) in appearance.

The size of bacteria ranges from about 0.2 to 5 µm. The smallest bacteria approximate the size of the largest viruses (poxviruses), whereas the longest bacilli attain the same length as some yeasts and human red blood cells (7 µm).
### Table 2.1  Differential characteristics of major groups of organisms

<table>
<thead>
<tr>
<th>Feature</th>
<th>Bacteria</th>
<th>Mycoplasmas</th>
<th>Rickettsiae</th>
<th>Chlamydiae</th>
<th>Virusesa</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible with light microscope</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Capable of free growth</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Both DNA and RNA present</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Muramic acid in cell wall</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Rigid cell wall</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>Variable</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Susceptible to penicillin</td>
<td>Variable</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Susceptible to tetracycline</td>
<td>Variable</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Reproduce essentially by binary fission</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Variable</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*Prions (agents responsible for Creutzfeldt–Jakob disease) are not included as their status is unclear.

### Table 2.2  Major differences among the three domains of life

<table>
<thead>
<tr>
<th>Feature</th>
<th>Bacteria</th>
<th>Archaea</th>
<th>Eucarya</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organization of the genetic material and replication</strong></td>
<td>DNA free in the cytoplasm</td>
<td>DNA free in the cytoplasm</td>
<td>DNA is contained with a membrane-bound nucleus. A nucleolus is also present</td>
</tr>
<tr>
<td>Only one chromosome</td>
<td>Only one chromosome</td>
<td>Only one chromosome</td>
<td>More than one chromosome. Two copies of each chromosome may be present (diploid)</td>
</tr>
<tr>
<td>DNA associated with histone-like proteins</td>
<td>DNA associated with histone-like proteins</td>
<td>DNA complexed with histone proteins</td>
<td></td>
</tr>
<tr>
<td>May contain extrachromosomal elements called plasmids</td>
<td>Plasmids may be found</td>
<td>Plasmids only found in yeast</td>
<td></td>
</tr>
<tr>
<td>Intron not found in mRNA</td>
<td>Intron not found in most genes</td>
<td>Intron not found in all genes</td>
<td></td>
</tr>
<tr>
<td>Cell division by binary fission: asexual replication only</td>
<td>Reproduce asexually and spores are not found</td>
<td>Cells divide by mitosis</td>
<td></td>
</tr>
<tr>
<td>Transfer of genetic information occurs by conjugation, transduction and transformation (see Chapter 3)</td>
<td>Processes similar to bacterial conjugation enable exchange of genetic material</td>
<td>Exchange of genetic information occurs during sexual reproduction. Meiosis leads to the production of haploid cells (gametes), which can fuse</td>
<td></td>
</tr>
<tr>
<td><strong>Cellular organization</strong></td>
<td>Cytoplasmic membrane contains hopanoids</td>
<td>Membranes contain isoprenes</td>
<td>Cytoplasmic membrane contains sterols</td>
</tr>
<tr>
<td>Lipopolysaccharides and teichoic acids found</td>
<td>No lipopolysaccharides or teichoic acids found</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy metabolism associated with the cytoplasmic membrane</td>
<td></td>
<td>Mitochondria present in most cases</td>
<td></td>
</tr>
<tr>
<td>Photosynthesis associated with membrane systems and vesicles in cytoplasm</td>
<td>Chloroplasts present in algal and plant cells</td>
<td>Internal membranes, endoplasmic reticulum and Golgi apparatus present associated with protein synthesis and targeting</td>
<td></td>
</tr>
<tr>
<td>Flagella consist of one protein, flagellin</td>
<td>Contains flagella that derive energy from proton pumps</td>
<td>Flagella have a complex structure with 9 + 2 microtubular arrangement</td>
<td></td>
</tr>
<tr>
<td>Ribosomes: 70S</td>
<td>Ribosomes behave more like eucarya when exposed to inhibitors</td>
<td>Ribosomes: 80S (mitochondrial and chloroplast ribosomes are 70S)</td>
<td></td>
</tr>
<tr>
<td>Peptidoglycan cell walls</td>
<td>Cell walls lack peptidoglycan</td>
<td>Polysaccharide cell walls, where present, are generally either cellulose or chitin</td>
<td></td>
</tr>
</tbody>
</table>
Fimbriae and pili

Fimbriae and pili are fine, hair-like filaments, shorter than flagella, that extend from the cell surface. Pili, found mainly on Gram-negative organisms, are composed of subunits of a protein, pilin, and mediate the adhesion of bacteria to receptors on the human cell surface, a necessary first step in the initiation of infection. A specialized type of pilus, the sex pilus, forms the attachment between the male (donor) and the female (recipient) bacteria during conjugation, when genes are transferred from one bacterium to another.

Glycocalyx (slime layer)

The glycocalyx is a polysaccharide coating that covers the outer surfaces of many bacteria and allows the bacteria to adhere firmly to various structures, for example, oral mucosa, teeth, heart valves and catheters, and contribute to the formation of biofilms. This is especially true in the case of Streptococcus mutans, a major cariogenic organism, which has the ability to produce vast quantities of extracellular polysaccharide in the presence of dietary sugars such as sucrose.
Capsule

An amorphous, gelatinous layer (usually more substantial than the glycocalyx) surrounds the entire bacterium; it is composed of polysaccharide, and sometimes protein (e.g., anthrax bacillus). The sugar components of the polysaccharide vary in different bacterial species and frequently determine the serological type within a species (e.g., 84 different serological types of Streptococcus pneumoniae can be distinguished by the antigenic differences of the sugars in the polysaccharide capsule). The capsule is important because:

- it mediates the adhesion of bacteria to human tissues or prosthesis such as dentures or implants, a prerequisite for colonization and infection.
- it hinders or inhibits phagocytosis; hence the presence of a capsule correlates with virulence.
- it helps in laboratory identification of organisms (in the presence of antiserum against the capsular polysaccharide the capsule will swell greatly, a phenomenon called the quellung reaction).
- its polysaccharides are used as antigens in certain vaccines because they elicit protective antibodies (e.g., polysaccharide vaccine of S. pneumoniae).

Cell wall

The cell wall confers rigidity upon the bacterial cell. It is a multilayered structure outside the cytoplasmic membrane. It is porous and permeable to substances of low molecular weight.

The inner layer of the cell wall is made of peptidoglycan and is covered by an outer membrane that varies in thickness and chemical composition, depending upon the Gram-staining property of the bacteria (Fig. 2.4). The term 'peptidoglycan' is derived from the peptides and the sugars (glycan) that make up the molecule. (Synonyms for peptidoglycan are murein and mucopeptide.)

The cell walls of Gram-positive and Gram-negative bacteria have important structural and chemical differences (Fig. 2.5):

- The peptidoglycan layer is common to both Gram-positive and Gram-negative bacteria but is much thicker in the Gram-positive bacteria.
- By contrast, the Gram-negative organisms have a complex outer membrane composed of lipopolysaccharide (LPS), lipoprotein and phospholipid. These form porins, through which hydrophilic molecules are transported in and out of the organism. The O antigen of the LPS and the lipid A component are also embedded in the outer membrane. Lying between the outer membrane and the cytoplasmic membrane of Gram-negative bacteria is the periplasmic space. It is in this space that some bacterial species produce enzymes that destroy drugs such as penicillins (e.g., β-lactamases).
- The LPS of Gram-negative bacteria, which is extremely toxic, has been called the endotoxin. (Hence, by definition, endotoxins cannot be produced by Gram-positive bacteria as they do not have LPS in their cell walls.) LPS is bound to the cell surface and is only released when it is lysed. It is responsible for many of the features of disease, such as fever and shock (see Chapter 5).

Bacteria with defective cell walls

Some bacteria can survive with defective cell walls. These include mycoplasmas, L-forms, spheroplasts and protoplasts. Mycoplasmas do not possess a cell wall and do not need hypertonic media for their survival. They occur in nature and may cause human disease (e.g., pneumonia). L-forms are usually produced in the laboratory and may totally or partially lack cell walls. They may be produced in patients treated with penicillin and, like mycoplasmas, can replicate on ordinary media.

Both spheroplasts (derived from Gram-negative bacteria) and protoplasts (derived from Gram-positive bacteria) lack cell walls, cannot replicate on laboratory media and are unstable and osmotically fragile. They require hypertonic conditions for maintenance and are produced in the laboratory by the action of enzymes or antibiotics.

Cytoplasmic membrane

The cytoplasmic membrane lies just inside the peptidoglycan layer of the cell wall and is a ‘unit membrane’ composed of a
phospholipid bilayer similar in appearance to that of eukaryotic cells. However, eukaryotic membranes contain sterols, whereas prokaryotes generally do not (the only exception being mycoplasmas). The membrane has the following major functions:

- active transport and selective diffusion of molecules and solutes in and out of the cell
- electron transport and oxidative phosphorylation, in aerobic species
- synthesis of cell wall precursors
- secretion of enzymes and toxins
- supporting the receptors and other proteins of the chemotactic and sensory transduction systems.

### Mesosome
This is a convoluted invagination of the cytoplasmic membrane that functions as the origin of the transverse septum that divides the cell in half during cell division. It is also the binding site of the DNA that will become the genetic material of each daughter cell.

### Cytoplasm
The cytoplasm comprises an inner, nucleoid region (composed of DNA), which is surrounded by an amorphous matrix that contains ribosomes, nutrient granules, metabolites and various ions.

### Nuclear material or nucleoid
Bacterial DNA comprises a single, supercoiled, circular chromosome that contains about 2000 genes, approximately 1 mm long in the unfolded state. (It is analogous to a single, haploid chromosome.) During cell division, it undergoes semiconservative replication bidirectionally from a fixed point.

### Ribosomes
Ribosomes are the sites of protein synthesis. Bacterial ribosomes differ from those of eukaryotic cells in both size and chemical composition. They are organized in units of 70S, compared with eukaryotic ribosomes of 80S. These differences are the basis of the selective action of some antibiotics that inhibit bacterial, but not human, protein synthesis.

### Cytoplasmic inclusions
The cytoplasm contains different types of inclusions, which serve as sources of stored energy; examples include polyphosphate, polysaccharide and β-hydroxybutyrate.

### Bacterial spores
Spores are formed in response to adverse conditions by the medically important bacteria that belong to the genus *Bacillus* (which includes the agent of anthrax) and the genus *Clostridium* (which includes the agents of tetanus and botulism). These bacteria sporulate (form spores) when nutrients, such as sources of carbon and nitrogen, are scarce (Fig. 2.6). The spore develops at the expense of the vegetative cell and contains bacterial DNA, a small amount of cytoplasm, cell membrane, peptidoglycan, very little water and, most importantly, a thick, keratin-like coat. This coat, which contains a high concentration of calcium dipicolinate, is remarkably resistant to heat, dehydration, radiation and chemicals. Once formed, the spore is metabolically inert and can remain dormant for many years. Spores are called either terminal or subterminal, depending on their position in relation to the cell wall of the bacillus from which they developed.

When appropriate conditions supervene (i.e., water, nutrients), there is enzymatic degradation of the coat, and the spore transforms itself into a metabolizing, reproducing bacterial cell once again (Fig. 2.6).

### Clinical significance of bacterial spores
The clinical importance of spores lies in their extraordinary resistance to heat and chemicals. Hence they can survive in a dormant state for many years in adverse habitats such as soil, and cause infections once they are implanted into an
unsuspecting host through, say a penetrative injury. Trauma from road traffic accidents and even an innocuous garden fork injury may lead to such infections when sporulation ensues with exotoxin production (e.g., tetanus caused by Clostridium tetani; Chapter 13).

The extraordinary ability of bacterial spores to withstand high temperatures is also exploited for evaluating the sterilization efficacy of autoclaves. In this case, the spores of Bacillus stearothermophilus and related species are used as ‘biological monitors’ to check the efficacy of the autoclaving process (Chapter 38).

---

**Taxonomy**

The systematic classification and categorization of organisms into ordered groups are called taxonomy. A working knowledge of taxonomy is useful for diagnostic microbiology and for studies in epidemiology and pathogenicity.

As mentioned at the beginning of this chapter, organisms encountered in medical microbiology fall into the domains of Bacteria, Archaea and Eucarya. Although this system of classification is based on the evolutionary relatedness or the genetic homogeneity of the species represented in each domain, a more pragmatic means of classification is employed in the clinical microbiology laboratory. Such bacterial classification is somewhat artificial in that they are categorized according to phenotypic (as opposed to genotypic) features, which facilitate their laboratory identification. These comprise:

- **morphology** (cocc, bacilli, spirochaetes)
- **staining properties** (Gram-positive, Gram-negative)
- **cultural requirements** (aerobic, facultative anaerobic, anaerobic)
- **biochemical reactions** (saccharolytic and asaccharolytic, according to sugar fermentation reactions)
- **antigenic structure** (serotypes).

Most of the medically and dentally important bacteria are classified according to their morphology, Gram-staining characteristics and atmospheric requirements. A simple classification of medically important bacteria is given in Figs 2.7 and 2.8.

---

**Genotypic taxonomy**

In contrast to the classical phenotypic classification methods outlined earlier, genotypic classification and speciation of organisms are becoming increasingly important and useful. Genotypic taxonomy exploits the genetic characteristics, which are more stable than the sometimes transient phenotypic features of organisms. These methods essentially evaluate the degree of DNA homology of organisms in order to speciate them, for example, by assessing molecular guanine and cytosine (GC) content, ribotyping, random amplification of polymorphic DNA (RAPD) analysis and pulsed-field gel electrophoresis.
Taxonomy

Organisms are named according to a hierarchical system, beginning with the taxonomic rank domain, followed by kingdom, phylum, class, order, family, genus and species (Table 2.3). The scientific name of an organism is classically a binomial

<table>
<thead>
<tr>
<th>Table 2.3 Hierarchical ranks in classification of organisms (e.g., Lactobacillus acidophilus)*</th>
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<tbody>
<tr>
<td><strong>Taxonomic rank</strong></td>
</tr>
<tr>
<td>Domain</td>
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<td>Kingdom</td>
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<td>Genus</td>
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<tr>
<td>Species</td>
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</tbody>
</table>

*Note: This is the basic classification although more modern and detailed classifications are also available with further subcategorization of the taxonomic ranks.

How do organisms get their names?

Organisms are named according to a hierarchical system, beginning with the taxonomic rank **domain**, followed by **kingdom**, **phylum**, **class**, **order**, **family**, **genus** and **species** (Table 2.3). The scientific name of an organism is classically a binomial

(PFGE). Novel bacterial typing methods based on the nucleotide sequences of ribosomal RNA (rRNA) genes have become a robust way of assessing bacterial identity. Further details of these methods are given in Chapter 3.

Additionally, recent research indicates that endogenous bacterial habitats in humans, including the oral cavity, harbour a flora that cannot be cultured using routine laboratory techniques. These so-called **unculturable species** comprise both bacteria and archaea, mentioned earlier, and can only be detected by molecular techniques or **metagenomics** (e.g., by direct amplification of 16S RNA). The role of these totally new **phylogenotypes** of bacteria in either disease or health awaits clarification.

Both the culturable and unculturable organisms (i.e., the total microbial community) including biomolecules within a defined habitat, such as the human body is given the term ‘**core microbiome**’. The total collection of resident microbes within the core microbiome is termed the **microbiota**. The analysis of this microbiome has been greatly facilitated by novel techniques such as **pyrosequencing** (a method of DNA sequencing) and **next-generation sequencing** (NGS). The data from such studies have revealed that the oral cavity in health may contain more than 1000 different phylotypes (Fig. 2.9; also see Chapter 31)!
Bacterial structure and taxonomy

Key facts

Note: clinically relevant facts and practice points are italicized; key words are in bold.

- The word ‘microorganism’ (microbe) is used to describe an organism that cannot be seen without the use of a microscope.
- The main groups of microbes are algae, protozoa, fungi, bacteria and viruses, with progressively decreasing size.
- All living cells are either prokaryotic (Archaea and Bacteria) or eukaryotic.
- Prokaryotes such as bacteria are simple cells with no internal membranes or organelles.
- Eukaryotes have a nucleus, organelles such as mitochondria and complex internal membranes (e.g., fungi, human cells).
- Bacteria are divided into two major classes according to staining characteristics: Gram-positive (purple) and Gram-negative (pink).
- Structures external to the cell wall of bacteria are flagella (whip-like filaments), fimbiae or pili (fine, short, hair-like filaments), glyccalyx (slime layer) and capsule.
- Flagella are used for movement, the fimbiae and pili for adhesion and the glyccalyx for adhesion, protection and biofilm formation.
- Cell wall peptidoglycan is common to both Gram-positive and Gram-negative bacteria but thicker in the former; it gives rigidity and shape to the organism.
- Peptidoglycan comprises long chains of N-acetylmuramic acid and N-acetylglucosamine cross-linked by peptide side chains and cross-bridges.
- Lipopolysaccharides (LPS) are integral components of the outer membranes of Gram-negative (but not Gram-positive) bacteria; LPS is the endotoxin and therefore Gram-positive bacteria cannot produce endotoxin.

Fig. 2.9 A schematic overview of the uses of bioinformatics for functional metagenome analysis. Microbial community contains numerous bacterial and other species. Once the total DNA has been extracted, the composition of the community is determined by amplifying and sequencing 16S ribosomal RNA (rRNA) gene. Highly similar sequences are then grouped as operational taxonomic units (OTUs), which are then recognized by comparisons with databases of already recognized organisms. OTUs are then analyzed to determine the biomolecular and metabolic functions of the community. (Adapted with permission from Elsevier from Morgan, X.C., Segata N., Huttenhower C. (2013). Biodiversity and functional genomics in the human microbiome. Trends in Genetics, 29(1), 51–58.)
• Cell walls of some bacteria such as the mycobacteria contain lipids (mycolic acids) that are resistant to Gram staining; these bacteria are called acid-fast organisms.

• Bacterial cytoplasm contains chromosomal nuclear material: nucleoid, ribosomes, inclusions/storage granules.

• Spore formation or sporulation is a response to adverse conditions in Bacillus spp. and Clostridium spp.

• Taxonomy (systematic classification of organisms) into groups can be performed according to morphology, staining reactions, cultural requirements, biochemical reactions, antigenic structure and DNA composition.

• The total microbial community including biomolecules within a defined habitat, such as the human body, is called the core microbiome.

• The total collection of resident microbes within the core microbiome is termed the microbiota.

Review questions (answers on p. 363)

Please indicate which answers are true, and which are false.

2.1 Prokaryotes are different from eukaryotes in that
A. have ribosomes
B. possess Golgi apparatus
C. have their genetic material organized in the cytoplasm
D. reproduce by binary fission only
E. do not have introns in their mRNA

2.2 Bacterial capsule:
A. mediates adhesion to surfaces
B. hinders the action of phagocytes
C. helps in identification
D. is antigenic
E. in all species is made up of polysaccharides

2.3 From the following list of bacterial structural components (A–G) match the best fit/association to the descriptors (1–8) given below:
A. cytoplasmic membrane
B. ribosomes
C. cytoplasmic inclusions
D. spores
E. nucleoid
F. fimbriae
G. flagella
1. associated with oxidative phosphorylation
2. mediates cell motility
3. a source of stored energy
4. protein synthesis
5. enables survival under harsh environmental conditions
6. mediates host attachment
7. enables selective transfer of molecule in and out of the cell
8. resembles a single chromosome

Further reading


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
Bacterial physiology and genetics

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*).

**Bacterial genetics**

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

![Fig. 3.3 The structure of DNA.](image-url)

**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 $2 \times 10^9$.

**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.](image-url)
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 later text).

**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, for example, 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 \(2 \times 10^9\) and composed of approximately \(5 \times 10^6\) 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.

**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.

**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).
Bacterial genetics

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.

Transmissible plasmids can be transferred from cell to cell by conjugation. They contain about 10–12 genes responsible for synthesis of the sex pilus and for the enzymes required for transfer; because of their large size, they are usually present in a few (one to three) copies per cell.

Non-transmissible plasmids are small and do not contain the transfer genes. However, they can be mobilized by co-resident plasmids that do contain the transfer gene. Many copies (up to 60 per cell) of these small plasmids may be present.

Clinical relevance of plasmids

A number of medically important functions of bacteria are attributable to plasmids (i.e., are plasmid coded). The plasmid-coded bacterial attributes include:

**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 aforementioned 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.

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Bacterial physiology and genetics

For their insertion, transposons do not require extensive homology between the terminal repeat sequences of the transposon (which mediate integration) and the site of insertion in the recipient DNA.

Transposons can code for metabolic or drug-resistance enzymes and toxins. They may also cause mutations in the gene into which they insert, or alter the expression of nearby genes.

In contrast to plasmids or bacterial viruses, transposons cannot replicate independently of the recipient DNA. More than one transposon can be located in the DNA; for example, a plasmid can contain several transposons carrying drug-resistance genes. Thus transposons can jump from:

- the host genomic DNA to a plasmid
- one plasmid to another
- a plasmid to genomic DNA.

Transposons, also called jumping genes, are pieces of DNA that move readily from one site to another, either within or between the DNAs of bacteria, plasmids and bacteriophages. In this manner, plasmid genes can become part of the chromosomal complement of genes. Interestingly, when transposons transfer to a new site, it is usually a copy of the transposon that moves, while the original remains in situ (like photocopying). For their insertion, transposons do not require extensive homology between the terminal repeat sequences of the transposon (which mediate integration) and the site of insertion in the recipient DNA.

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- one plasmid to another
- a plasmid to genomic DNA.
Recombinant DNA technology in microbiology

By definition, every classified species must have somewhere on its genome a unique DNA or RNA sequence that distinguishes it from another species. In diagnostic microbiology, this attribute is used to identify microbes where the DNA sequence of the offending pathogen can be identified by means of a number of clever techniques, using clinical samples from the patient.

Gene cloning

Gene cloning is the artificial incorporation of one or more genes into the genome of a new host cell by various genetic recombination techniques.

The candidate DNA is first extracted from the source, purified and cut or cleaved into small fragments by restriction enzymes, leaving ‘sticky ends’. These are then inserted into a vector DNA, first by cutting the vector DNA with the same enzyme so as to produce complementary sticky ends. The sticky ends of the vector and the candidate DNA are then tied or ligated together using enzymes called ‘DNA ligases’ to produce a recombinant DNA molecule. This process can also be used for cloning RNA, when complementary copies of DNA are produced by reverse transcription using reverse transcriptase enzymes. The vector used for gene transfer is usually a plasmid or a virus.

The vector with the integrated DNA has to be inserted into a cell in order to obtain multiple copies of the organism that expresses the selected gene. This can be done by:

- transformation (see above): very popular owing to its simplicity, but competent cells need to be found
- electroporation: here an electric current induces pores on the cell membrane for vector entry
- gene gun: tungsten or gold particles are coated with the vector and propelled into cells by a helium burst
- microinjection: direct manual injection of the vector into a cell by a glass micropipette.

The insertion of the vector containing the recombinant DNA does not necessarily mean that all the progeny bacteria will contain the inserted element, because the vector integration process is somewhat random. In order to select the clone of bacteria that expresses the recombinant gene, other devious manoeuvres have to be adopted. For instance, one can choose a plasmid vector that carries resistance to antibiotics A and B. If the foreign DNA is inserted in the middle of gene \( A \), then this gene will be inactivated as a consequence. In this manner, bacteria with the cloned foreign DNA can be selected and are called the gene library.

Gene probes

DNA probes

Used extensively in diagnostic microbiology, gene probes are pieces of DNA that are labelled radioactively or with a chemiluminescent marker. The probes carry a single strand of DNA analogous to the pathogen that is sought in the clinical sample. There are different types of DNA probe:

- Whole DNA probes are derived from chromosomal DNA and are used to seek organisms where the genome is not well characterized. Owing to their relatively large size, non-specific reactions are common and the method is not very reliable.
- Cloned DNA probes are similar but are smaller, and the reaction is more specific. These are generally targeted at genes unique to the organism sought.

Oligonucleotide probes

Oligonucleotide probes are based on the variable region of the 16S ribosomal RNA (rRNA) genes. The nucleotide sequences of the latter gene of a number of microbes have been well characterized, and are known to be well preserved across species, except for several small variable regions. This property is helpful in the construction of specific oligonucleotide probes of about 18–30 bases, which are much more specific than the aforementioned DNA probes.

RNA probes

Cellular protein synthesis is dependent on rRNA, and any mutation of the rRNA leads to cell death. Further, rRNA is highly species specific, and this property is exploited to produce RNA probes that are useful for both diagnostic microbiology and taxonomic studies. The most commonly used are the 5S, 16S and 23S probes.

DNA/RNA probes and oral microbiology

Cultivation of the complex mixture of bacteria residing in the oral cavity is fraught with problems, and it is now recognized that a number of bacterial genera are difficult or almost impossible to culture. The introduction of DNA and RNA probes has helped us to obtain a more complete picture of the oral flora. For example, commercially available probes can now be used in diagnostic laboratories not only to identify but also to quantify periodontopathic flora in subgingival plaque samples obtained from a periodontal pocket (Fig. 3.7). Further, the samples, say in paper points, could be simply sent by post to distant laboratories for identification without the fear of death of organisms and the associated cumbersome culture procedures.

Polymerase chain reaction (PCR)

Gene-cloning techniques revolutionized the molecular biological advances in the 1970s. The analogous event that took place in the late 1980s was the invention of the PCR. It is a simple technique in which a short region of a DNA molecule, a single gene, for instance, is copied repetitiously by a DNA polymerase enzyme (Fig. 3.8). This technique, in combination with a number of others described in the following section, is used to identify unculturable bacteria from the oral cavity and other body sites (Fig. 3.9).

Materials

The following materials are required:

- the region of the DNA molecule to be amplified
- Taq polymerase (a heat-stable enzyme from *T. aquaticus* (hence *Taq*), a bacterium that lives in hot springs)
- deoxyribonucleoside 5′-triphosphate (dNTP): adenine, guanine, cytosine and thymine
- primers (with a known DNA sequence).
visualize the product, which manifests as a discrete band after staining with ethidium bromide (Fig. 3.8).

10. The latter step is obviated in newer variations of PCR such as real-time PCR where the amplicon can be identified using labelled probes and labelled fluorophores (see the following section).

**PCR and its variations**

The basic PCR methodology is now modified to provide sophisticated analytical tools. The main features of three commonly used variations of PCR, namely, nested, multiplex and real-time PCR, are as follows.

**Nested PCR**

Here, two sets of primers are used: the first set is used for the primary amplification round. The second primer set, specifically chosen to anneal with an internal sequence of the amplicon, re-amplifies the latter ‘specific’ sequence; nested PCR has increased sensitivity than the conventional PCR.

**Multiplex PCR**

In this method, more than one locus of the nucleotide is simultaneously amplified using multiple sets of primers, thus saving time and resources; multiplex PCR has increased specificity and can identify organisms more accurately.

**Real-time PCR**

Conventional PCR requires gel electrophoresis for analysis of the amplicons. In real-time PCR, this step is automatically performed in real time, and the target sequence is identified within a
Recombinant DNA technology in microbiology

diagnosis of viral, bacterial and fungal and other diseases. For instance, amplification of viral DNA in a patient sample could be made within hours, and sometimes even before the onset of symptoms.

- **Amplification of RNA.** Here, the RNA molecule has to be first converted to single-strand complementary DNA (cDNA) with an enzyme called reverse transcriptase (as it transcribes the RNA code into DNA in a reverse manner). Once this initial step is carried out, the PCR primers and Taq polymerase are added; afterwards, the experimental procedure is identical to the standard technique.

- **Comparison of different genomes.** Random amplification with short lengths of primers can be used in phylogenetics, the study of evolutionary history and lines of descent of species or groups of organisms. This technique is called random amplification of polymorphic DNA (RAPD).

**Why is PCR so widely used?**

Some reasons why the use of PCR is so widespread:

- **To study minuscule quantities of DNA,** as a single DNA molecule is adequate for an amplification reaction (hence its use in forensic studies, archaeology and palaeontology).

- **Use in rapid clinical diagnostic procedures.** The sensitivity of the PCR has resulted in its use in rapid diagnosis of viral, bacterial and fungal and other diseases. For instance, amplification of viral DNA in a patient sample could be made within hours, and sometimes even before the onset of symptoms.

**Fig. 3.8** The polymerase chain reaction (PCR). dNTP, deoxyribonucleoside 5'-triphosphate.
Bacterial physiology and genetics

Restriction fragment length polymorphism (RFLP)

In RFLP, the DNA is first cleaved using restriction endonucleases and separated on an agarose gel. Afterwards, the separated fragments are transferred by blotting onto a nitrocellulose or nylon membrane by a method called Southern blotting, and DNA probes constructed from genes of known organisms (species or strains) are then hybridized to the membrane; these will bind to complementary sequences in the DNA fragments on the membrane, revealing the species or strain identity.

Pulsed-field gel electrophoresis (PFGE)

PFGE is similar to RFLP. Here, the chromosomal DNA of an organism is cut into relatively large fragments using a method called PFGE.

Other techniques for genetic typing of microorganisms

Restriction enzyme analysis

A genetic ‘fingerprint’ of the organism is obtained by extracting its DNA and cutting or cleaving the DNA at specific points by restriction endonucleases. The DNA fragments so generated are run on an agarose electrophoresis gel and viewed under ultraviolet illumination after staining with ethidium bromide. The profiles of the bands produced on the gel (the ‘fingerprints’) can be compared or contrasted with those from other strains. This was the original molecular method used for genotyping organisms, but has been supplanted by newer methods that are more discriminatory.

Restriction fragment length polymorphism

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Pulsed-field gel electrophoresis

PFGE is similar to RFLP. Here, the chromosomal DNA of an organism is cut into relatively large fragments using a method called PFGE.
pieces by restriction enzymes and the resultant fragments are separated in an agarose gel with the help of a pulsed electric field, in which the polarity is regularly reversed. Large pieces of chromosomes usually do not separate in conventional agarose gels, hence the necessity of the pulsed/reversed electric field.

**Pyrosequencing**

Pyrosequencing is one of the most novel and reliable techniques of DNA sequencing. It is based on the ‘sequencing by synthesis’ principle. So called as it relies on the detection of pyrophosphate release on nucleotide incorporation, rather than chain termination with dideoxynucleotides used in PCR techniques. It uses chemiluminescence enzyme reactions and photodetection techniques that are highly automated, rapid and sensitive.

**Next-generation sequencing**

Next-generation sequencing (NGS), also known as high-throughput sequencing, is the catch-all term used to describe a number of different modern sequencing technologies (e.g., Illumina, Ion Torrent). NGS permits sequencing DNA and RNA much more quickly and cheaply than other previously developed technologies and has revolutionized microbiology. NGS techniques are now widely used in the analysis of the oral microbiome.

**The era of ‘-omics’**

With the advent of the new millennium, there has been an explosion of digital and computer technology, the use of which has led to a parallel advancement of the knowledge of our biosphere. This in turn has led to focal developments of sub-disciplines such as genomics, proteomics and metabolomics: the so-called -omics era. These new technologies have had a significant impact on the identification of microbes, particularly those that could not be cultured in the laboratory (unculturable bacteria), and on the elucidation of their pathogenic mechanisms such as resistance to antibiotics. A brief introduction to the various -omics domains are as follows.

**Genomics**

This refers to the study of the identity of all genes within the chromosome of a cell. The human animal and microbial genome sequencing projects have thus far provided a rich genetic resource to better understand human diseases including oral diseases. As mentioned in Chapter 2, the development of technologies such as microarray analysis has helped microbiologists to explore patterns of gene expression in various infectious diseases, and their pathogenic mechanisms, for example, in periodontal disease. The sub-category of functional genomics deals with the organization of the genes and their expression patterns under defined conditions.

The development of computer models for high throughput analyses of genomic data has simplified the exploration of gene expression profiles in both eukaryotes and prokaryotes. Furthermore, **DNA microarray technologies** help investigators evaluate gene expression on a genome-wide basis, providing a ‘global’ perspective of how an organism responds to a specific stress, drug or toxin.

**Proteomics**

This is defined as the study of the myriad of proteins expressed by the genome of either an organism, cell or tissue type. Proteomics builds on and complements the knowledge gained from genomics by revealing the levels, activities, regulation and interactions of every protein in an organism or a cell. Study of the proteome is more complex than that of the genome as the number of proteins in an organism/cell is considered many orders of magnitude greater than that of the number of genes.

Such complexity is further confounded by the dynamic changes in the proteome in response to the environment and also the multiple possible interactive combinations among proteins. **Protein chips** that can simultaneously identify large numbers of proteins are helpful in unravelling such complexity.

**Transcriptomics**

This is a related branch of molecular biology that deals with the study of messenger RNA molecules produced in an individual or population of a particular cell type.

**Metabolomics**

This is defined as the scientific study of chemical processes involving metabolites of a cell or an organism. While proteomic analyses do not tell the whole story of what might be happening in a cell, metabolic profiling can give an instantaneous snapshot of the physiology of that organism. This has led to the development of a further domain known as **interactomics**. The latter is defined as a discipline involving the intersection of bioinformatics and biology that deals with studying both the interactions and the consequences of those interactions between and among proteins, and other molecules within an organism. The network of all such interactions is called the interactome. In essence, interactomics aims to compare networks of interactions (i.e., interactomes) between and within species in order to elucidate how the traits of such networks are either preserved or varied.

One of the current challenges of science is to integrate proteomic, transcriptomic, metabolic and interactomic data to provide a more complete picture of living organisms.

**Bioinformatics**

Bioinformatics is an essential component of the -omics era. The avalanche of information spewed out, for instance, by NGS and similar techniques cannot be sorted using traditional methods. Hence new computational methods and their application to the solution of biological problems, often via the mining of information databases, have been developed over the last decade or so. This rapidly developing field is called bioinformatics, and forms a crucial pivotal point for the -omics technology. As bioinformatics occupies a central role in a broad spectrum of biological research, its analytical toolkits are equally diverse and complex.
Key facts

- Bacteria, like all living organisms, require oxygen, hydrogen, carbon, inorganic ions and organic nutrients for survival.
- Other factors that modify growth are end product inhibition and catabolite repression, and the temperature and pH of the medium.
- Bacteria reproduce by binary fission, leading to logarithmic growth of cell numbers; the doubling or mean generation time of bacteria can vary from minutes to hours or days.
- Bacterial growth in laboratory media can be divided into a lag phase, log phase, stationary phase and decline phase.
- Depending on their oxygen requirements, bacteria can be divided into obligate aerobes, facultative anaerobes, obligate anaerobes and microaerophiles.
- Bacterial chromosomes comprise a single, continuous strand of DNA with a closed, circular structure attached to the cell membrane.
- DNA replication is the synthesis of new strands of DNA using the original DNA strands as templates.
- DNA replicates by a process called semiconservative replication; DNA-dependent DNA polymerase is the main enzyme that mediates DNA replication.
- Restriction enzymes of bacteria delete foreign nucleotides from their genomes. These enzymes are therefore extremely useful in molecular biological techniques.
- Genetic variations in bacteria can occur by either mutation or gene transfer.
- Mutation, a change in the base sequence of the DNA, can be due to either base substitution frame shifts or insertion of additional pieces of DNA.
- Gene transfer in bacteria may occur by conjugation, transduction, transformation or transposition.
- Plasmids are extrachromosomal, double-stranded circular DNA molecules capable of independent replication within the bacterial host.
- The clinical relevance of plasmids lies in the fact that they code for antibiotic resistance, resistance to heavy metals, exotoxin production and pli formation.
- Transposons are ‘jumping genes’ that move from one site to another either within or between the DNA molecules.
- Gene cloning is the introduction of foreign DNA into another cell where it can replicate and express itself.
- Gene probes used in diagnostic microbiology are labelled (with chemicals or radioactively) pieces of DNA that can be used to detect specific sequences of DNA of the pathogen (in the clinical sample) by pairing with the complementary bases.
- The polymerase chain reaction (PCR) is a widely used technique that enables multiple copies of a DNA molecule to be generated by enzymatic amplification of the target DNA sequence.
- Pyrosequencing is a rapid, reliable sequencing method of relatively short DNA templates based on real-time (quantitative) pyrophosphate release and is a valuable tool for identification of bacteria (particularly unculturable).
- Next-generation sequencing (NGS) is a general term used to describe a number of different modern sequencing technologies.

Review questions (answers on p. 363)

Please indicate which answers are true, and which are false.

3.1 With regard to bacterial growth, which of the following statements are true?
A. autotrophic bacteria can use carbon dioxide as the sole source of carbon
B. the growth of facultative anaerobes is arrested in the presence of oxygen
C. a new progeny of cells are formed as a result of sporulation
D. the logarithmic growth phase of bacteria precedes the lag phase
E. some bacteria can grow at 80°C

3.2 Plasmid-coded bacterial attributes include:
A. antibiotic resistance
B. production of exotoxins
C. resistance to disinfectants
D. transfer of genetic material
E. production of endotoxins

3.3 With regard to transposons, which of the following are true?
A. they are also called jumping genes
B. they can replicate independently of the chromosome or the plasmid
C. they can cause mutations
D. they mediate antimicrobial resistance
E. a bacterial chromosome can have only one transposon

Further reading

Viruses are one of the smallest forms of microorganism and infect most other forms of life: animals, plants and bacteria. They can also cause severe acute oral and orofacial disease, produce oral signs of systemic infection and be transmitted to patients and dental staff. The main features that characterize viruses are:

- **small size** (10–100 nm), averaging about one-tenth the size of a bacterium
- **genome** consisting of either DNA or RNA but never both; single- (ss) or double-stranded (ds); linear or circular (the encoding of the whole of the genetic information as RNA in RNA viruses is a situation unique in biology)
- **metabolic inactivity** outside the cells of susceptible hosts; viruses lack ribosomes, the protein-synthesizing apparatus (the corollary of this is that viruses can only multiply inside living cells, i.e., they are **obligate intracellular parasites**).

### Structure

Viruses consist of a nucleic acid core containing the viral genome, surrounded by a protein shell called a **capsid** (Figs 4.1 and 4.2). The entire structure is referred to as the **nucleocapsid**. This may be ‘naked’, or it may be ‘enveloped’ within a lipoprotein sheath derived from the host cell membrane. In many viruses (e.g., orthomyxoviruses, paramyxoviruses), the ensheathment begins by a budding process at the plasma membrane of the host cell, whereas others, such as herpesviruses, ensheathe at the membrane of the nucleus or endoplasmic reticulum.

The protein shell or capsid consists of repeating units of one or more protein molecules; these protein units may go on to form structural units, which may be visualized by electron microscopy as morphological units called **capsomeres** (Fig. 4.1). Genetic economy dictates that the variety of viral proteins be kept to a minimum as viral genomes lack sufficient genetic information to code for a large array of different proteins. In enveloped viruses, the protein units, which comprise the envelopes and are visualized electron microscopically, are called peplomers (loosely referred to as ‘spikes’).

### Viral nucleic acid

Viral nucleic acid may be either DNA or RNA. The RNA, in turn, may be ss or ds, and the genome may consist of one or several molecules of nucleic acid. If the genome consists of a single molecule, this may be linear or have a circular configuration. The DNA viruses all have genomes composed of a single molecule of nucleic acid, whereas the genomes of many RNA viruses consist of several different molecules or segments, which are probably loosely linked together in the virion.

### Viral protein

In terms of volume, the major bulk of the virion is protein, which offers a protective sheath for the nucleic acid. The viral protein is made up of two or three different polypeptide chains, although in some only one kind of polypeptide chain may be present. Virion surface proteins may have a special affinity for receptors on the surface of susceptible cells and may bear antigenic determinants.

Although most viral proteins have a structural function, some have enzymatic activity. For instance, many viruses such as the human immunodeficiency virus (HIV) contain a reverse transcriptase, whereas several enzymes (e.g., neuraminidase, lysozyme) are found in larger, more complex viruses.

### Viral lipid and carbohydrate

In general, lipids and carbohydrates of viruses are only found in their envelopes and are mostly derived from the host cells. About 50%–60% of the lipids are phospholipids; most of the remainder is cholesterol.

### Virus symmetry

The nucleocapsids of viruses are arranged in a highly symmetrical fashion (symmetry refers to the way in which the
protein units are arranged). Three kinds of symmetry are recognized (Fig. 4.3):

- **Icosahedral symmetry.** The protein molecules are symmetrically arranged in the shape of an icosahedron (i.e., a 20-sided solid, each face being an equilateral triangle). Herpesviruses are an example (Figs 4.1 and 4.2).

- **Helical symmetry.** The capsomeres surround the viral nucleic acid in the form of a helix or spiral to form a tubular nucleocapsid. Most mammalian RNA viruses have this symmetry, where the nucleocapsid is arranged in the form of a coil and enclosed within a lipoprotein envelope.

- **Complex symmetry.** This is exhibited by a few families of viruses, notably the retroviruses and poxviruses.

### Taxonomy

Vertebrate viruses are classified into families, genera and species. The attributes used in classification are their symmetry, the presence or absence of an envelope, nucleic acid composition (DNA or RNA), the number of nucleic acid strands and their polarity. Classification of some of the recognized families of RNA and DNA viruses is given in Table 4.1. (Note: to memorize which viruses contain DNA, remember the acronym ‘PHAD’: P is for papova and pox, H for herpes and AD for adenoviruses. Most of the remainder are RNA viruses, including the self-evident picornaviruses.)

The following is a concise description of the families of mammalian viruses.

### DNA viruses

#### Papovaviruses

Papovaviruses are small, icosahedral DNA viruses with a capacity to produce tumours in vivo and to transform cultured