Part 1

Basic microbiology

1

Basic bacteriology

Peter Lambert
Aston University, Birmingham, UK

Bacterial structure

Bacteria are single-celled prokaryotic microorganisms, and their DNA is not contained within a separate nucleus as in eukaryotic cells. They are approximately 0.1–10.0 µm in size (Figure 1.1) and exist in various shapes, including spheres (cocci), curves, spirals and rods (bacilli) (Figure 1.2). These characteristic shapes are used to classify and identify bacteria. The appearance of bacteria following the Gram stain is also used for identification. Bacteria which stain purple/blue are termed Gram-positive, whereas those that stain pink/red are termed Gram-negative. This difference in response to the Gram stain results from the composition of the cell envelope (wall) (Figure 1.3), which are described below.

Cell envelope

Cytoplasmic membrane

A cytoplasmic membrane surrounds the cytoplasm of all bacterial cells and are composed of protein and phospholipid; they resemble the membrane surrounding mammalian (eukaryotic) cells but lack sterols. The phospholipids form a bilayer into which proteins are embedded, some spanning the membrane. The membrane carries out many functions, including the synthesis and export of cell-wall components, respiration, secretion of extracellular enzymes and toxins, and the uptake of nutrients by active transport mechanisms.

Mesosomes are intracellular membrane structures, formed by folding of the cytoplasmic membrane. They occur more frequently in Gram-positive than in Gram-negative bacteria. Mesosomes present at the point of cell division of Gram-positive bacteria are involved in chromosomal separation; at other sites they may be associated with cellular respiration and metabolism.

Cell wall

Bacteria maintain their shape by a strong rigid outer cover, the cell wall (Figure 1.3).

Gram-positive bacteria have a relatively thick, uniform cell wall, largely composed of peptidoglycan, a complex molecule consisting of linear repeating sugar subunits cross-linked by peptide side chains (Figure 1.4a). Other cell-wall polymers, including teichoic acids, teichuronic acids and proteins, are also present.

Gram-negative bacteria have a thinner peptidoglycan layer and an additional outer membrane that differs in structure from the cytoplasmic membrane (Figure 1.4b). The outer membrane contains lipopolysaccharides on its outer face, phospholipids on its inner face, proteins and lipoproteins which anchor it to the peptidoglycan. Porins are a group of proteins that form channels through which small hydrophilic molecules, including nutrients, can cross the outer membrane. Lipopolysaccharides are

Medical Microbiology and Infection Lecture Notes, Fifth Edition. Edited by Tom Elliott, Anna Casey, Peter Lambert and Jonathan Sandoe.

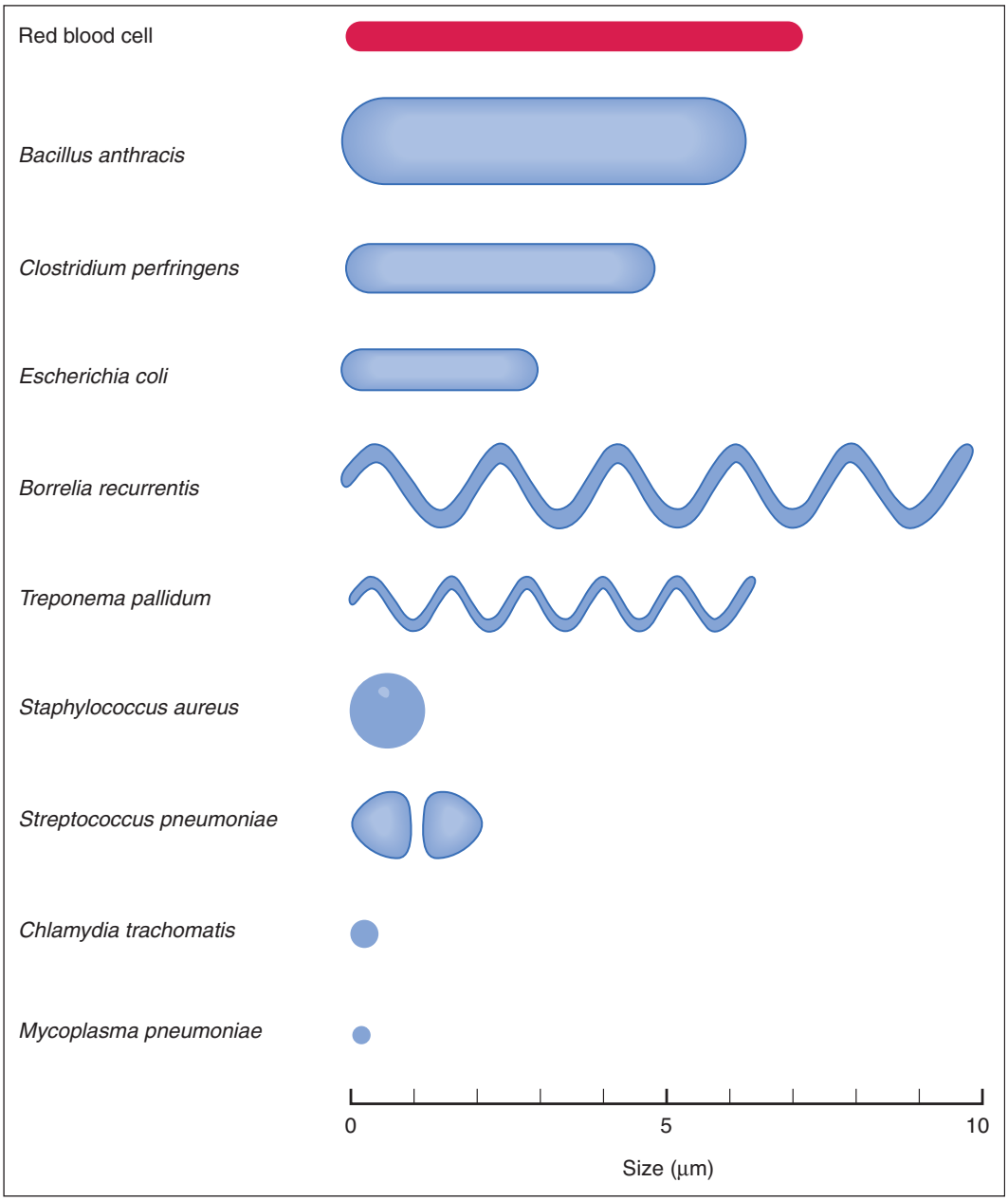

Figure 1.1 Shape and size of some clinically important bacteria.

a characteristic feature of Gram-negative bacteria and are also termed 'endotoxins' or 'pyrogen'. Endotoxins are released on cell lysis and have important biological activities involved in the pathogenesis of Gram-negative infections; they activate macrophages, clotting factors and complement, leading to disseminated intravascular coagulation and septic shock (Chapter 33).

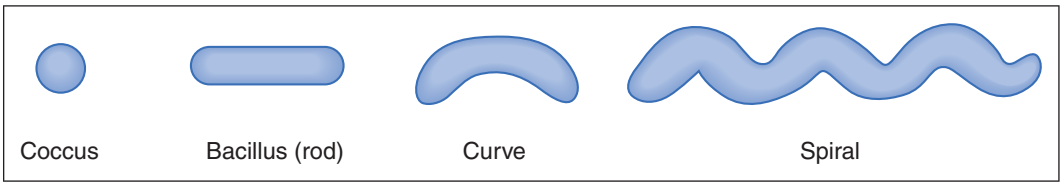

Figure 1.2 Some bacterial shapes.

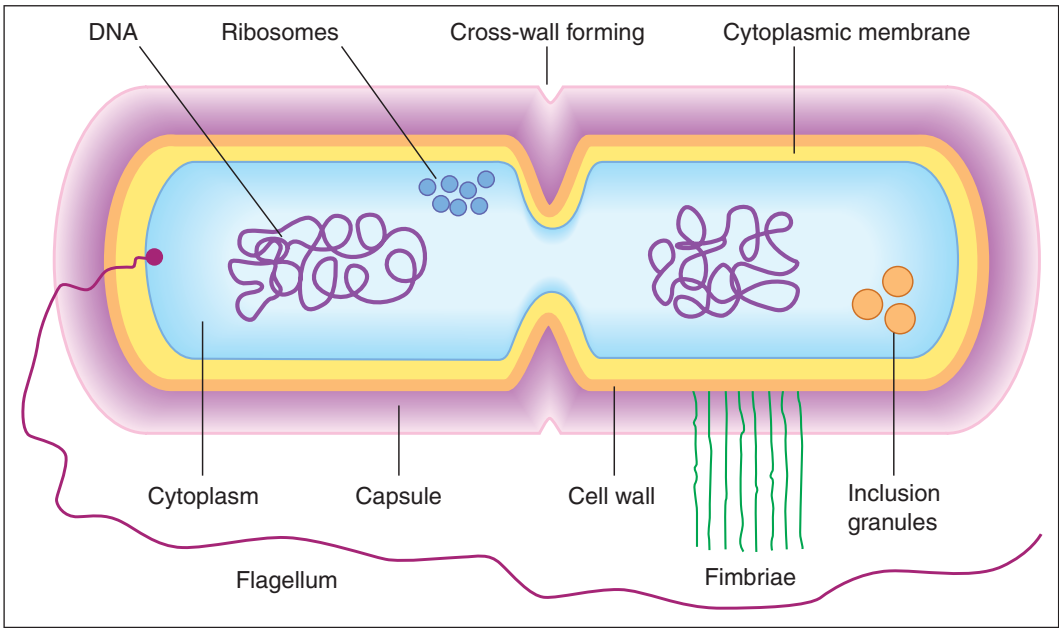

Figure 1.3 A section of a typical bacterial cell.

Mycobacteria have a distinctive cell wall structure and composition that differs from that of Gram-positive and Gram-negative bacteria. It contains peptidoglycan but has large amounts of high molecular weight lipids in the form of long chain length fatty acids (mycolic acids) attached to polysaccharides and proteins. This high lipid content gives the mycobacteria their acid fast properties (retaining a stain on heating in acid), which allows them to be distinguished from other bacteria (e.g. positive Ziehl-Neelsen stain).

The cell wall is important in protecting bacteria against external osmotic pressure. Bacteria with damaged cell walls, e.g. after exposure to β-lactam antibiotics such as penicillin, often rupture. However, in an osmotically balanced medium, bacteria deficient in cell walls may survive in a spherical form called protoplasts. Under certain conditions some protoplasts can multiply and are referred to as L-forms. Some bacteria, e.g. mycoplasmas, have no cell wall at any stage in their life cycle.

The cell wall is involved in bacterial division. After the nuclear material has replicated and separated, a cell wall (septum) forms at the equator of the parent cell. The septum grows in, produces a cross-wall and eventually the daughter cells may separate. In many species the cells can remain attached, forming groups, e.g. staphylococci form clusters and streptococci form long chains (Figure 1.5).

Capsules

Some bacteria have capsules external to their cell walls (Figure 1.3). These structures are bound

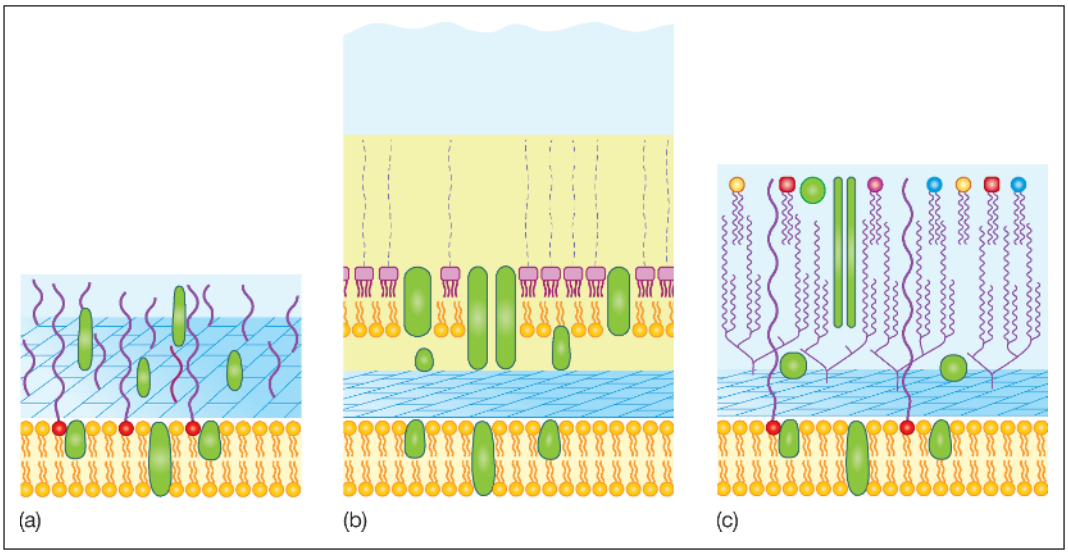

Figure 1.4 Cell wall and cytoplasmic membrane of (a) Gram-positive bacteria, (b) Gram-negative bacteria and (c) mycobacteria. The Gram-positive bacterial cell wall has a thick peptidoglycan layer with associated molecules (teichoic acids, teichuronic acids and proteins). The Gram-negative bacterial cell wall contains lipopolysaccharides, phospholipids and proteins in an outer membrane linked to a thin inner peptidoglycan layer. The mycobacterial cell wall contains long chain length fatty acids (mycolic acids).

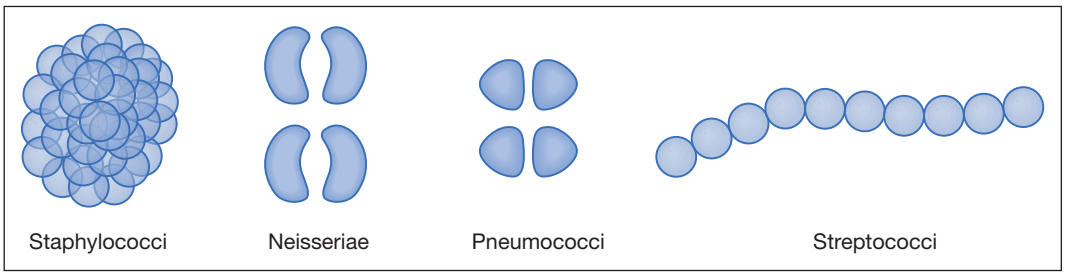

Figure 1.5 Some groups of bacteria.

to the bacterial cell and have a clearly defined boundary. They are usually polysaccharides with characteristic compositions that can be used to distinguish between microorganisms of the same species (e.g. in serotyping). Capsular antigens can be used to differentiate between strains of the same bacterial species, e.g. in the typing of *Streptococcus pneumoniae* for epidemiological purposes. The capsules are important virulence determinants in both Gram-positive and Gram-negative bacteria, because they may protect the bacteria from host defences and, in some bacteria, aid attachment to host cells.

Bacterial slime and biofilm

Extracellular slime layers are produced by some bacteria. They are more loosely bound to the cell surface than capsules and do not form a clearly defined surface boundary. The slime layer is composed predominantly of complex polysaccharides (glycocalyx), which acts as a virulence

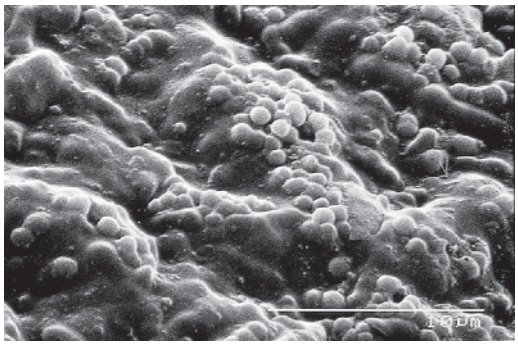

Figure 1.6 Scanning electronmicrograph of *Staphylococcus epidermidis* embedded in slime attached to a catheter.

factor through the formation of biofilm, e.g. by facilitating the attachment of *Staphylococcus epidermidis* onto artificial surfaces, such as intravascular cannulae (Figure 1.6), replacement joints and heart valves. Once formed, biofilms present a major problem for treatment and may require removal of the biomedical device.

Flagella

Bacterial flagella are spiral-shaped surface filaments consisting mainly of the protein, flagellin. They are attached to the cell envelope as single (monotrichous) or multiple (peritrichous) forms (Figure 1.7).

Flagella facilitate movement (motility) in bacteria by rapid rotation. They can be observed under the light microscope with special stains. Flagella are usually detected for diagnostic purposes by observing motility in a bacterial suspension or by spreading growth on solid media. The antigenic nature of the flagella may be used to differentiate between and identify strains of *Salmonella* spp.

Fimbriae

Fimbriae (also termed pili) are thin, hair-like appendages on the surface of many Gram-negative, and some Gram-positive, bacteria (Figure 1.3). They are approximately half the width of flagella, and are composed of proteins called pilins. In some bacteria they are distributed over the entire cell surface.

Fimbriae are virulence factors enabling bacteria to adhere to particular mammalian cell surfaces, an important initial step in colonisation of mucosal surfaces, e.g. *Neisseria gonorrhoeae* produce fimbriae that bind to specific receptors of cervical epithelial cells, whereas *Streptococcus pyogenes* have fimbriae containing 'M' protein, which facilitates adhesion to human cells in the pharynx.

Specialised fimbriae are involved in genetic material transfer between bacteria, a process called conjugation.

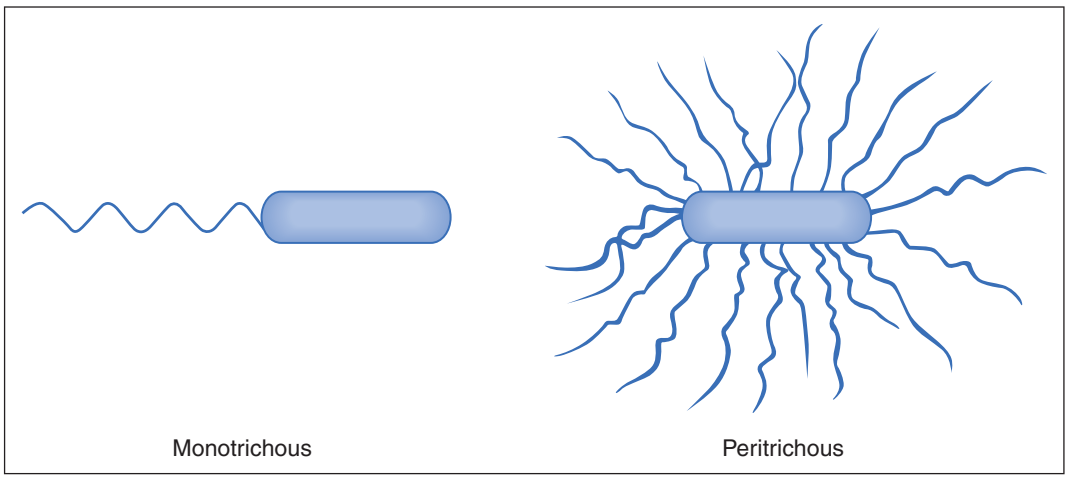

Figure 1.7 Arrangements of bacterial flagella.

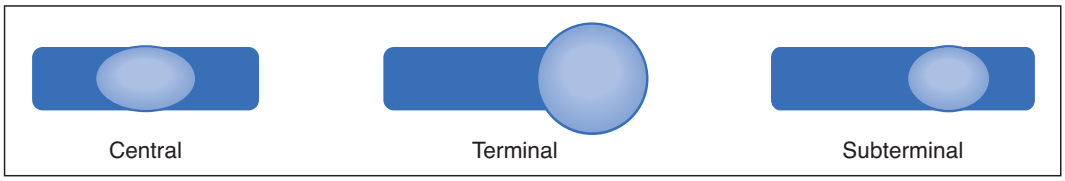

Figure 1.8 Size, shape and position of bacterial spores (from left to right): non-projecting, oval, central, e.g. *Bacillus anthracis*; projecting, spherical, terminal, e.g. *Clostridium tetani*; non-projecting, oval, subterminal, e.g. *C. perfringens*.

Intracellular structures

Nuclear material

The bacterial chromosome consists of a single circular molecule of double-stranded DNA, which is maintained in a compact form within the cell by supercoiling. When released from the cell and uncoiled the DNA would be about 1 mm long (10 to 100-times the length of the cell). Additional smaller extra-chromosomal DNA molecules, called plasmids, may also be present in bacteria. The chromosome usually codes for all the essential functions required by the cell; some plasmids control important phenotypic properties of pathogenic bacteria, including antibiotic resistance and toxin production. Extracellular nuclear material for encoding virulence and antibiotic resistance may also be transferred between bacteria and incorporated into the recipient's chromosome or plasmid. Transfer of genes encoding for virulence or antibiotic resistance may account for bacteria becoming resistant to antibiotics and for low-virulent bacteria becoming pathogenic.

Ribosomes

The cytoplasm has many ribosomes, which contain both ribonucleic acid (RNA) and proteins. Ribosomes are involved in protein synthesis.

Inclusion granules

Various cellular inclusions, which serve as energy and nutrient reserves, may be present in the bacterial cytoplasm. The size of these inclusions may increase in a favourable environment and decrease when conditions are adverse, e.g. *Corynebacterium diphtheriae* may contain high-energy phosphate reserves in inclusions termed 'volutin granules'.

Endospores

Endospores (spores) are small, metabolically dormant cells with a thick, multi-layered coat, formed intracellularly by members of the genera *Bacillus* and *Clostridium* (Figure 1.8). They are highly resistant to adverse environmental conditions and may survive desiccation, disinfectants or boiling water for several hours.

Spores are formed in response to limitations of nutrients by a complex process (sporulation) involving at least seven stages. When fully formed, they appear as oval or round cells within the vegetative cell. The location is variable, but is constant in any one bacterial species (Figure 1.9). Spores can remain dormant for long periods of time. However, they are able to revert to actively-growing cells (i.e. germinate) relatively rapidly in response to certain conditions such as the presence of specific sugars, amino acids or bile salts.

Spores also have an important role in the epidemiology of certain human diseases, such as anthrax, tetanus, gas gangrene and infection caused by *Clostridium difficile*.

The eradication of spores is of particular importance in some processes, e.g. the production of sterile products including pharmaceuticals and surgical instruments, in routine hospital ward and care centre cleaning, and in food preservation.

Bacterial growth

Most bacteria will grow on artificial culture media prepared from extracts from animal or plant tissues, which supply pre-formed nutrients and vitamins. However, some bacteria, e.g. *Mycobacterium leprae* (leprosy) and *Treponema pallidum*

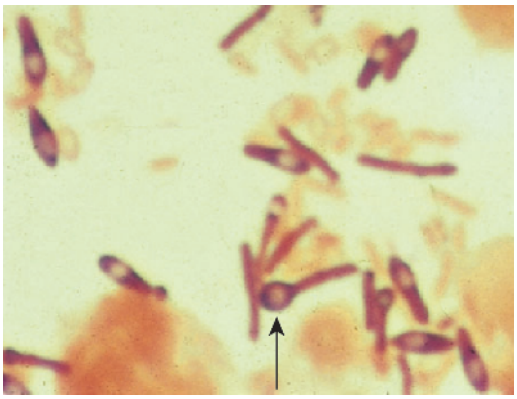

Figure 1.9 Gram-stain of *Clostridium sporogenes* (showing oval subterminal spores) and a *Clostridium tetani* with a terminal spore (arrowed).

(syphilis), cannot yet be grown *in vitro*; other bacteria, e.g. *Chlamydia* spp. and *Rickettsia* spp., only replicate intracellularly within host cells and are therefore grown in tissue culture.

Under suitable conditions (nutrients, temperature and atmosphere) a bacterial cell will increase in size and then divide by binary fission into two identical cells. These two cells are able to grow and divide at the same rate as the parent cell, provided that conditions including nutrient supply remain stable. This results in an exponential or logarithmic growth rate. The time required for the number of bacteria in a culture to double is called the generation time, e.g. *Escherichia coli* has a generation time of about 20 minutes under optimal conditions. By contrast, *Mycobacterium tuberculosis* has a generation time of 24 hours.

Requirements for bacterial growth

Most bacteria of medical importance require carbon, nitrogen, water, inorganic salts and a source of energy for growth. They have various gaseous, temperature and pH requirements, and can utilise a range of carbon, nitrogen and energy sources. Some bacteria also require special growth factors, including amino acids and vitamins.

Growth requirements are important in selecting the various culture media required in diagnostic microbiology and in understanding the tests for identifying bacteria.

Carbon and nitrogen sources

Bacteria are classified into two main groups according to the type of compounds that they can utilise as a carbon source:

1 *Autotrophs* utilise inorganic carbon from carbon dioxide and nitrogen from ammonia, nitrites and nitrates; they are of minor medical importance.
2 *Heterotrophs* require organic compounds as their major source of carbon and energy; they include most bacteria of medical importance.

Atmospheric conditions

Carbon dioxide

Bacteria require CO_2 for growth; adequate amounts are present in the air or are produced during metabolism by the microorganisms themselves. A few bacteria, however, require additional CO_2 for growth, e.g. *Neisseria meningitidis*, *Campylobacter jejuni*.

Oxygen

Bacteria may be classified into four groups according to their O_2 requirements:

1 *Obligate (strict) aerobes*: grow only in the presence of oxygen, e.g. *Pseudomonas aeruginosa*.
2 *Microaerophilic bacteria*: grow best in low oxygen concentrations, e.g. *Campylobacter jejuni*.
3 *Obligate (strict) anaerobes*: grow only in the absence of free oxygen, e.g. *Clostridium tetani*.
4 *Facultative anaerobes*: grow in the presence or absence of oxygen, e.g. *Escherichia coli*.

Temperature

Most pathogenic bacteria grow best at 37 °C. However, the optimum temperature for growth is occasionally higher, e.g. for *C. jejuni*, it is 42 °C. The ability of some bacteria to grow at low temperatures (0–4 °C) is important in food microbiology; *Listeria monocytogenes*, a cause of food poisoning, will grow slowly at 4 °C and has resulted in outbreaks of food poisoning associated with cook-chill products.

pH

Most pathogenic bacteria grow best at a slightly alkaline pH (pH 7.2–7.6). There are a few exceptions: *Lactobacillus acidophilus*, present in the

vagina of post-pubescent females, prefers an acid medium (pH 4.0). It produces lactic acid, which keeps the vaginal secretions acid, thus preventing many pathogenic bacteria from establishing infection. *Vibrio cholerae*, the cause of cholera, prefers an alkaline environment (pH 8.5).

Growth in liquid media

When bacteria are added (inoculated) into a liquid growth medium, subsequent multiplication can be followed by determining the total number of live microorganisms (viable counts) at various time intervals. The growth curve produced normally has four distinct phases (Figure 1.10):

1 *Lag phase* (*A*): the interval between inoculation of a fresh growth medium with bacteria and the commencement of growth;
2 *Log phase* (*B*): the phase of exponential growth; the growth medium becomes visibly turbid at approximately 1×10^6 cells/ml;
3 *Stationary phase* (*C*): the growth rate slows as nutrients become exhausted, waste products accumulate, and the rate of cell division equals the rate of death; the total viable count remains relatively constant;
4 *Decline phase* (*D*): the rate of bacterial division is slower than the rate of death, resulting in a decline in the total viable count.

Note that the production of waste products by bacteria, particularly CO_2, and the uptake of O_2 have been utilised in the development of semi-automated instruments to detect bacterial growth in blood samples obtained from patients with suspected bloodstream infection.

Growth on solid media

Liquid growth media containing the nutrients needed for bacterial growth can be solidified with agar, a polysaccharide extracted from seaweed. Heating during sterilisation of the medium melts the agar, which then remains liquid until the temperature falls to approximately 40 °C, when it produces a transparent solid gel. Solid media are normally set in Petri dishes ('agar plates'). When spread across the surface of an agar plate, most bacteria grow as visible colonies. Each colony comprises millions of bacterial cells that emanated from either a single cell or a cluster of cells. The appearance of the bacterial colony (colonial morphology) assists in identification.

Growth on laboratory media

To grow bacteria *in vitro*, the microbiologist has to take into account the physiological requirements. Various types of liquid and solid media have been

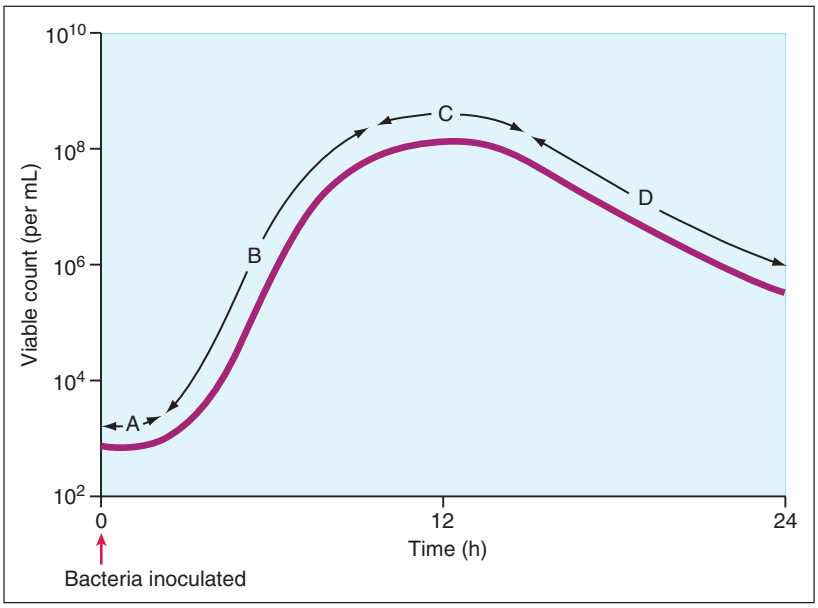

Figure 1.10 Bacterial growth curve showing the four phases: (A) lag; (B) log or exponential; (C) stationary; and (D) decline (death).

developed for the diagnostic microbiology laboratory.

Simple media

Many bacteria will grow in or on simple media, e.g. nutrient broth/nutrient agar that contains 'peptone' (polypeptides and amino acids from the enzymatic digestion of meat) and 'meat extract' (water-soluble components of meat containing mineral salts and vitamins).

Enriched media

These contain additional nutrients for the isolation of more fastidious bacteria that require special conditions for growth, e.g. agar containing whole blood (blood agar) or agar containing lysed blood (chocolate agar).

Selective media

These are designed to facilitate growth of some bacteria, while suppressing the growth of others, and include:

- *mannitol salt agar* which contains increased NaCl (salt) concentration for the recovery of staphylococci;
- *MacConkey agar*, which contains bile salts and allows the growth of bile-tolerant bacteria only; and
- *antibiotics*, which are frequently added to media to allow only certain bacteria to grow while suppressing or killing others.

Indicator media

These are designed to aid the detection and recognition of particular pathogens. They are often based on sugar fermentation reactions that result in production of acid and the subsequent colour change of a pH indicator, e.g. MacConkey agar contains lactose and a pH indicator (neutral red); lactose-fermenting bacteria (e.g. *Escherichia coli*) produce acid and form pink colonies, whereas non-lactose fermenting bacteria (e.g. *Salmonella* spp.) do not produce acid and form pale yellow colonies. This property facilitates the recognition of possible *Salmonella* colonies among normal bowel flora. Note that indicator media may also contain selective agents including antibiotics or substances such as bile salts and crystal violet to suppress growth of most Gram-positive microorganisms. MacConkey agar is therefore both a selective medium and an indicator medium.