# 1

# An introduction to the human–microbe symbiosis

The first 9 months of our existence – the time we spend in our mother's womb – is the only period of our life during which we are free of microbes. Our delivery from this parasitic existence into the outside world exposes us to an enormous range of microbes from a variety of environments – our first encounter with life forms which have an anatomy, physiology, and metabolism very different from those of our own. Hence, our immediate companions on life's long journey include organisms from (1) the vagina, gastrointestinal tract (GIT), skin, oral cavity, and respiratory tract of our mother; (2) the skin, respiratory tract, and oral cavity of other individuals present at the delivery; (3) the instruments and equipment used during delivery; and (4) the immediate environment. These will include, therefore, not only microbes from other human beings, but also organisms from soil, water, and vegetation that may be present. All of the studies that have been carried out on neonates have shown that, within a very short time following delivery, microbes are detectable on most of those surfaces of the baby that are exposed to the external environment (i.e., the skin, respiratory tract, GIT, and oral cavity). Despite the fact that we are exposed to a wide variety of microbes at birth, only a limited number of species are able to permanently colonise the various body sites available, and each site is colonised predominantly by only certain microbial species (i.e., the microbes display "tissue tropism"). The organisms found at a particular site constitute what is known as the indigenous (or "normal") microbiota of that site. It is important to note that the term "indigenous microbiota" will include all of the bacteria, viruses, fungi, and protoctists that are able to colonise any of the body surfaces. However, the vast majority of studies undertaken so far have been concerned with identifying only the bacteria present at a particular site, and so we know very little about the distribution or frequency of occurrence of Archaea, viruses, fungi, or protoctists on healthy individuals. This book, therefore, is concerned only with the bacterial members of the indigenous microbiota and with those fungi (e.g., *Candida albicans* and *Malassezia* spp.) which the available data suggest are also indigenous to humans.

It is appropriate at this point to define what is meant by "symbiosis". Strictly speaking, the term means "living together" and so can be applied to any association between two (or more) organisms. However, it is possible to recognise at least three types of symbiosis: (1) mutualism – when both members of the association benefit, (2) commensalism – when one member benefits while the other is unaffected, and (3) parasitism – when one member suffers at the expense of the other. Confusingly, however, many scientists now use the term "symbiosis" to mean only the first of these three

Table 1.1. | Mass of the microbial communities associated with various body sites

| Organ/system | Associated microbiota (grams wet weight) |
|---|---|
| eyes | 1 |
| nose | 10 |
| mouth | 20 |
| lungs | 20 |
| vagina | 20 |
| skin | 200 |
| intestines | 1000 |

possibilities (i.e., a mutually beneficial interaction between two (or more) organisms). In this book, symbiosis will be used in this sense while the term "mutualism" will be reserved for those mutually-beneficial relationships in which the association is obligatory (see Section 1.2.1). When the species comprising a symbiosis differ in size, the larger member is known as the host whereas the smaller is termed a "symbiont".

One of the many remarkable features of the microbiota of a particular anatomical location is the similarity of its composition among human beings worldwide despite the huge variations in the climate they are exposed to, the diet they consume, the clothes they wear, the hygiene measures they practice, and the lifestyle they have adopted. It would appear, therefore, that over many millennia humankind has co-evolved with some of the microbial life forms present on earth to form a symbiosis that is usually of mutual benefit to all of the organisms involved. However, this relationship between the indigenous microbiota and its human host is delicately balanced and can break down, resulting in an "endogenous" or "opportunistic" infection. This book is concerned with the indigenous microbiota of humans and describes (1) its development and composition at various body sites, (2) how its composition can be affected by various human activities, (3) the benefits it confers on its human host, (4) the diseases which it is able to cause, and (5) how its composition may be manipulated for the host's benefit.

## 1.1 Overview of the distribution and nature of the indigenous microbiota of humans

The indigenous microbiota of humans consists of a number of microbial communities, each with a composition characteristic of a particular body site. With few exceptions (the stomach and duodenum being two examples), the communities consist of large numbers of microbes and have a complex composition. As can be seen from Table 1.1, the microbial component of the average human being weighs approximately 1.25 kg. In terms of cell numbers, the figures are even more astonishing, with microbes outnumbering mammalian cells by a factor of 10 – the average human consists of $10^{13}$ mammalian cells and $10^{14}$ microbial cells. Some appreciation of the complexity of the indigenous microbiota can be gained by considering the number of different taxa (or phylotypes) that have been detected at various sites. Hence, the number of microbial taxa that are able to colonise the oral cavity has been estimated to be between

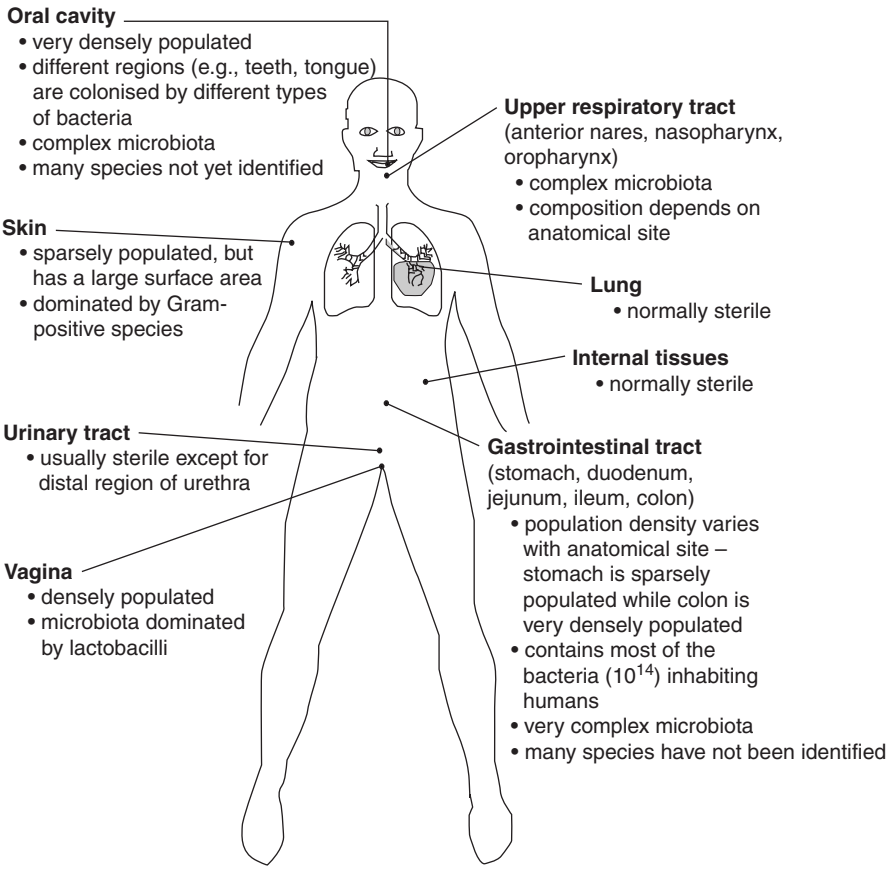



**Figure 1.1** The nature of the microbial communities found inhabiting various sites on the human body. Reproduced with permission from *Bacterial disease mechanisms; an introduction to cellular microbiology*. Wilson, M., McNab, R., and Henderson, B. 2002. Cambridge: Cambridge University Press.

500 and 700, whereas, for the colon, the number lies between 500 and 1,000 – these figures, however, are continually being revised upwards as detection methods improve. Fortunately, the numbers of different organisms detected in an individual at any one time are usually considerably lower – no more than approximately 100 in the more complex communities such as those found in the colon, dental plaque, and vagina.

Although many of those body surfaces that are exposed to the external environment are colonised by microbes, some are not (e.g., the lungs), and the population density of those sites that are colonised varies markedly from site to site (Figure 1.1). Hence, the oral cavity, the colon, and the vagina are densely colonised, whereas the eyes, stomach, and urethra have much sparser microbial communities. The density of colonisation and the community composition can vary enormously at different sites within an organ system. For example, the upper regions of the respiratory tract are more densely populated than the lower regions – in fact, the bronchi and alveoli are usually sterile. The skin is generally rather sparsely populated, but regions such as the axillae and the perineum support more substantial microbial communities. In the GI tract, the stomach, duodenum, and ileum have low population densities, whereas the jejunum, caecum, and colon are densely populated.

Table 1.2. Problems with defining the indigenous microbiota of a body site

| |
|---|
| technical problems due to complexity of the microbial community |
| generally only small numbers of samples can be processed – limits the statistical reliability of the data obtained |
| difficulty in obtaining appropriate, uncontaminated samples from many body sites |
| variations between individuals related to genotype, age, sex, diet, hygiene practices, health status, type of clothing, occupation, prevailing climate, etc. |
| difficulties in comparing results obtained using different methodologies |
| changes in microbial nomenclature – renders comparisons with previous studies difficult |

### 1.1.1 Difficulties associated with determining microbial community composition

Communities with a large diversity pose considerable technical problems when it comes to identifying all of the species present, and herein lies one of the problems associated with trying to define the indigenous microbiota of a body site. Until relatively recently, analysis of such communities relied on the cultivation of the species present. Such an approach is fraught with problems, and these are described in greater detail in Section 1.4.2. The application of modern molecular means of identifying microbes has added greatly to our knowledge (but not necessarily to our understanding) of the composition of the microbial communities inhabiting humans (Section 1.4.3). Unfortunately, however, few such studies have been carried out to date, and most of these have been restricted to samples taken from the oral cavity and the colon. It is important to emphasise at this point that, in addition to the technical difficulties associated with analysing such complex communities, there are a number of other problems inherent in attempting to determine the indigenous microbiota of a body site (Table 1.2). Firstly, regardless of whether culture-based or culture-independent methodologies are being used in a study, the work involved in processing a single sample is considerable, and this limits the number of samples that can be handled which, in turn, reduces the statistical reliability of the results obtained. Secondly, comparisons between studies are often difficult because of the different methodologies involved – not only between culture-based and culture-independent studies, but also among studies using similar approaches. Hence, culture-based studies often use different media with differing abilities to grow or select different species, whereas culture-independent studies often use primers or probes with different specificities. Changes in microbial nomenclature and taxonomy (particularly among the anaerobic Gram-positive cocci and rods and the anaerobic Gram-negative rods) have exacerbated the problem by making comparisons with previous studies difficult. While obtaining samples from some sites (e.g., the skin) is relatively easy, it can be extremely difficult to obtain samples from other sites. Hence, obtaining samples from the stomach and duodenum that are uncontaminated by microbes inhabiting adjacent sites is very difficult. This can be exacerbated by problems arising from the attitude of the individuals being sampled who are, naturally, reluctant to undergo any procedure that is uncomfortable, painful, or embarrassing. Studies have shown that the numbers and types of microbes present at a site may be affected by the age, gender, sexual maturity, diet, hygiene practices, type of clothing worn,

occupation, prevailing climate, and so forth. This means that a properly designed study should minimise such variations between the participants in the study – this is seldom done because of the difficulty in recruiting sufficient numbers. Even if all of the previously described problems can be overcome, the scientific community is then faced with the problem of deciding whether or not a particular organism detected should be regarded as being a member of the indigenous microbiota of the site under investigation. This can be a very difficult and – because there are no rigid rules – controversial issue. If an organism A is isolated in large proportions from a particular body site in every participant in a large group of age- and gender-matched individuals and similar results are obtained on a number of different sampling occasions, then it would be reasonable to regard it as being a member of the indigenous microbiota of that site. However, what should be the status of organisms B and C if they are isolated from 50% and 5% of these individuals, respectively? Or what if B and C are isolated from all individuals on one occasion but not on another occasion? Attempts have been made to distinguish between microbes that are "residents" of a site and those that are "transients". Residents of a site should be able to grow and reproduce under the conditions operating at the site, whereas organisms that cannot do so, but are found at the site, are regarded as transients. However, the complexity of the microbial communities at many sites, the paucity of longitudinal studies of most sites, and the difficulties associated with trying to establish whether an organism is actively growing or reproducing at a site often make such distinctions difficult to make.

Once an organism has been designated as being a member of the indigenous microbiota of a body site, it is important to try and understand why it is present at that site. It is reasonable to assume that the organism must be adhering to some substratum within the site – this may be a host cell, the extracellular matrix, some molecule secreted by the host, some structure produced by the host (e.g., a tooth or hair), or another microbe. The predilection of many organisms for a particular host site has been known for many years, and this phenomenon is termed "tissue tropism". The presence of a receptor on a host tissue able to recognise the complementary adhesin on the bacterium is considered to be the mechanism underlying tissue tropism. However, this alone cannot explain the presence of an organism at a specific body site because it does not take into account the fact that, as well as acting as a substratum for adhesion, the site must also be able to satisfy all of the nutritional and other needs of the organism. Furthermore, the organism must also be able to withstand any antimicrobial defences being mounted by the host at that site. An understanding of such host–microbe interactions can be gained only by considering the anatomy and physiology of the site which are largely responsible for creating the unique environment existing there. As Pasteur remarked more than 120 years ago, "The germ is nothing. It is the terrain in which it is found that is everything". The author has tried, therefore, to provide information on the environmental factors operating at each of the body sites colonised by microbes. Unfortunately, in many cases, such data do not appear to be available – this being due to the difficulties in accessing the site or in analysing the small quantities of fluid and/or tissue that can be obtained from the site. Although the environment provided by the host is the dominant factor dictating whether or not an organism can colonise a particular site, once colonisation has occurred, the environment is altered by microbial activity. This results in the phenomenon of microbial succession in which organisms previously unable to colonise the original site are now provided with an

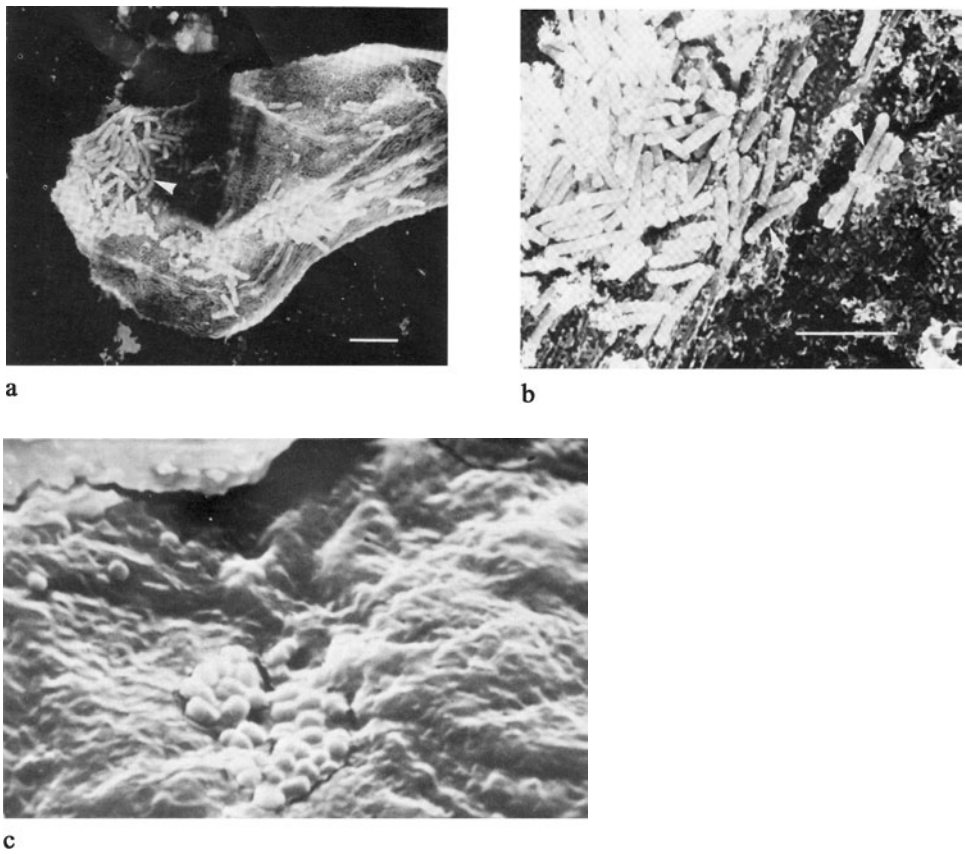
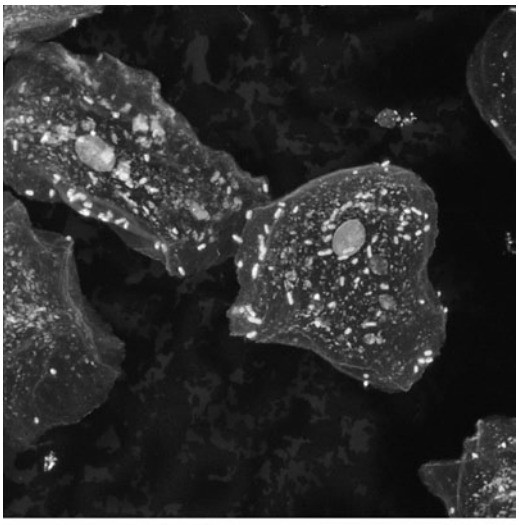



**Figure 1.2** (a) Scanning electron micrograph showing a microcolony (arrowhead) of a *Lactobacillus* sp. on a uroepithelial cell. (b) Higher magnification image. The extracellular matrix enclosing the microcolony has collapsed during the dehydration stages essential for sample preparation and can be seen as accretions on the surface of some bacteria (arrowheads). Bar = 5 $\mu$m. (c) Microcolony on the surface of human skin. (a,b) Reproduced with permission of Lippincott Williams & Wilkins from: Adherence of cervical, vaginal and distal urethral normal microbial flora to human uroepithelial cells and the inhibition of adherence of Gram-negative uropathogens by competitive exclusion. Chan, R.C.Y., Bruce, A.W., and Reid, G. *Journal of Urology* 1983;131: 596–601. (c) Reprinted from: *Microbiology of human skin*. Noble, W.C. Copyright © 1974, with permission from Elsevier.

environment suitable for their growth and reproduction. This process is fundamental to understanding the development of microbial communities at the various body sites and will be referred to repeatedly throughout this book.

## 1.1.2 Structural aspects of residential microbial communities

As well as determining the numbers and types of microbes found at a particular anatomical site, it is also important to consider the structural organisation of the communities inhabiting these sites. Adhesion of an organism to a substratum is followed by its growth and reproduction – if the habitat has a suitable environment and can satisfy the nutritional requirements of the organism. This may result in the production of adherent microbial aggregates known as microcolonies, which are often enclosed within some microbial extracellular polymer (see Figures 1.2, 2.12, and 6.13). Microcolonies



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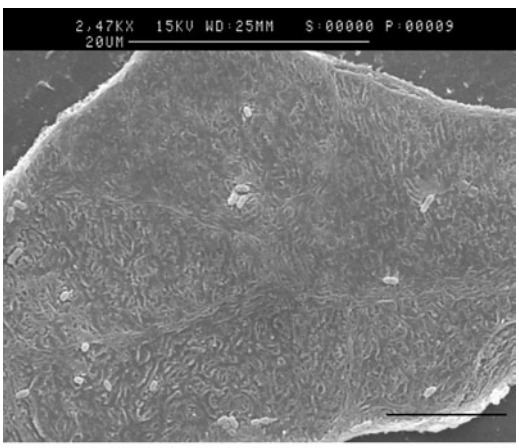



b

**Figure 1.3** Epithelial cells from the cheek mucosa viewed by (a) confocal laser scanning microscopy and (b) scanning electron microscopy (bar = 10 $\mu$m). Pairs of bacteria and individual cells can be seen attached to the epithelial cells. Images kindly supplied by: (a) Dr. Chris Hope and (b) Mrs. Nicola Mordan, Eastman Dental Institute, University College London.

have been detected on the surface of the skin and on mucosal surfaces such as the respiratory, urogenital, and intestinal tracts. However, this does not happen in all cases, as the development of such aggregates is often limited by mechanical and hydrodynamic forces tending to disrupt or dislodge such structures (see Section 1.2.3). Furthermore, if the organism is motile, reproduction often leads to one or more of the daughter cells detaching and moving to another site within the habitat. Many epithelial cells, therefore, may only have small numbers of individual microbial cells on their surfaces (Figure 1.3). Another factor limiting the growth of microbial aggregates is that most of the surfaces exposed to the external environment (apart from the teeth) consist of epithelial cells which are continually being shed, taking the aggregates with them.

Sometimes however, the microcolony produced can grow further and develop into a larger structure known as a "biofilm" (Figure 1.4) – this occurs particularly on the non-shedding surfaces of the teeth and on mucosal surfaces with suitable anatomical features (e.g., the crypts of the tongue and tonsils and in the vagina). They are

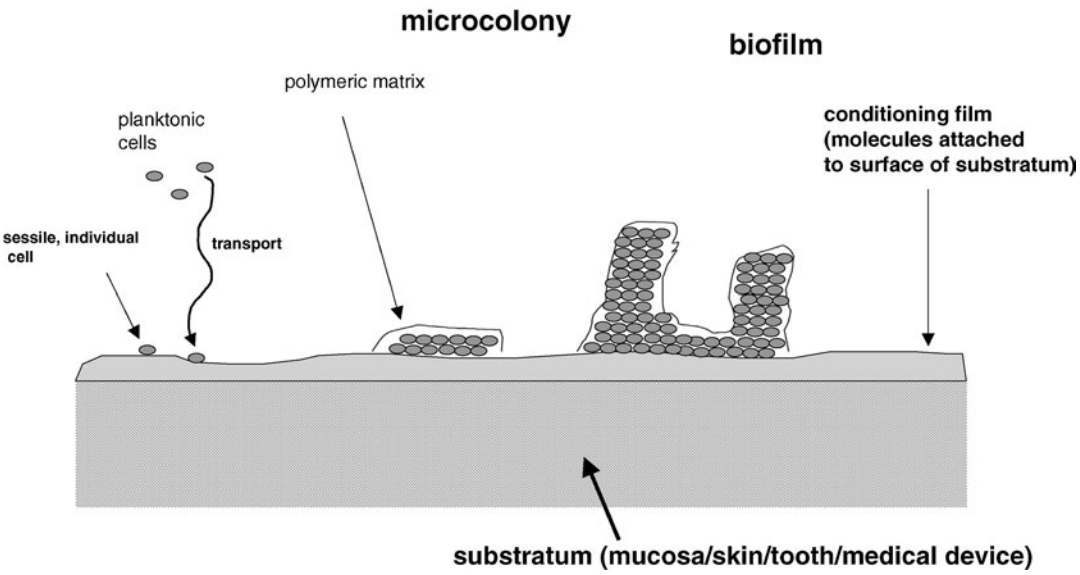



**Figure 1.4** The various patterns of microbial colonisation that may be found on host tissues. Adhesion of single cells to the host tissue may lead to the production of microcolonies or biofilms.

also found on particulate matter in the colon and on medical devices and prostheses (e.g., catheters, artificial joints, limbs, and heart valves). A biofilm is defined as a matrix-enclosed microbial community attached to a surface. Because most surfaces in nature are coated with an adsorbed layer of macromolecules, the biofilm is usually attached to this layer (termed a "conditioning film") rather than directly to the surface itself. The matrix consists of polymers produced by the constituent microbes, as well as molecules derived from the host. An organism growing within a biofilm has a phenotype different from that which it displays when it grows planktonically (i.e., in an aqueous suspension) and the collective properties of a biofilm differ considerably from those of a simple aqueous suspension of the same organism(s) (Table 1.3). Furthermore, the utilisation of oxygen and nutrients from the environment by cells in the outermost layers of the biofilm, together with impeded diffusion of such molecules by the biofilm matrix, results in chemical and physicochemical gradients within the biofilm (Figure 1.5). Other gradients will be generated with respect to metabolites produced by the organism present inside the biofilm. Within the biofilm, therefore, an enormous variety of microhabitats exist, thereby providing conditions suitable for colonisation by a variety of physiological types of microbes.

Table 1.3. General properties of biofilms

| |
|---|
| reduced susceptibility to antimicrobial agents |
| reduced susceptibility to host defence mechanisms |
| contain a range of microhabitats due to chemical and physico-chemical gradients |
| constituent organisms display novel phenotypes |
| facilitates nutritional interactions between constituent organisms |
| facilitates quorum sensing |

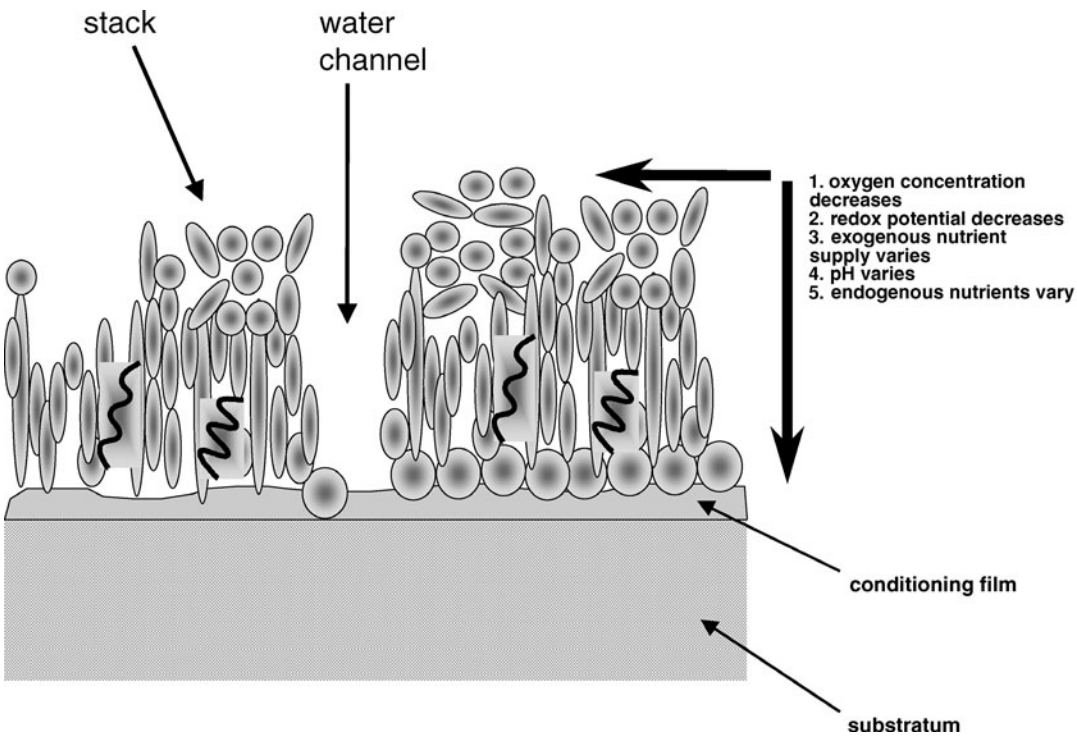
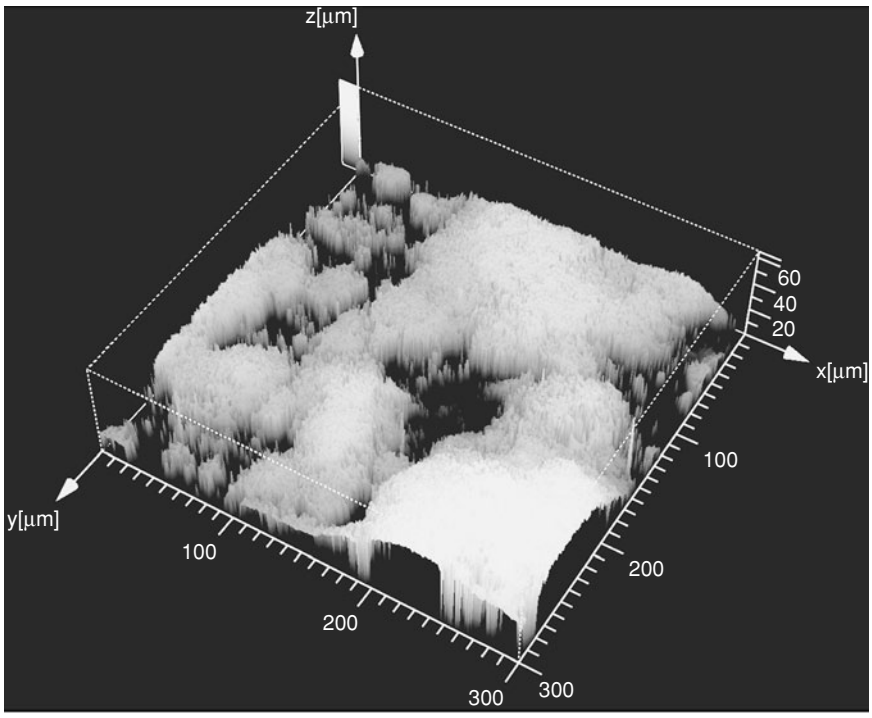



**Figure 1.5** A wide range of microhabitats exist within a biofilm due to gradients in physicochemical factors (e.g., pH, redox potential), the partial pressure of gases such as oxygen and $CO_2$, the concentration of exogenous nutrients, and the concentration of metabolic end-products of the organism(s) within the biofilm.

Biofilms are highly hydrated structures, and the bacteria within them may occupy only between 10% and 50% of the total volume. This means that the staining and dehydration techniques used to prepare biofilms for examination by light and/or electron microscopy grossly distort their structure. Fortunately, the advent of confocal laser scanning microscopy (CLSM) – which enables the examination of biofilms in their native, hydrated state – has enabled a more accurate estimation of their structure and dimensions. Until CLSM began to be used for studying biofilm structure, there was little evidence that biofilms displayed any organised structure – bacteria were thought to be more or less randomly distributed throughout the matrix. However, CLSM (and other modern microscopic techniques such as differential interference contrast microscopy) has enabled us to view biofilms in their living, hydrated state, and this has revealed structures that are both complex and beautiful (Figure 1.6). Because a number of factors can affect biofilm structure, there is no single, unifying structure that can be said to characterise all biofilms. The key variables involved include the nature of the organism (or community), the concentration of nutrients present, the hydrodynamic properties of the environment, and the presence (and nature) of any mechanical forces operating at the site. Hence, the structure of a biofilm can range from the relatively featureless, flat type to one consisting of a more complex organisation involving tower-like "stacks" (consisting of microbes enclosed in an extracellular matrix) separated by water channels (Figure 1.6). The latter are characteristic of biofilms formed under the




a

b

**Figure 1.6** Confocal laser scanning micrograph (low magnification) of multi-species oral biofilms grown in the laboratory under conditions similar to those which exist *in vivo*. A fluorescent dye was used to stain the constituent organisms but not the extracellular biofilm matrix. (a) Three-dimensional image showing stacks of bacteria (up to 60 $\mu$m high) separated by water channels. (b) A section (160 $\mu$m $\times$ 160 $\mu$m) of a different part of the biofilm viewed from above. Grey-scale coding denotes the height of the constituent bacteria above the surface – the darker the colour, the nearer the bacteria are to the substratum. Images kindly supplied by: Dr. Chris Hope, Eastman Dental Institute, University College London.