CHAPTER 2

# Micro-organisms and Food Materials

Foods, by their very nature, need to be nutritious and metabolizable and it should be expected that they will offer suitable substrates for the growth and metabolism of micro-organisms. Before dealing with the details of the factors influencing this microbial activity, and their significance in the safe handling of foods, it is useful to examine the possible sources of micro-organisms in order to understand the ecology of contamination.

## 2.1 DIVERSITY OF HABITAT

Viable micro-organisms may be found in a very wide range of habitats, from the coldest of brine ponds in the frozen wastes of polar regions, to the almost boiling water of hot springs. Indeed, it is now realized that actively growing bacteria may occur at temperatures in excess of 100 °C in the thermal volcanic vents, at the bottom of the deeper parts of the oceans, where boiling is prevented by the very high hydrostatic pressure (see Section 3.2.5). Micro-organisms may occur in the acidic wastes draining away from mine workings or the alkaline waters of soda lakes. They can be isolated from the black anaerobic silts of estuarine muds or the purest waters of biologically unproductive, or oligotrophic, lakes. In all these, and many other, habitats microbes play an important part in the recycling of organic and inorganic materials through their roles in the carbon, nitrogen and sulfur cycles (Figure 2.1). They thus play an important part in the maintenance of the stability of the biosphere.

The surfaces of plant structures such as leaves, flowers, fruits and especially the roots, as well as the surfaces and the guts of animals all have a rich microflora of bacteria, yeasts and filamentous fungi. This natural, or normal flora may affect the original quality of the raw ingredients used in the manufacture of foods, the kinds of contamination which may occur during processing, and the possibility of food spoilage or food associated illness. Thus, in considering the possible sources of

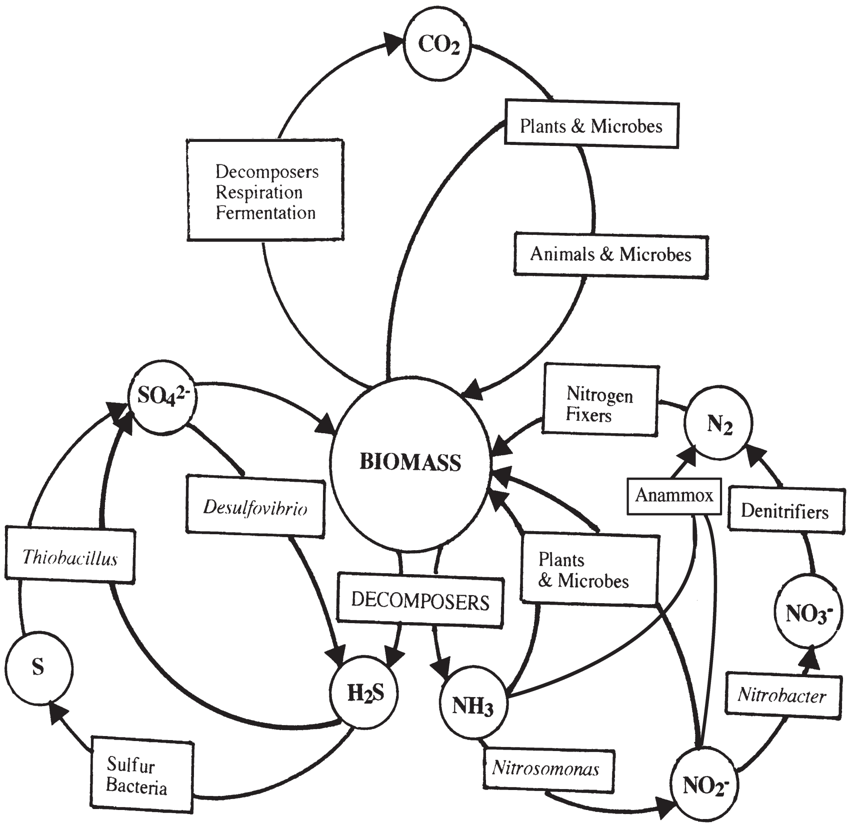


**Figure 2.1** *Micro-organisms and the carbon, nitrogen and sulfur cycles*

micro-organisms as agents of food spoilage or food poisoning, it will be necessary to examine the natural flora of the food materials themselves, the flora introduced by processing and handling, and the possibility of chance contamination from the atmosphere, soil or water.

## 2.2 MICRO-ORGANISMS IN THE ATMOSPHERE

Perhaps one of the most hostile environments for many micro-organisms is the atmosphere. Suspended in the air, the tiny microbial propagule may be subjected to desiccation, to the damaging effects of radiant energy from the sun, and the chemical activity of elemental gaseous oxygen ($O_2$) to which it will be intimately exposed. Many micro-organisms, especially Gram-negative bacteria, do indeed die very rapidly when suspended in air and yet, although none is able to grow and multiply in the atmosphere, a significant number of microbes are able to survive and use the turbulence of the air as a means of dispersal.

### 2.2.1 Airborne Bacteria

The quantitative determination of the numbers of viable microbial propagules in the atmosphere is not a simple job, requiring specialized sampling equipment, but a qualitative estimate can be obtained by simply exposing a Petri dish of an appropriate medium solidified with agar to the air for a measured period of time. Such air exposure plates frequently show a diverse range of colonies including a significant number which are pigmented (Figure 2.2).

The bacterial flora can be shown to be dominated by Gram-positive rods and cocci unless there has been a very recent contamination of the air by an aerosol generated from an animal or human source, or from water. The pigmented colonies will often be of micrococci or corynebacteria and the large white-to-cream coloured colonies will frequently be of aerobic sporeforming rods of the genus *Bacillus*. There may also be small raised, tough colonies of the filamentous bacteria belonging to *Streptomyces* or a related genus of actinomycetes. The possession of pigments may protect micro-organisms from damage by both visible and ultraviolet radiation of sunlight and the relatively simple, thick cell walls of Gram-positive bacteria may afford protection from desiccation. The endospores of *Bacillus* and the conidiospores of *Streptomyces* are especially resistant to the potentially damaging effects of suspension in the air.

The effects of radiation and desiccation are enhanced by another phenomenon, the 'open air factor' which causes even more rapid death

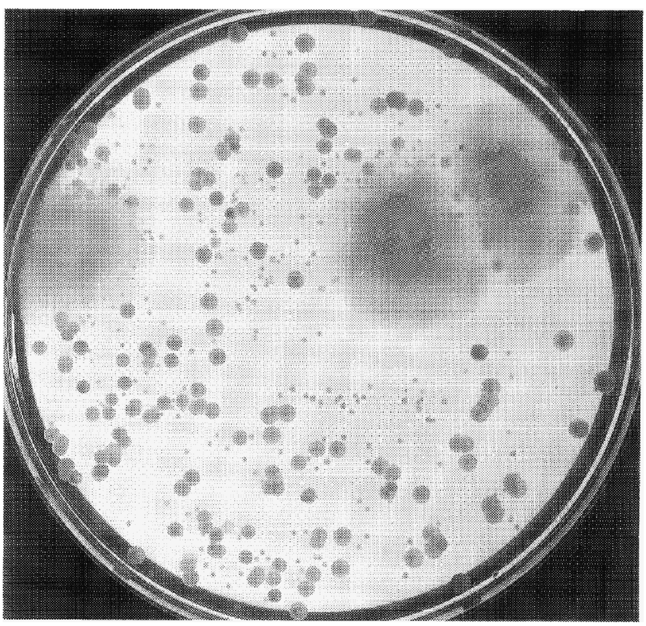

**Figure 2.2** *Exposure plate showing air flora*

rates of sensitive Gram-negative organisms such as *Escherichia coli*. It can be shown that these organisms may die more rapidly in outdoor air at night time than they do during the day, in spite of reduced light damage to the cells. It is possible that light may destroy this 'open-air factor', or that other more complex interactions may occur. Phenomena such as this, alert us to the possibility that it can be very difficult to predict how long micro-organisms survive in the air and routine monitoring of air quality may be desirable within a food factory, or storage area, where measures to reduce airborne microbial contamination can have a marked effect on food quality and shelf-life. This would be particularly true for those food products such as bakery goods that are subject to spoilage by organisms that survive well in the air.

Bacteria have no active mechanisms for becoming airborne. They are dispersed on dust particles disturbed by physical agencies, in minute droplets of water generated by any process which leads to the formation of an aerosol, and on minute rafts of skin continuously shed by many animals including humans. The most obvious mechanisms for generating aerosols are coughing and sneezing but many other processes generate minute droplets of water. The bursting of bubbles, the impaction of a stream of liquid onto a surface, or taking a wet stopper out of a bottle are among the many activities that can generate aerosols, the droplets of which may carry viable micro-organisms for a while.

One group of bacteria has become particularly well adapted for air dispersal. Many actinomycetes, especially those in the genus *Streptomyces*, produce minute dry spores which survive well in the atmosphere. Although they do not have any mechanisms for active air dispersal, the spores are produced in chains on the end of a specialized aerial structure so that any physical disturbance dislodges them into the turbulent layers of the atmosphere. The air of farmyard barns may contain many millions of spores of actinomycetes per cubic metre and some species, such as *Thermoactinomyces vulgaris* and *Micropolyspora faeni*, can cause the disabling disease known as farmer's lung where individuals have become allergic to the spores. Actinomycetes are rarely implicated in food spoilage but geosmin-producing strains of *Streptomyces* may be responsible for earthy odours and off-flavours in potable water, and geosmin (Figure 2.3) may impart earthy taints to such foods as shellfish.

### 2.2.2 Airborne Fungi

It is possible to regard the evolution of many of the terrestrial filamentous fungi (the moulds) as the development of increasingly sophisticated mechanisms for the air dispersal of their reproductive propagules. Some of the most important moulds in food microbiology do not have active spore dispersal mechanisms but produce large numbers of small

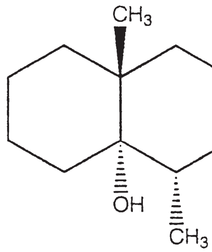


**Figure 2.3** *Geosmin*

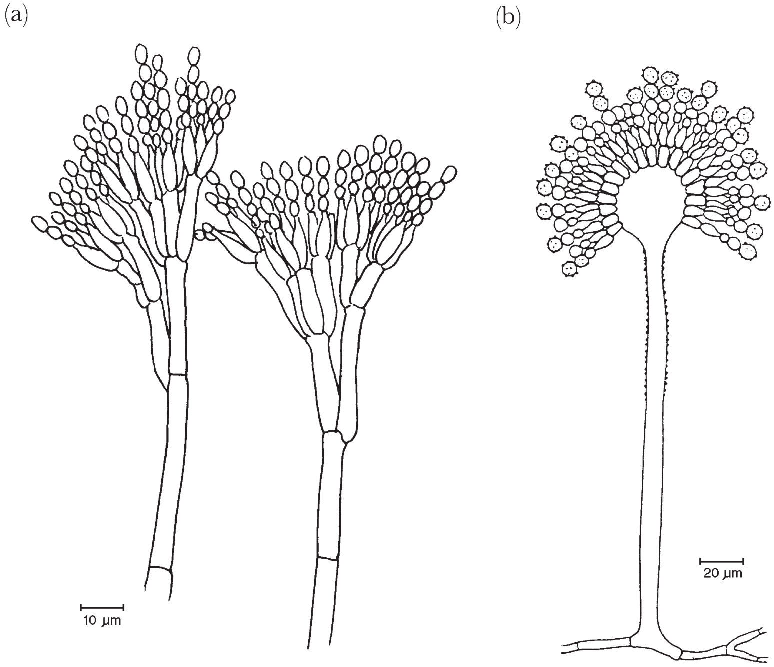


**Figure 2.4** *(a) Pencillium expansum and (b) Aspergillus flavus*

unwettable spores which are resistant to desiccation and light damage. They become airborne in the same way as fine dry dust particles by physical disturbance and wind. Spores of *Penicillium* and *Aspergillus* (Figure 2.4) seem to get everywhere in this passive manner and species of these two genera are responsible for a great deal of food spoilage. The individual spores of *Penicillium* are only 2–3 $\mu$m in diameter, spherical to sub-globose (*i.e*. oval), and so are small and light enough to be efficiently dispersed in turbulent air.

Some fungi, such as *Fusarium* (Figure 2.5), produce easily wettable spores which are dispersed into the atmosphere in the tiny droplets of water which splash away from the point of impact of a rain drop and so may become very widely distributed in field crops during wet weather.

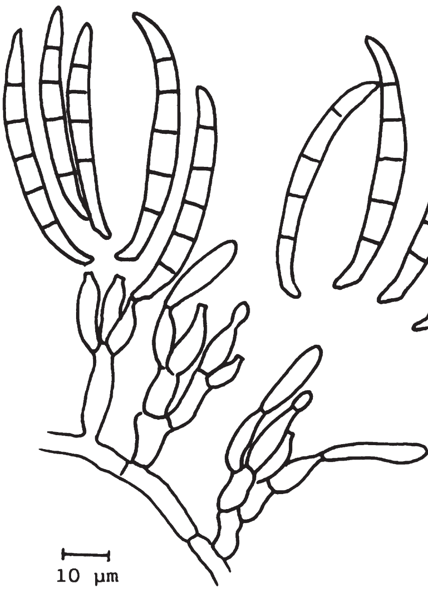


**Figure 2.5** *Fusarium graminearum*

Such spores rarely become an established part of the long-term air spora and this mechanism has evolved as an effective means for the short-term dispersal of plant pathogens.

As the relative humidity of the atmosphere decreases with the change from night to day, the sporophores of fungi such as *Cladosporium* (Figure 2.6) react by twisting and collapsing, throwing their easily detached spores into the atmosphere. At some times of the year, especially during the middle of the day, the spores of *Cladosporium* may be the most common spores in the air spora. Species such as *Cladosporium herbarum* grow well at refrigeration temperatures and may form unsightly black colonies on the surface of commodities such as chilled meat.

Many fungi have evolved mechanisms for actively firing their spores into the atmosphere (Figure 2.7), a process which usually requires a high relative humidity. Thus the ballistospores of the mirror yeasts, which are frequently a part of the normal microbial flora of the leaf surfaces of plants, are usually present in highest numbers in the atmosphere in the middle of the night when the relative humidity is at its highest.

The evolutionary pressure to produce macroscopic fruiting bodies, which is seen in the mushrooms and toadstools, has produced a structure which provides its own microclimate of high relative humidity so that these fungi can go on firing their spores into the air even in the middle of a dry day.

In our everyday lives we are perhaps less aware of the presence of micro-organisms in the atmosphere than anywhere else, unless we

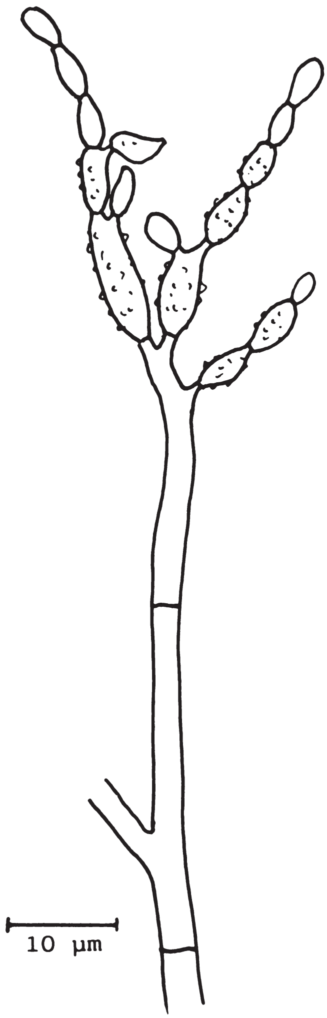


**Figure 2.6** *Cladosporium cladosporioides*

happen to suffer from an allergy to the spores of moulds or actinomycetes, but, although they cannot grow in it, the atmosphere forms an important vehicle for the spread of many micro-organisms, and the subsequent contamination of foods.

## 2.3 MICRO-ORGANISMS OF SOIL

The soil environment is extremely complex and different soils have their own diverse flora of bacteria, fungi, protozoa and algae. The soil is such a rich reservoir of micro-organisms (Figure 2.8) that it has provided many of the strains used for the industrial production of antibiotics, enzymes, amino acids, vitamins and other products used in both the

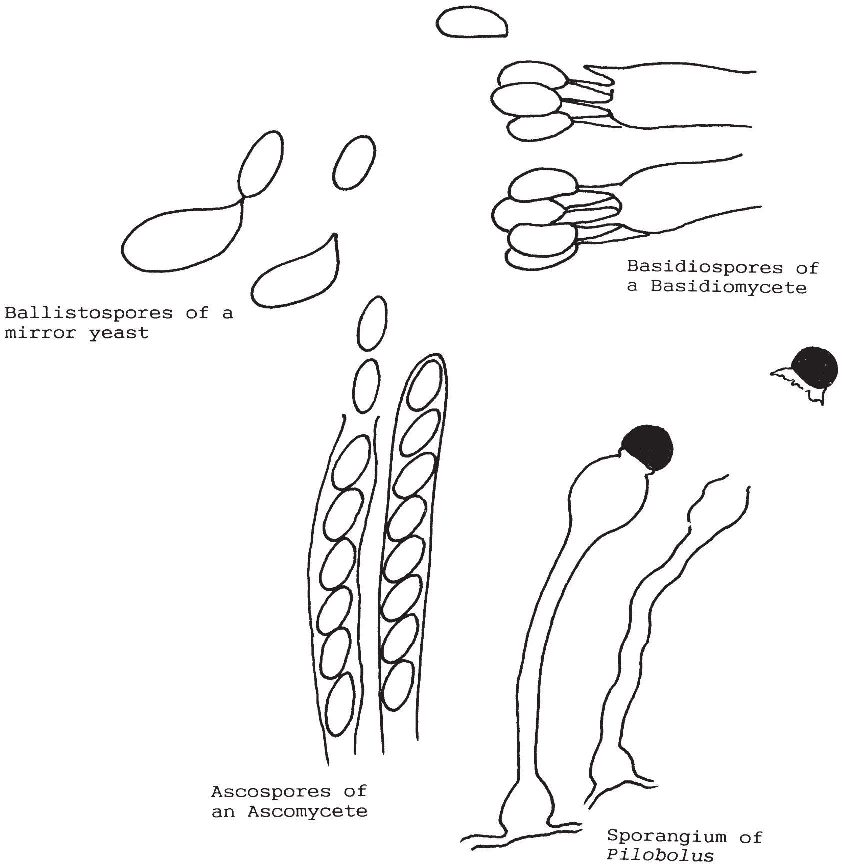


**Figure 2.7** *Mechanisms for active dispersal of fungal propagules*

pharmaceutical and food industries. Soil micro-organisms participate in the recycling of organic and nitrogenous compounds which is essential if the soil is to support the active growth of plants, but this ability to degrade complex organic materials makes these same micro-organisms potent spoilage organisms if they are present on foods. Thus the commonly accepted practice of protecting food from 'dirt' is justified in reducing the likelihood of inoculating the food with potential spoilage organisms.

The soil is also a very competitive environment and one in which the physico-chemical parameters can change very rapidly. In response to this, many soil bacteria and fungi produce resistant structures, such as the endospores of *Bacillus* and *Clostridium*, and chlamydospores and sclerotia of many fungi, which can withstand desiccation and a wide range of temperature fluctuations. Bacterial endospores are especially resistant to elevated temperatures, indeed their subsequent germination is frequently triggered by exposure to such temperatures, and their

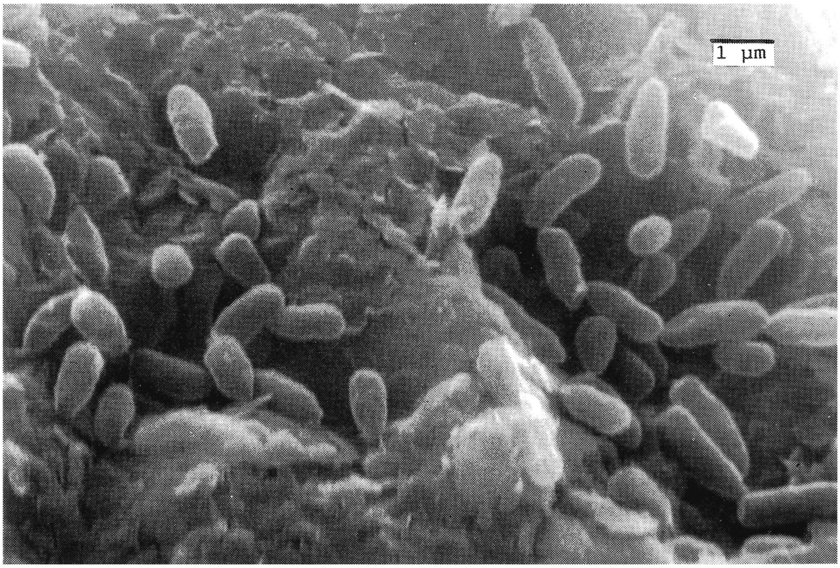


**Figure 2.8** *Electron micrograph of micro-organisms associated with soil particles*

common occurrence in soil makes this a potent source of spoilage and food poisoning bacilli and clostridia.

## 2.4 MICRO-ORGANISMS OF WATER

The aquatic environment represents in area and volume the largest part of the biosphere and both fresh water and the sea contain many species of micro-organisms adapted to these particular habitats. The bacteria isolated from the waters of the open oceans often have a physiological requirement for salt, grow best at the relatively low temperatures of the oceans and are nutritionally adapted to the relatively low concentrations of available organic and nitrogenous compounds. Thus, from the point of view of a laboratory routinely handling bacteria from environments directly associated with man, marine bacteria are usually described as oligotrophic psychrophiles with a requirement for sodium chloride for optimum growth.

The surfaces of fish caught from cold water in the open sea will have a bacterial flora which reflects their environment and will thus contain predominantly psychrophilic and psychrotrophic species. Many of these organisms can break down macromolecules, such as proteins, polysaccharides and lipids, and they may have doubling times as short as ten hours at refrigeration temperatures of 0–7 °C. Thus, in ten days, *i.e.* 240 hours, one organism could have become $2^{24}$ or between $10^7$ and $10^8$ under such conditions. Once a flora has reached these numbers it could be responsible for the production of off-odours and hence spoilage. Of course, during the handling of a commodity such as fish, the natural flora of the environment will be contaminated with organisms associated with man, such as members of the Enterobacteriaceae and *Staphylococcus*, which can grow well at 30–37 °C. It is readily possible to distinguish the environmental flora from the 'handling' flora by comparing the numbers of colonies obtained by plating-out samples on nutrient agar and incubating at 37 °C with those from plates of sea water agar, containing a lower concentration of organic nutrients, and incubated at 20 °C.

The seas around the coasts are influenced by inputs of terrestrial and freshwater micro-organisms and, perhaps more importantly, by human activities. The sea has become a convenient dump for sewage and other waste products and, although it is true that the seas have an enormous capacity to disperse such materials and render them harmless, the scale of human activity has had a detrimental effect on coastal waters. Many shellfish used for food grow in these polluted coastal waters and the majority feed by filtering out particles from large volumes of sea water. If these waters have been contaminated with sewage there is always the risk that enteric organisms from infected individuals may be present and will be concentrated by the filter feeding activities of shellfish. Severe diseases such as hepatitis or typhoid fever, and milder illnesses such as gastroenteritis have been caused by eating contaminated oysters and mussels which seem to be perfectly normal in taste and appearance. In warmer seas even unpolluted water may contain significant numbers of *Vibrio parahaemolyticus* and these may also be concentrated by filter-feeding shellfish, indeed they may form a stable part of the natural enteric flora of some shellfish. This organism may be responsible for outbreaks of food poisoning especially associated with sea foods.

The fresh waters of rivers and lakes also have a complex flora of micro-organisms which will include genuinely aquatic species as well as components introduced from terrestrial, animal and plant sources. As with the coastal waters of the seas, fresh water may also act as a vehicle for bacteria, protozoa and viruses causing disease through contamination with sewage effluent containing human faecal material. These organisms do not usually multiply in river and lake water and may be present in very low, but nonetheless significant, numbers making it difficult to demonstrate their presence by direct methods. It is usual to infer the possibility of the presence of such organisms by actually looking for a species of bacterium which is always present in large numbers in human faeces, is unlikely to grow in fresh water, but will survive at least as long as any pathogen. Such an organism is known as an 'indicator organism' and the species usually chosen in temperate climates is *Escherichia coli*.

Fungi are also present in both marine and fresh waters but they do not have the same level of significance in food microbiology as other micro-organisms. There are groups of truly aquatic fungi including some which are serious pathogens of molluscs and fish. There are fungi which have certainly evolved from terrestrial forms but have become morphologically and physiologically well adapted to fresh water or marine habitats. They include members of all the major groups of terrestrial fungi, the ascomycetes, basidiomycetes, zygomycetes and deuteromycetes and there is the possibility that some species from this diverse flora could be responsible for spoilage of a specialized food commodity associated with water such as a salad crop cultivated with overhead irrigation from a river or lake, but this is speculation.

Of the aquatic photosynthetic micro-organisms, the cyanobacteria, or blue-green algae, amongst the prokaryotes and the dinoflagellates amongst the eukaryotes, have certainly had an impact on food quality and safety. Both these groups of micro-organisms can produce very toxic metabolites which may become concentrated in shellfish without apparently causing them any harm. When consumed by humans, however, they can cause a very nasty illness such as paralytic shellfish poisoning (see Chapter 8).

## 2.5 MICRO-ORGANISMS OF PLANTS

All plant surfaces have a natural flora of micro-organisms which may be sufficiently specialized to be referred to as the phylloplane flora, for that of the leaf surface, and the rhizoplane flora for the surface of the roots. The numbers of organisms on the surfaces of healthy, young plant leaves may be quite low but the species which do occur are well adapted for this highly specialized environment. Moulds such as *Cladosporium* and the so-called black yeast, *Aureobasidium pullulans*, are frequently present. Indeed, if the plant is secreting a sugary exudate, these moulds may be present in such large numbers that they cover the leaf surface with a black sooty deposit. In the late summer, the leaves of such trees as oak and lime may look as though they are suffering from some form of industrial pollution, so thick is the covering of black moulds. *Aureobasidium* behaves like a yeast in laboratory culture but develops into a filamentous mould-like organism as the culture matures.

There are frequently true yeasts of the genera *Sporobolomyces* and *Bullera* on plant leaf surfaces. These two genera are referred to as mirror yeasts because, if a leaf is attached to the inner surface of the lid of a Petri dish containing malt extract agar, the yeasts produce spores which they actively fire away from the leaf surface. These ballistospores hit the agar surface and germinate to eventually produce visible colonies in a pattern which forms a mirror image of the leaf. An even richer yeast flora is found in association with the nectaries of flowers and the surfaces of fruits and the presence of some of these is important in the spontaneous fermentation of fruit juices, such as that of the grape in the production of wine. The bacterial flora of aerial plant surfaces which is most readily detected is made up predominantly of Gram-negative rods, such as *Pectobacterium, Erwinia, Pseudomonas* and *Xanthomonas* but there is usually also present a numerically smaller flora of fermentative Gram-positive bacteria such as *Lactobacillus* and *Leuconostoc* which may become important in the production of such fermented vegetable products as sauerkraut (see Chapter 9).

The specialized moulds, yeasts and bacteria living as harmless commensals on healthy, young plant surfaces are not usually any problem in the spoilage of plant products after harvest. But, as the plant matures, both the bacterial and fungal floras change. The numbers of pectinolytic bacteria increase as the vegetable tissues mature and a large number of mould species are able to colonize senescent plant material. In the natural cycling of organic matter these organisms would help to break down the complex plant materials and so bring about the return of carbon, nitrogen and other elements as nutrients for the next round of plant growth. But, when humans break into this cycle and harvest plant products such as fruits, vegetables, cereals, pulses, oilseeds and root crops, these same organisms may cause spoilage problems during prolonged periods of storage and transport.

Plants have evolved several mechanisms for resisting infection by micro-organisms but there are many species of fungi and bacteria which overcome this resistance and cause disease in plants and some of these may also cause spoilage problems after harvesting and storage. Amongst the bacteria, *Pectobacterium caratovorum* subsp. *caratovorum* (previously known as *Erwinia carotovora* var. *atroseptica*) is a pathogen of the potato plant causing blackleg disease of the developing plant. The organism can survive in the soil when the haulms of diseased plants fall to the ground and, under the right conditions of soil moisture and temperature, it may then infect healthy potato tubers causing a severe soft rot during storage. One of the conditions required for such infection is a film of moisture on the tuber for this species can only infect the mature tuber through a wound or via a lenticel in the skin of the potato. This process may be unwittingly aided by washing potatoes and marketing them in plastic bags so that, the combination of minor damage and moisture trapped in the bag, favours the development of *Pectobacterium* soft rot.

Amongst the fungi, *Botrytis cinerea* (Figure 2.9) is a relatively weak pathogen of plants such as the strawberry plant where it may infect the flower. However, this low pathogenicity is often followed by a change to an aggressive invasion of the harvested fruit, usually through the calyx into the fruit tissue. Once this 'grey mould' has developed on one fruit, which may have been damaged and infected during growth before harvest, the large mass of spores and actively growing mould readily infects neighbouring fruit even though they may be completely sound.

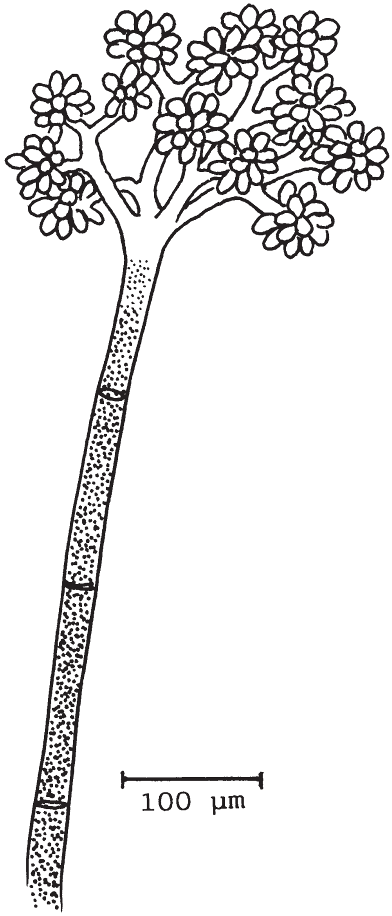


**Figure 2.9** *Botrytis cinerea*

The cereals are a group of plant commodities in which there is a pronounced and significant change in the microbial flora following harvesting. In the field the senescent plant structures carrying the cereal grain may become infected by a group of fungi, referred to as the field fungi, which includes such genera as *Cladosporium, Alternaria, Helminthosporium* and *Chaetomium*. After harvest and reduction of the moisture content of the grain, the components of the field flora decrease in numbers and are replaced by a storage flora which characteristically includes species of the genera *Penicillium* and *Aspergillus*. Some genera of fungi, such as *Fusarium*, contain a spectrum of species, some of which are specialized plant pathogens, others saprophytic field fungi and others capable of growth during the initial stages of storage. Indeed, the more that is learnt about the detailed ecology of individual species, the more it is realized that it may be misleading to try to pigeon hole them into simple categories such as field fungi and storage fungi. Thus it is now known that *Aspergillus flavus*, a very important species because of its ability to produce the carcinogenic metabolite known as aflatoxin, is not just a storage mould as was once believed, but may infect the growing plant in the field and produce its toxic metabolites before harvesting and storage (see Chapter 8). Indeed, it is now recognized that many plants carry fungal endophytes in their naturally healthy state.

## 2.6 MICRO-ORGANISMS OF ANIMAL ORIGIN

All healthy animals carry a complex microbial flora, part of which may be very specialized and adapted to growth and survival on its host, and part of which may be transient, reflecting the immediate interactions of the animal with its environment. From a topological point of view, the gut is also part of the external surface of an animal but it offers a very specialized environment and the importance of the human gut flora will be dealt with in Chapter 6.

### 2.6.1 The Skin

The surfaces of humans and other animals are exposed to air, soil and water and there will always be the possibility of contamination of foods and food handling equipment and surfaces with these environmental microbes by direct contact with the animal surface. However, the surface of the skin is not a favourable place for most micro-organisms since it is usually dry and has a low pH due to the presence of organic acids secreted from some of the pores of the skin. This unfavourable environment ensures that most micro-organisms reaching the skin do not multiply and frequently die quite quickly. Such organisms are only 'transients' and would not be regularly isolated from the cleaned skin surface.

Nevertheless, the micro-environments of the hair follicles, sebaceous glands and the skin surface have selected a specialized flora exquisitely adapted to each environment. The bacteria and yeasts making up this 'normal' flora are rarely found in other habitats and are acquired by the host when very young, usually from the mother. The micro-organisms are characteristic for each species of animal and, in humans, the normal skin flora is dominated by Gram-positive bacteria from the genera *Staphylococcus, Corynebacterium* and *Propionibacterium*. For animals which are killed for meat, the hide may be one of the most important sources of spoilage organisms while, in poultry, the micro-organisms associated with feathers and the exposed follicles, once feathers are removed, may affect the microbial quality and potential shelf-life of the carcass.

### 2.6.2 The Nose and Throat

The nose and throat with the mucous membranes which line them represent even more specialized environments and are colonized by a different group of micro-organisms. They are usually harmless but may have the potential to cause disease, especially following extremes of temperature, starvation, overcrowding or other stresses which lower the resistance of the host and make the spread of disease more likely in both humans and other animals. *Staphylococcus aureus* is carried on the mucous membranes of the nose by a significant percentage of the human population and some strains of this species can produce a powerful toxin capable of eliciting a vomiting response. The food poisoning caused by this organism will be dealt with in Chapter 7.

## 2.7 CONCLUSIONS

In this chapter we have described some of the major sources of micro-organisms which may contaminate food and cause problems of spoilage or create health risks when the food is consumed. It can be seen that most foods cannot be sterile but have a natural flora and acquire a transient flora derived from their environment. To ensure that food is safe and can be stored in a satisfactory state, it is necessary to either destroy the micro-organisms present, or manipulate the food so that growth is prevented or hindered. The manner in which environmental and nutritional factors influence the growth and survival of micro-organisms will be considered in the next chapter. The way in which this knowledge can be used to control microbial activity in foods will be considered in Chapter 4.