

1

Bacteria as plant pathogens

Bacteria–plant associations

The origin and evolutionary development of higher plants has occurred in environments that were already colonised by bacteria, resulting in the co-evolution of a range of bacteria–plant associations. The associated microbes may be broadly considered in two main categories: epiphytic bacteria (present on the outside of the plant) and internal bacteria (infecting the plant tissue).

Epiphytic bacteria

These are associated with the plant surface, which is generally divided into root (rhizosphere) and aerial (phyllosphere) regions. A wide range of bacteria are adapted to various microenvironments at the soil and air interface, and are important in such aspects as nutrient uptake, frost damage, and biological control of plant pathogens. Many of these epiphytic bacteria are saprophytes, obtaining complex nutrients from the plant. Some epiphytic bacteria are also parasites, spending part of their life cycle on the plant surface, and part within the plant tissue.

Infective bacteria: parasites and symbionts

Parasitic bacteria are able to invade plant tissue, where they grow and multiply, and cause localised or general deterioration in the health of the plant. The great majority of these parasites are extracellular, multiplying within intercellular spaces but not penetrating plant cell walls or entering protoplasts. The relatively few parasitic bacteria that are able to penetrate the higher plant cell include members of the genus *Agrobacterium* (with the ability to transfer part of the genome into the plant cell) and *Rhizobium* (where the

whole organism enters the plant cell). These associations ultimately lead to symbiotic interaction, where both partners benefit from the combination, and where the distinction between parasitism and symbiosis is not always clear. Thus, although agrobacteria typically have an adverse effect on their infected plants, some strains of *Agrobacterium rhizogenes* have no overt effect on their host. Similarly, some strains of the normally symbiotic *Rhizobium japonicum* produce toxins that adversely affect plant growth.

This book is concerned primarily with bacteria that are parasites and act as plant pathogens. Epiphytic bacteria are also considered specifically where they are potential plant pathogens (facultative parasites) or where they are important in the biological control of plant pathogens.

Evolution of bacterial plant pathogens and the origins of disease

One of the surprising aspects of bacterial plant pathology is that very few species of bacteria have evolved the ability to invade and grow in healthy plant tissues. These bacteria are present in only five major taxonomic groups, comprising the genera *Agrobacterium*, *Erwinia*, *Pseudomonas*, *Xanthomonas* and the coryneform bacteria (*Arthrobacter*, *Clavibacter*, *Curtobacterium* and *Rhodococcus*). Gram-positive bacteria in particular, which constitute a substantial part of soil microflora, have no pathogens of significance – with the exception of the coryneform bacteria. The ability of bacteria to cause disease is thus a relatively unusual event, and the origin of this type of bacteria–plant interaction depends on quite separate evolutionary pressures on bacteria and plants.

Evolution of bacterial pathogenicity

The internal environment of healthy plants (e.g. leaf mesophyll tissue) is not particularly conducive to bacterial growth, since, although it is humid and protected from environmental extremes, it is low in nutrient. Bacterial growth can only occur when the nutrient status is increased, and this has been achieved during evolution in various ways:

1. Plant cells may be perturbed by bacteria in such a way as to release water and nutrients without killing the cells. This is effected by altering the permeability of the plasmalemma, and is mediated by special pathogenicity (*hrp*) genes (see Chapter 8). These *hrp* genes are highly conserved and appear to have arisen on a discrete number of occasions, which may explain why the number of pathways leading to bacterial phytopathogenicity seems to be limited.

2. Bacteria in the genus *Agrobacterium* are able to alter the nutrient level of the internal plant environment in a very specialised way by redirecting the plant synthetic machinery to produce and secrete specific microbial nutrients (opines). This is achieved by injecting bacterial DNA into the plant cells via a highly evolved and complex sequence of events (see Chapter 8).
3. Some prokaryotes (Mollicutes and some bacteria) are adapted to survive permanently within plant conducting tissues (wet and high nutrient), and have evolved specific invertebrate vector associations for direct transmission between vascular sites.

Other plant pathogenic bacteria (particularly those causing soft rot diseases) have evolved a role as secondary invaders, being able to survive in plant tissue only when natural degeneration or other pathogens have caused cell damage and the release of nutrients.

The ability of bacteria to grow in plant tissue has further evolved with the acquisition and development of many features which promote a more rapid colonisation of the plant tissue: such as cell wall degrading enzymes and toxins. These features lead to more pronounced disease symptoms and are referred to as virulence factors (see Chapter 7).

Evolution of plant resistance: compatibility and incompatibility

As with other types of host–parasite interaction, success of the parasite depends on causing minimal damage during its growth in the host, so there is intense selection pressure for compatibility on the part of the parasite. Survival of the plant, on the other hand, depends on its ability to isolate and restrict the growth of the parasite by localised reaction against it, so there is pronounced selection pressure for incompatibility on the part of the host.

In the case of the pathogen, evolution of compatibility has occurred in relation to a particular host or group of host plants, and has involved the development of a set of genes which closely relates to these plants. Host range varies considerably between different bacteria, being highly specific with some of the foliar phytopathogens (e.g. *Pseudomonas syringae* pv. *tabaci*/*Nicotiana tabacum*), but quite broad in other cases (e.g. *Erwinia amylovora*/over 130 species in the family Rosaceae).

Whereas evolution of plant pathogenic bacteria has involved adaptation to specific hosts, evolution of plants has involved the development of a general incompatibility mechanism against a very wide range of phytopathogenic bacteria. This incompatibility mechanism (resistant response) shows some similarity to the immune system of higher animals, with recognition of the foreign organism followed by the production of anti-bacterial compounds

(see Chapter 6). In the case of plants, however, the resistant response is localised, directed specifically against phytopathogens (i.e. not all foreign bodies) and plant anti-pathogen compounds (phytoalexins) are very different from the antibodies of the vertebrate immune system.

The association between bacteria and plants is a continuously evolving interaction, with the periodic origin of new types of phytopathogen, leading to new types of plant disease or more virulent forms of existing disease. In 1984, for example, a new foliar disease of citrus (citrus bacterial spot) caused by *Xanthomonas campestris* was identified in a Florida nursery, and has since been widely isolated and implicated in extensive outbreaks of the citrus disease (Graham & Gottwald, 1990).

Bacteria and plant disease

A plant pathogen (phytopathogen) may be defined in very broad terms as a biological agent which causes a deterioration in the health of the plant. Where this deterioration is apparent as clear and reproducible symptoms it is typically referred to as a named plant disease.

A wide range of biological agents, plus various environmental chemical and physical factors, are capable of causing disease in plants. Some indication of this diversity is provided by recent lists of named diseases (with their causal agents) of agricultural and ornamental crops published by the American Phytopathological Society (see Hansen, 1985; Smiley, 1988). Table 1.1 summarises information from these lists for four different types of crop. The percentage occurrence of different categories of disease agent followed a similar pattern in different crops, with approximately 50–65% of all diseases caused by fungi, 10–20% caused by viruses and 5–10% caused by bacteria. Other biological pathogens include Mollicutes (mycoplasma-like organisms and *Spiroplasma*), nematodes, algae and the higher plant *Cuscuta*. Other factors causing disease include genetic defects and various environmental aspects such as pollution (e.g. photochemical oxidants, sulphur oxides), physical factors (e.g. extremes of temperature, pH) and mineral deficiency (e.g. lack of calcium, boron or manganese). A small but variable proportion of diseases were caused by completely unknown factors.

Bacterial and fungal pathogens

Although fungi are generally more important than bacteria as plant pathogens – both in terms of number of diseases caused and overall economic losses – bacterial phytopathogens are highly successful as disease agents,

Bacteria as plant pathogens

5

Table 1.1. *Percentage occurrence of different agents in lists of named diseases of crop plants*

	Soft fruit	Cereals	Root crop	Ornamentals
<i>Biological agents</i>				
Fungi	49	58	55	64
Bacteria	6	6	9	8
Mollicutes	2	1	2	1
Viruses	14	15	12	8
Graft-transmissible	4	—	—	3
Nematodes	13	20	17	16
Algae	*1	—	—	—
Higher plant (<i>Cuscuta</i>)	*1	—	—	1
<i>Genetic defects</i>	2	*1	—	—
<i>Physical agents</i>				
Pollution	—	*1	*1	—
Physical	3	—	2	—
Mineral deficiency	1	*1	*1	—
<i>Cause unknown</i>	4	*1	2	—

*1, less than 0.5% of total list.

Data are collated from Hansen (1985) for soft fruit (apple, citrus, pear, tomato), cereals (barley, corn, oats, wheat), root crops (beet, potato, onion, sweet potato) and ornamentals (rhododendron, rose, carnation, chrysanthemum). Graft-transmissible diseases in this listing are probably caused by undetermined virus or Mollicute pathogens.

causing disease throughout the whole range of families of higher plants. In comparison with fungal pathogens they have a number of apparent biological disadvantages:

1. The bacterial cell is thin-walled and fragile and is easily damaged by desiccation, irradiation and high temperature. Plant pathogenic bacteria are therefore relatively susceptible to adverse conditions outside the host plant, although many can be found in the general environment throughout most of the year (see Chapter 4).
2. Plant pathogenic bacteria do not generally form spores and thus differ from fungi in not being able to form resistant structures. Under unfavourable conditions, bacteria depend instead on protection provided by the host plant.
3. Plant pathogenic bacteria generally lack an efficient means of long-distance dispersal (independent of vectors). Unlike fungal spores, which are typically light and windblown, bacteria are sticky and are not easily airborne. Physical dispersal of bacteria is normally via aerosols or rain-splash, and there is greater dependence on biological dispersion via vectors or transport of infected plant tissue.
4. Unlike fungi, bacteria are not able to directly penetrate the plant cuticle. Entry

into the host plant can therefore only occur via natural openings (such as stomata) and wounds.

These limitations are counterbalanced by a number of advantages that bacteria have over fungal pathogens. These include a rapid rate of reproduction, rapid entry into infection courts and independent motility. In general, once bacterial pathogens are established in a particular area or host plant, the progression of disease and the localised spread of pathogen are very rapid.

Criteria of pathogenicity

One of the first steps in determining whether a particular disease is bacteria-induced might be to attempt the isolation and culture of bacterial cells from diseased tissue. However, plant pathogenic bacteria are not restricted in their occurrence to diseased plants, but are widely present in the environment as epiphytes, so that association of a particular organism with a diseased plant does not imply causality of disease. Further experimental evidence is required before a particular bacterial isolate can be designated as the responsible agent.

The need for some experimental confirmation of pathogenicity was recognised by Burrill (1880), the first investigator to identify a bacterium as the causal agent of a plant disease. Working on fireblight disease of pear, Burrill observed motile bacteria within mucilaginous fluid from diseased tissue, and published a description of the bacterial species, which he named *Micrococcus amylovorus* and which he thought was the cause of the disease. Burrill went on to demonstrate that fireblight could be transferred by inoculation of healthy plants with diseased material, showing that it was caused by a transmissible agent.

Criteria for the designation of a disease agent were initially defined by Koch (1880), and are subsequently referred to as **Koch's postulates**. These are:

1. The organism must be consistently associated with the diseased tissue.
2. The organism must be isolated and grown in pure culture, with no other organisms present.
3. The cultured organism must be inoculated into healthy plants of the same species from which isolation originally occurred, and must produce the same disease as originally observed.
4. The organism must be reisolated and reinoculated into healthy plants to produce the same disease.

A recent example of the use of these criteria to confirm bacterial phytopathogenicity is provided by the work of Brown and Michelmore (1988) and Van

Bruggen *et al.* (1988) on corky root of lettuce. This is a serious disease of lettuce in California, causing deterioration in the root system of infected plants, and has been variously attributed to a variety of fungal and bacterial pathogens as well as different abiotic factors. Studies by the above workers (Fig. 1.1) led to the isolation of rod-shaped Gram-negative bacteria with a single lateral flagellum, which were grown in pure culture and reinoculated to reproduce the original disease in accordance with Koch's postulates. Procedures for the isolation and *in vitro* culture of bacteria are clearly a major aspect of disease investigation, and are discussed in Chapter 3.

Although Koch's postulates are generally applicable to bacterial diseases, and have been widely used in confirming the pathogenic status of many bacteria, a number of problems may arise. They are not strictly applicable, for example, in the case of obligate pathogens, where culture *in vitro* is not possible and where inoculation of healthy plants by a suitable direct isolate must be substituted. Problems with implementing Koch's postulates may also arise where symptoms are not well defined and clear diagnosis of disease is difficult. An example of this is given by ratoon stunting disease of sugar-cane caused by *Clavibacter xyli* subsp. *xyli* (Davis *et al.*, 1988), where stunting is the only overt symptom of the disease, but is also typical of a number of other diseases.

Other criteria may also be useful in determining pathogenic status in addition to those proposed by Koch. These include:

1. Microscopic examination of diseased tissue. This will reveal multiplication and invasion of particular organisms. It may also demonstrate a localisation of the pathogen in the tissue which relates directly to the symptoms, e.g. the localisation of bacteria to xylem vessels in bacterial wilt diseases.
2. Remission of symptoms with antibiotic therapy, where specific elimination of an organism relates to loss of particular disease symptoms. This approach is especially useful where a bacterial pathogen is difficult to isolate and culture, and was employed by Bennett *et al.* (1987) in confirmation that Sumatra disease of cloves was caused by a xylem-limited bacterium.

Plant pathogenic bacteria and crop monoculture

In natural environments there is typically a great diversity of species within the plant community and a corresponding diversity of plant pathogens, all at relatively low level. The destruction of such communities, and the development by man of large-scale culture of single crops (monoculture) has led to the selection of particular crop-related pathogens and major outbreaks of

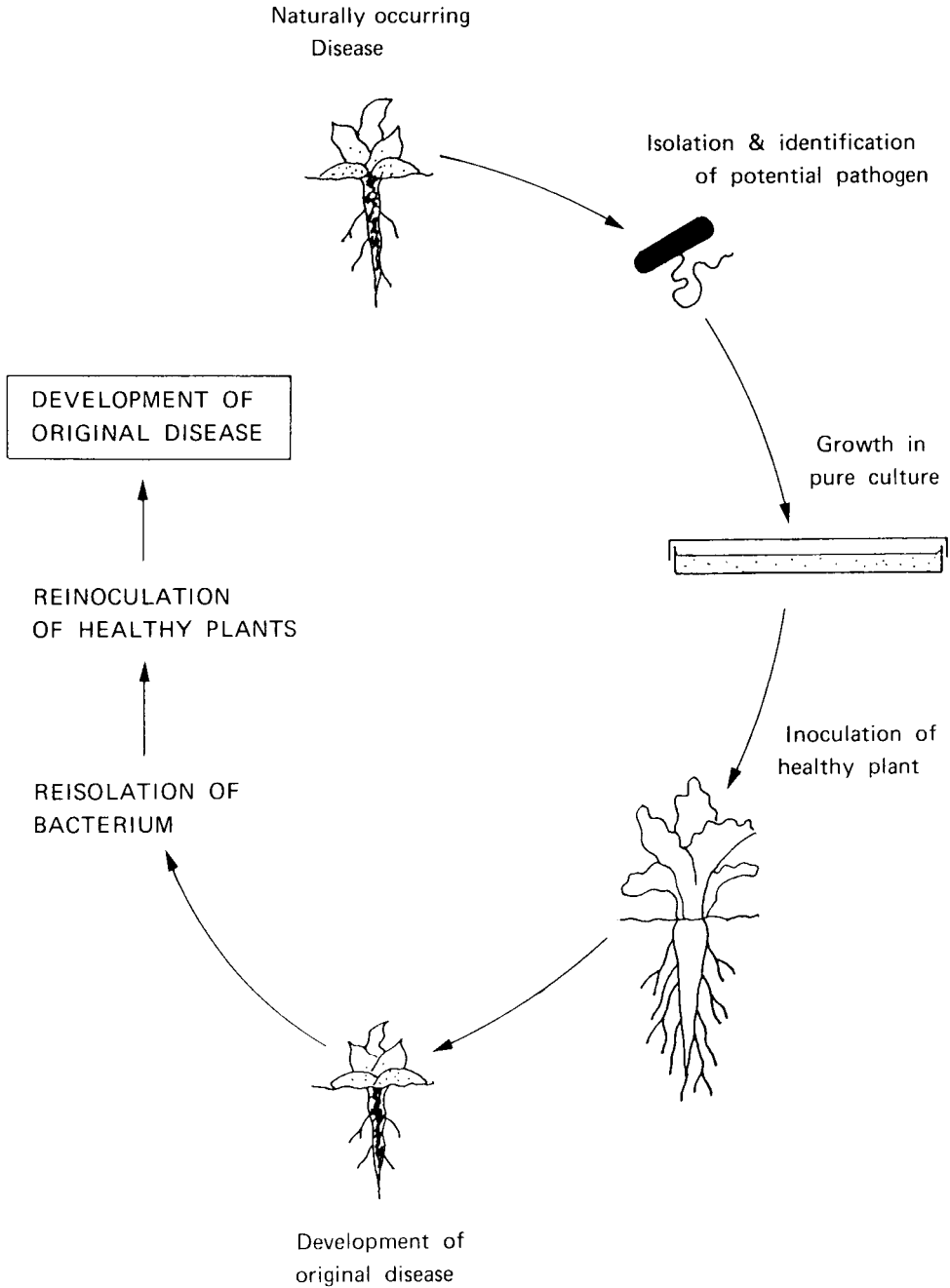
Bacterial plant pathology

Fig. 1.1 Identification of the causal agent of corky root disease of lettuce using Koch's postulates. Control inoculation of healthy lettuce plants with sterile medium (water or nutrient broth) did not result in the development of corky root disease, and the disease bacterium could not be isolated from these plants.

disease (epidemics). Monoculture not only leads to larger quantities of host plant being available for infection, but also promotes spread of the pathogen over both long distances (wind or vector transport from major sites of infection to major sites of potential infection) and short distances (spread from plant to plant within a crop). The latter is particularly relevant to bacterial pathogens, where localised dispersal is of prime importance (see above).

With many bacterial diseases, the region and date of origin (or initial observation) can be historically defined, and the geographical spread of the pathogen within a particular crop documented. A good example of this is provided by bacterial leaf blight of rice, caused by *Xanthomonas campestris* pv. *oryzae* and one of the most destructive diseases of rice in Asia (Ou, 1984). This disease was initially observed in Japan in 1884. By 1908 it was common in the south-west part of the country, and in 1926 was recorded in the north-west. After 1950, the disease increased markedly and by 1960 was known to occur throughout Japan, with the exception of the island of Hokkaido. In India, bacterial leaf blight was initially recorded in the Bombay area in 1951, and has since become widespread throughout the country with the introduction and cultivation of new high yielding but susceptible rice cultivars. In recent times, bacterial leaf blight has also been reported as a major disease of rice in China, Korea, the Philippines, Taiwan, Thailand and Vietnam, and is also present in Australia and the Caribbean.

Other examples where the origin and spread of a particular bacterial pathogen has been recorded include *Erwinia amylovora* (see below) and *Pseudomonas avenae*. The latter pathogen, which is an important agent of foliar disease in oats and maize in warmer climates, was first observed as a series of epidemics in the USA (1890–1908) and has now spread world-wide (Shakya *et al.*, 1985).

Economic importance

A number of bacterial diseases are of major economic importance, with direct financial loss due to decreased agricultural production and indirect loss due to the implementation of expensive control measures. The financial loss in agricultural production typically arises both due to a direct effect on the quality and quantity of the agricultural product as well as an overall deleterious effect on the plant itself. Post-harvest damage to stored plant products by bacterial pathogens (particularly soft rot bacteria) is also an area of major economic importance.

Table 1.2. *Economically important bacterial plant pathogens*

Bacterium	Host	Disease	Geographic location
<i>Agrobacterium tumefaciens</i>	Rosaceae, chrysanthemum grapevine	Crown gall	Temperate & mediterranean regions
<i>Erwinia amylovora</i>	Apples, pears & some ornamentals	Fireblight	World-wide in temperate zones
<i>Erwinia carotovora</i>	Potato	Soft rot	Temperate zones
<i>Pseudomonas solanacearum</i>	Over 200 species, e.g. banana, potato, tobacco, tomato	Bacterial wilt	World-wide in tropics & subtropics
<i>Xanthomonas campestris</i> pv. <i>citri</i>	Citrus fruit	Citrus canker	Asia, S. America, N. America (Florida) New Zealand
<i>Xanthomonas campestris</i> pv. <i>oryzae</i>	Rice	Leaf blight	Asia, S. & N. America, Australia, China

Some of the more widespread and economically important bacterial plant pathogens are shown in Table 1.2. As can be seen from this table, economically important phytopathogenic bacteria comprise a wide taxonomic range of organisms and result in a variety of diseases. More detailed descriptions of some of these pathogens are given below:

Agrobacterium tumefaciens This is a very common and economically important pathogen of dicots, with a very wide host range including many members of the Rosaceae (stone fruits, pome fruits and Rubus species). Symptoms are both localised (gall formation) and more general, with frequent loss of overall vigour and yield. Assessment of disease is often complicated by the fact that infected plants may be more susceptible to invasion by other pathogens (fungal, bacterial and insect), which may cause more damage than the crown gall disease itself.

Xanthomonas campestris pv. *oryzae* This bacterium causes bacterial leaf blight of rice (see above), and has the effect of reducing the overall growth and maturation of the host plant; resulting in poor development and lowered grain quality, with an increased number of undeveloped grains. According to Ou (1984), over 300 000 to 400 000 hectares have been affected annually in Japan in recent years, with yield losses of 20 to 30% in infected fields. Losses