CHAPTER

18

Beneficial and Pathogenic Microbes in Agriculture

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18.1 OVERVIEW

18.1.1 The Soil–Plant–Microorganism System

As we have seen, most normal soils do not contain abundant microbial nutrients, because microbial communities utilize any nutrients that are available (see Chapter 4). In contrast, the **rhizosphere** is a unique soil environment found in close proximity to plant roots, where nutrients are more abundant because of the influence of the plant itself. Increased nutrient availability in turn results in enhanced microbial activity and numbers. Thus the rhizosphere exists because of **soil–plant–microorganism interactions** (Fig. 18.1). Ultimately microbial gene expression in the rhizosphere is controlled by these interactions, which in turn are influenced by direct or indirect environmental factors. Overall, the microbial populations within the soil–plant–microorganism system can affect plant growth in beneficial or detrimental ways (Fig. 18.2). In this chapter we will examine the role of these microbes in the agricultural arena.

18.1.2 The Rhizosphere Environment

The term rhizosphere was coined by Hiltner in 1904 to describe the part of the soil that is influenced by plant roots. Originally the rhizosphere was thought to extend 2 mm outward from the root surface. Now it is recognized that the rhizosphere can extend 5 mm or more as a series of gradients of organic substrate, pH, O_2, CO_2, and H_2O (Fig. 18.3). Essentially two regions of the rhizosphere are now recognized: (1) the rhizosphere soil and (2) the soil in direct contact with the plant root, which is the **rhizoplane.** Microorganisms also inhabit the root itself and are known as **endophytes.** The portion of the root occupied by microbes was formerly known as the **endorhizosphere** but this term is no longer used by soil microbiologists. Finally, note that the rhizosphere effect occurs almost as soon as a seed is planted, with the area of increased microbial activity around a seed bring known as the **spermosphere.**

The rhizosphere effect is caused by the release of organic and inorganic compounds from the plant roots. In particular, the rhizosphere is influenced by living root border cells that are released by the root (see Section 18.3.4.3). Because of these releases and because of the influence of the plant roots themselves, rhizosphere soil is thought to be quite different from nonrhizosphere or bulk soil. However, despite hundreds of different studies, very little can actually be said with certainty about rhizosphere soil. Part of the problem lies in the methods used to sample rhizosphere soil. Despite even the most sophisticated of analyses performed on "rhizosphere soil," most studies are restricted due to the historically crude method of obtaining such a sample. Typically, this has involved extracting a plant from soil and shaking the roots until most of the soil particles fall off. To this day, this is still the method of choice. Other problems include the subtle interactions between specific plants and specific soils in specific environments. Thus, an infinite array of different "rhizosphere environments" is possible. This is evidenced by a perusal of rhizosphere litera-

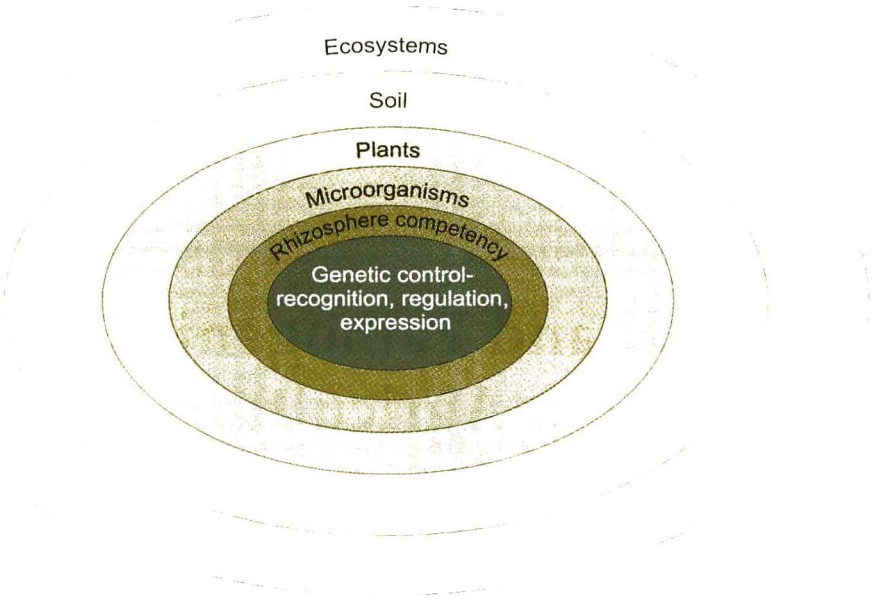


FIGURE 18.1 The soil–plant–microorganism system. Here, the influence of the environment on both soil and plant controls gene expression in the rhizosphere.

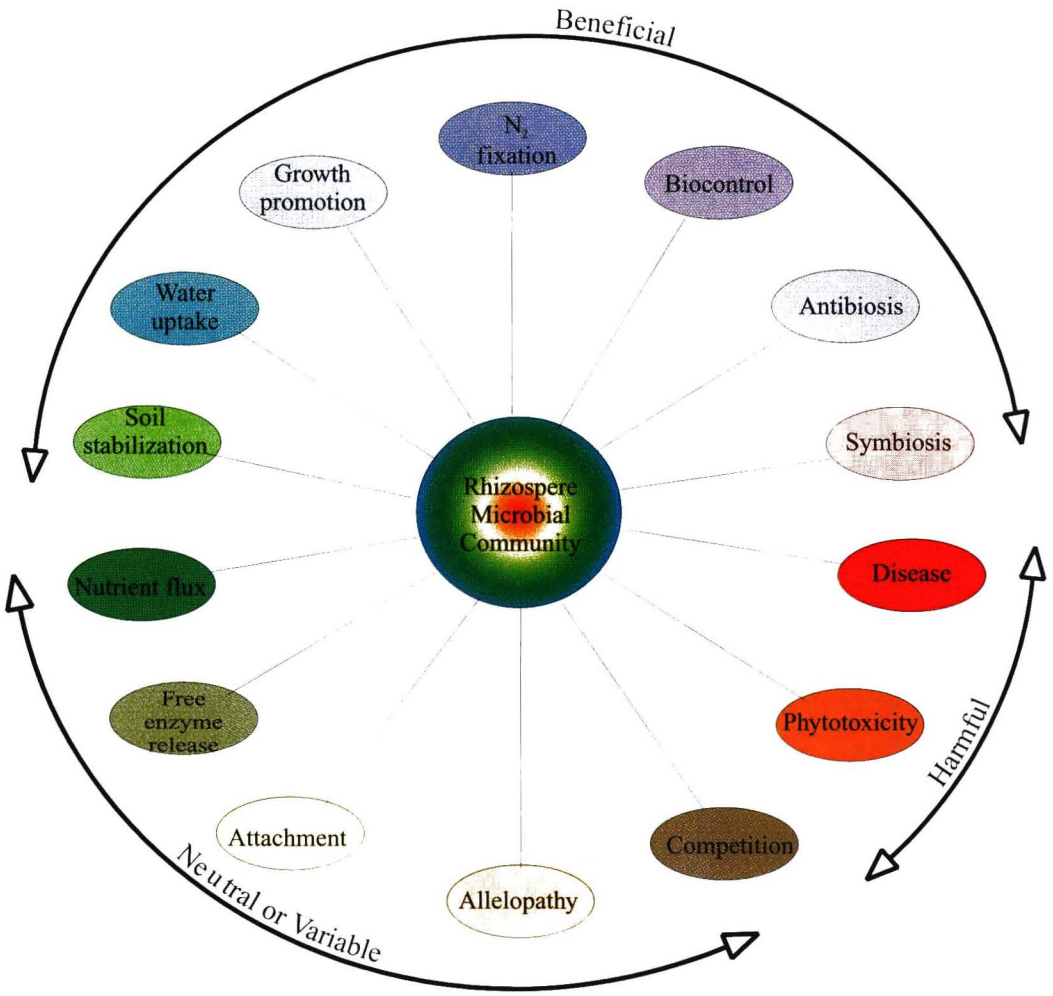
ture, which tends to be confusing and inconsistent. This is not an indictment of the scientists who have worked on the rhizosphere, rather it illustrates the difficulties of studying the complex rhizosphere ecosystem. As an example, rhizosphere soil can have pH values one unit higher or lower than those of the bulk soil, depending on nitrogen nutrition and other factors. It is, however, known that rhizosphere soil tends to be drier than bulk soil because of plant transpiration, and it also contains greater concentrations of organics because of plant-released compounds.

18.1.3 Organic Compounds Released by Plants

In natural vegetation systems, plant roots are in intimate contact with soil particles. Soil exists as a discontinuous environment with a matrix of organic and inorganic constituents combined in diverse conditions and is therefore a unique environment for many microorganisms. These organisms include viruses, bacteria, actinomycetes, fungi, algae, protozoa, and nematodes. Aerobic and anaerobic microsites exist in close proximity, allowing organic and inorganic substrates to be metabolized by organisms with different modes of nutrition. These conditions permit billions of organisms to coexist in soil. Populations vary with the soil, environment, and method of analysis, but reasonable values for "normal" soils are shown in Table 18.1.

Roots are therefore surrounded by organisms and exist as part of the soil–plant–microorganism system, which can be termed the rhizosphere. The complexity of the rhizosphere is shown in Fig. 18.1, where the two inner circles depict, respectively, the early events necessary for colonization. In addition, the figure shows subsequent factors that contribute to rhizosphere competence and the ability to metabolize and reproduce in the rhizosphere in the presence of other organisms. The components of this system (microorganisms, plant, and soil) interact with each other, which distinguishes the rhizosphere from the bulk soil. The activity of root microorganisms is affected by soil environmental factors or by environmental factors operating indirectly through the plant. Root microorganisms can affect the plant and plant nutrient uptake, directly by colonizing the root and modifying its structure or indirectly by modifying the soil environment around the root. The spokes of the wheel in Fig. 18.2 depict not only the significant processes that affect plant growth but also those that have potential for enhancement through improved cultural practices, genetic manipulation, and modeling. Substrates released from roots have many origins and were originally classified by Rovira *et al.* (1979) as

1. **Exudates**—compounds of low molecular weight that leak nonmetabolically from intact plant cells
2. **Secretions**—compounds metabolically released from active plant cells
3. **Lysates**—compounds released by the autolysis of older cells



The beneficial, harmful, and neutral or variable effects of the rhizosphere microbial community on plant growth.

FIGURE 18.2 Potential influences of the rhizosphere microbial community on plant growth. (Reprinted with permission from *Principles and Applications of Soil Microbiology* by Sylvia, D., *et al.*, © 1991, Prentice-Hall, Inc., Upper Saddle River, NJ.)

4. **Plant mucilages**—polysaccharides from the root cap, root cap cells, primary cell wall, and other cells
5. **Mucigel**—gelatinous material of plant and microbial origin

More recently, it has been demonstrated that living root border cells affect the rhizosphere ecology more than any other plant source of carbon substrate (see Section 18.1.3.2). The terms "exudates" and "exudation" were sometimes used collectively and perhaps incorrectly to include all of the organic compounds released from roots.

8.1.3.1 Exudates, Secretions, and Lysates

The release of soluble organic compounds (loosely known as root exudates) is also responsible for some of the rhizosphere effect. Loss of substrates from roots can change the pH, the structure of rhizosphere soil, the availability of inorganic nutrients, and can induce toxic or stimulatory effects on soil microorganisms (Hale *et al.*, 1978). The major mechanisms are leakage and secretion. **Leakage** involves simple diffusion of compounds because of the higher concentrations of compounds within the root as compared with the soil. **Secretion** can occur against concentration gradients but requires the expenditure of metabolic energy. Polysaccharides in particular are susceptible to secretion. Almost any plant metabolite has the potential to be exuded including carbohydrates, amino acids, organic acids and lipids, growth factors, enzymes, and miscellaneous compounds. Of these, the carbohydrates and the amino acids, which also represent a source of nitrogen, are particularly important as substrates. Organic acids and lipids reduce the pH of

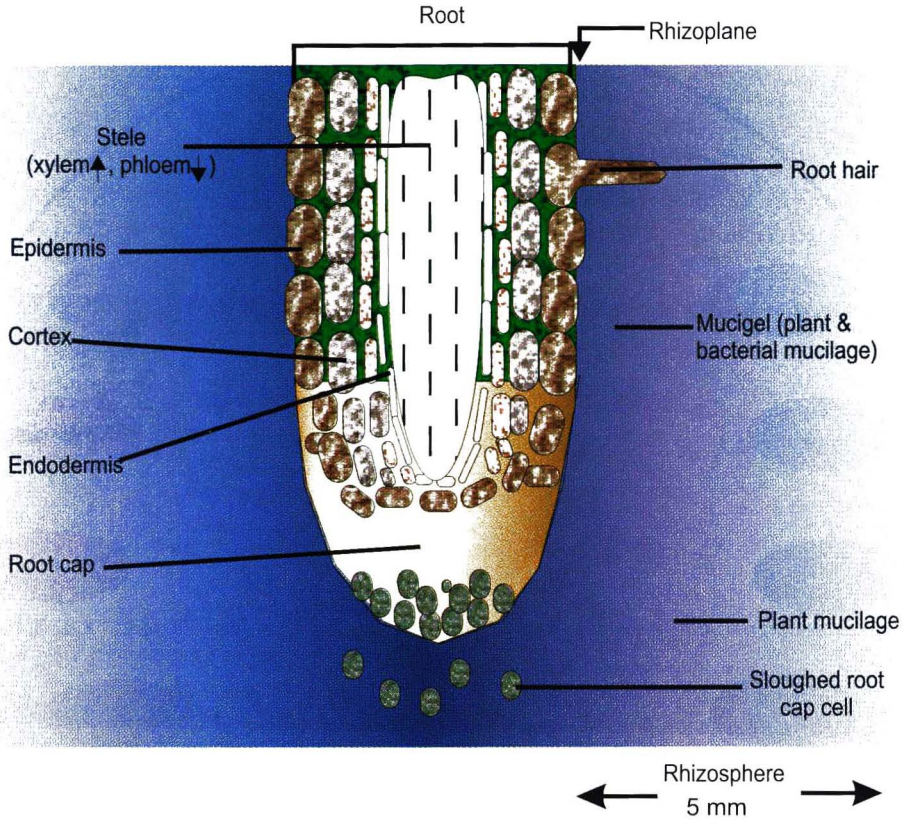


FIGURE 18.3 Structure of a root and corresponding rhizosphere.

the rhizosphere and also have a role in the chelation of metals (Curl and Truelove, 1985). Growth factors, including vitamins, and enzymes stimulate microbial activity and low growth of organisms with complex heterotrophic requirements. Miscellaneous compounds including volatiles can physiologically stimulate or inhibit organisms. When viewed collectively, it is apparent that the rhizosphere is a unique ecosystem in soil that provides a constant supply of substrate and growth factors for organisms.

TABLE 18.1 Numbers of Microorganisms in the Rhizosphere (R) of Wheat (*Triticum aestivum*) and Nonrhizosphere Soil (S) and Their Resultant *R/S* Ratio

	Rhizosphere	Nonrhizosphere	
Microorganisms	**CFU[a] g soil**		**R/S ratio**
Bacteria	120×10^7	5×10^7	24.0
Fungi	12×10^5	1×10^5	12.0
Protozoa	2.4×10^3	1×10^3	2.4
Ammonifiers	500×10^6	4×10^6	125.0
Denitrifiers	1260×10^5	1×10^5	1260.0

From Rouatt *et al.* (1960).
[a] CFU, colony-forming units.

18.1.3.2 Root Border Cells and Mucigel

As the root cap extends through soil, viable root border cells and some nonviable material (sloughed cells) are released into the soil. The role of border cells in controlling the rhizosphere ecosystem is discussed later in this chapter (see Section 18.3.4.3). The amount of sloughed material can be considerable. In solution culture, peanut plants released 0.15% of the plant's carbon, nitrogen, and hydrogen per week (Griffin *et al.*, 1976). One would predict that much more material would be lost in soil because of its abrasive nature. From the root tip to the root hair zone, the root is frequently covered with a layer composed of sloughed root border cells and polysaccharides of plant and microbial origin, which is termed mucigel (Miki *et al.*, 1980). These plant products are excellent substrates for microbial growth, in particular soil bacteria, which are extremely competitive at metabolizing simple sugars. Thus mucigel is in intimate contact with bacteria that consume the material, as well as bacteria that contribute bacterial polysaccharides to the mucigel. The amount of mucigel on a particular root depends on the net production and consumption of the material, so that in some instances parts of the root may have no mucigel. Mucigel may protect the root rip from injury

and desiccation as well as play a role in nutrient uptake through its pH-dependent cation-exchange capacity (COO^- groups).

18.1.3.3 Factors Affecting the Release of Compounds

Major factors affecting release of organic compounds include plant species and cultivar, age and stage of plant development, light intensity and temperature, soil factors, plant nutrient, plant injury, and soil microorganisms (Pepper and Bezdicek, 1990). Because so many factors affect the release of compounds, generalizations are difficult, including the actual rate of exudation (Kennedy, 1997). However, it is known that plant genes control the release of root border cells (Hawes *et al.*, 1996).

18.1.4 Rhizosphere Populations

Rhizosphere populations are influenced by many plant, soil, and environmental factors. Crop plant roots tend to have greater rhizosphere populations than tree roots (Dangerfield *et al.*, 1978). Different cultivars of the same plant species may have different rhizosphere populations.

Soils directly affect the growth and vigor of plants and therefore influence shoot growth, photosynthesis, and the amount of exudation into the rhizosphere. The concentration of oxygen in the rhizosphere is usually lower than in nonrhizosphere soil as a result of its utilization by large rhizosphere populations. Hence, in heavy-textured soils oxygen may become limiting, resulting in reduced rhizosphere populations compared with coarser-textured soils.

The physical environment around the plant and its roots also affects rhizosphere populations by affecting the amount of organic material released into the soil. Factors such as light, moisture, and temperature can all cause changes in plant metabolism and the rhizosphere effect. In summary, rhizosphere populations are dependent on many diverse interacting factors, and care must be taken when interpreting different studies.

Overall, a vast number of different kinds of microorganisms are found in the rhizosphere, and their numbers generally decrease from the rhizoplane outward toward bulk soil. The rhizosphere effect is often evaluated in terms of **R/S ratios,** where R = the number of microbes in the rhizosphere and S = the number of similar microbes in bulk soil. Thus the greater the *R/S* ratio, the more pronounced the rhizosphere effect (Table 18.1).

18.1.4.1 Microflora

Bacteria including actinomycetes are the most numerous inhabitants of the rhizosphere, and *R/S* ratios can typically be 20 : 1 (Table 18.1). Pseudomonads and other gram-negative bacteria are especially competitive in the rhizosphere. Typical actinomycete *R/S* ratios are 10 : 1 (Rouatt *et al.*, 1960). Overall *R/S* ratios are useful in delineating the rhizosphere effect, but they are only estimates and vary with different crop plants and different soil environments. The mechanisms that allow rhizosphere competence are discussed later (see Section 18.3.4.2).

Fungal plate counts are generally less than bacterial counts and in any case are often biased toward spore-forming species. However, fungal inhabitants of the rhizosphere are prevalent and can be extremely important because they can be beneficial, as in the case of mycorrhizal fungi, or harmful when they are pathogenic to plants.

18.1.4.2 Microfauna

Most research on the microfauna has centered on protozoa, nematodes, and the microarthropods. Soil protozoa are mostly rhizopods and flagellates, with smaller numbers of ciliates. Protozoan populations tend to mimic bacterial populations, because bacteria are their major food supply. Thus the rhizosphere should contain large populations of protozoa. Rouatt *et al.* (1960) reported *R/S* ratios for protozoa of 2 : 1 in wheat rhizospheres (Table 18.1). Darbyshire (1966) reported even higher protozoan populations in ryegrass rhizospheres. Many soil nematodes, including *Heterodera* and *Fylenchus,* are plant parasites that feed on underground roots. Little research has been conducted on nematodes in the rhizosphere, but populations have been reported to be higher in rhizosphere than in nonrhizosphere soil (Henderson and Katznelson, 1961). The Acari (mites) and Collembola (springtails) are important members of soil microarthropods. Mites are predatory on nematodes, and the rhizosphere would be expected to be a favorable habitat for the Acari, but studies in the rhizosphere have been limited in scope. Springtails have been shown to be abundant in cotton rhizospheres with *R/S* ratios of 4 : 1 in a sandy loam soil (Wiggins *et al.*, 1979), but the reasons for their attraction to roots are not clear.

18.2 BENEFICIAL ROOT–MICROBIAL INTERACTIONS

The fact that there are many beneficial root–microbial interactions can easily be demonstrated by growing plants in the laboratory in sterilized soil and comparing plant growth with that achieved in nonsterilized soil. Inevitably, growth in the nonsterilized

system is superior to that in the sterile system. There are many ways that microorganisms can beneficially influence plant growth, but two of the predominant mechanisms involve the macroelements nitrogen and phosphorus. The prokaryotic bacteria can enhance plant nitrogen uptake through the process of biological nitrogen fixation, whereas eukaryotic fungi enhance plant phosphorus uptake through mycorrhizal associations. Because of the importance of these two processes in agriculture, each will now be discussed in detail.

18.2.1 Biological Dinitrogen Fixation

Nitrogen is critical for plant growth and is often applied to plants as organic or inorganic fertilizers. However, organic forms of nitrogen can be converted to nitrate by the microbial processes of ammonification and nitrification and subsequently denitrified to an inorganic form, nitrogen gas or nitrous oxide (see Chapter 14.3). Clearly, some microbial process that converts nitrogen gas back to ammonia must be present, or all nitrogen would ultimately end up as nitrogen gas. This process, **biological dinitrogen fixation,** is mediated only by prokaryotes, including bacteria, cyanobacteria, and the actinomycete *Frankia*. These nitrogen-fixing organisms can exist as independent free-living organisms or as part of complex interactions with other microbes, plants, and animals. Organisms that can utilize atmospheric nitrogen gas as their sole source of nitrogen for growth are known as **diazotrophs.** In terms of benefits to agriculture, the following major systems can be delineated:

1. SYMBIOTIC RELATIONSHIPS

Symbiont	*Host*
Rhizobia (bacterium)	Legumes
Frankia (actinomycete)	Nonlegume
Anabaena (cyanobacterium)	Azolla (fern)

2. ASSOCIATIVE SYMBIOTIC RELATIONSHIPS INVOLVING FREE-LIVING DIAZOTROPHS

Microbe	*Benefitting crop*
Acetobacter	Sugarcane
Azotobacter	Tropical grasses

The preceding list is by no means inclusive of all of the possible associations between microbes and plants, but in terms of importance to agriculture, these are the major players. They are also the systems that have been most easily manipulated by human activity, including that of environmental microbiologists. Also note that there are about 100 true diazotrophs that can exist free living and that can contribute fixed nitrogen into the rhizosphere and other environments. Each of these nitrogen-fixing associations will be examined, with emphasis on the rhizobia–legume symbiosis, because it is the best studied system and is critical to agricultural crop production. First, however, we will examine the enzymatic process of biological nitrogen fixation, because many characteristics are common to all nitrogen-fixing associations.

18.2.1.1 The Process of Nitrogen Fixation

Nitrogen gas is a triple-bonded molecule that unites two nitrogen atoms. As such it is a very stable molecule that requires a large amount of energy (226 kcal per mole) to break bands and initiate nitrogen fixation. The overall reaction can be written as follows:

$$N_2 + 3H_2 \rightarrow 2NH_3$$

Energy for this process arises from the oxidation of carbon sources in the case of heterotrophs or from light in the case of photosynthetic diazotrophs. Central to biological nitrogen fixation is the enzyme complex **nitrogenase.** Initially it was thought that only one type of nitrogenase existed, but now it is clear that at least three different enzyme complexes are involved in different nitrogen systems. The classical nitrogenase complex was described by Evans and Burris (1992) and is illustrated in Fig. 18.4.

The overall nitrogenase complex consists of two protein components, which in turn consist of multiple subunits (Fig. 18.4). The iron protein termed **dinitrogenase reductase** is thought to function in the reduction of the molybdenum–iron protein **dinitrogenase,** which reduces nitrogen gas to ammonia. In the 1980s, it was shown that some nitrogenase enzymes did not contain molybdenum and that vanadium and perhaps other metals could substitute for molybdenum (Bishop and Premakumar, 1992).

A schematic of the nitrogen fixation process is shown in Fig. 18.5. Two Mg ATPs are required for each electron transferred from dinitrogenase reductase to dinitrogenase. Thus, under optimal conditions at least 16 molecules of ATP are required, illustrating the energy-intensive nature of the reaction. In practice perhaps 30 ATPs are needed because the overall process is not 100% efficient in the environment (Burris and Roberts, 1993). Initially the dinitrogenase reductase accepts electrons from a low-redox donor such as reduced ferredoxin (Fd_{red}) and binds two Mg ATPs. The dinitrogenase reductase and dinitrogenase form a complex during which an electron is transferred and the two Mg ATPs are hydrolyzed to Mg ADP + inorganic phosphate (P_i). The two proteins then dissociate

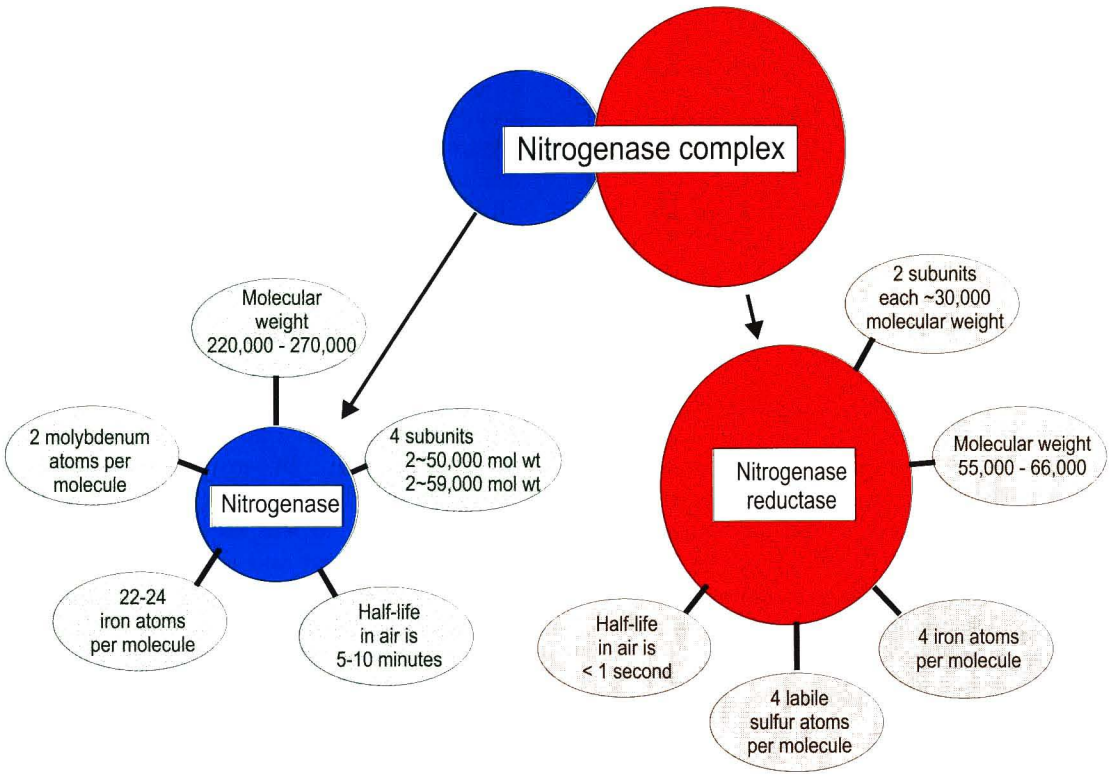


FIGURE 18.4 Characteristics of dinitrogenase (the Mo–Fe protein) and dinitrogenase reductase (the Fe protein).

and the process repeats. After the dinitrogenase protein has collected sufficient electrons, it binds a molecule of nitrogen gas and reduces it, producing ammonia and hydrogen gas. Thus, during reduction of one N_2 molecule, the two proteins must complex and then dissociate a total of eight times. This is the rate-limiting step of the process and takes considerable time. In fact, it takes 1.25 seconds for a molecule of enzyme to reduce one molecule of N_2 (Zubener, 1997). This is why nitrogen-fixing bacteria require a great deal of the nitrogenase enzyme, which can constitute 10–40% of the bacterial cell's proteins (Postgate, 1994).

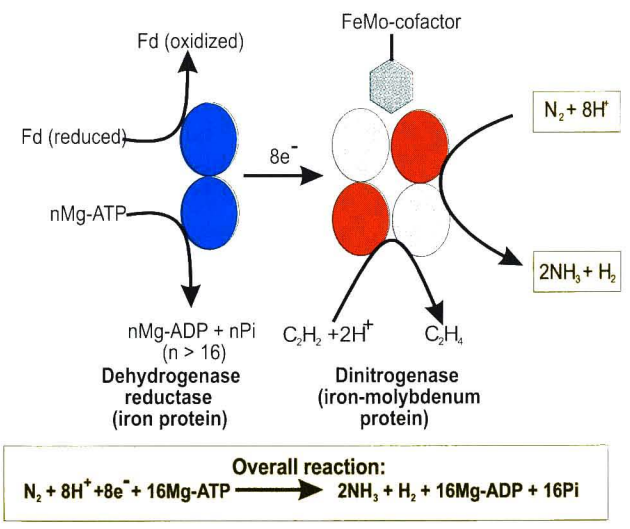


FIGURE 18.5 The nitrogen fixation process. (Adapted from Sylvia *et al.*, 1997.)

Figure 18.5 also shows that one H_2 molecule is released for each N_2 reduced to $2NH_3$. Thus, of the 16 theoretical ATPs required, 4 are spent on the formation of H_2 (25%). Some diazotrophs contain an uptake hydrogenase that reoxidizes some of the H_2 and regains some of the 25% of energy lost during nitrogen fixation (Zuberer, 1997).

An important point to note is that the nitrogenase enzyme is inactivated by molecular oxygen because of the sensitivity of dinitrogenase reductase. Thus, many organisms have developed unique strategies to protect the nitrogenase enzyme. Finally, note that the enzyme can reduce many substrates including H^+, N_2, N_2O, cyanide, carbon monoxide, and acetylene. The reduction of acetylene to ethylene and subsequent measurement of the ethylene led to the development of the **acetylene reduction assay** for nitrogenase activity,

which has been used extensively as an indicator of nitrogen fixation (Burris, 1974).

18.2.2 Free-Living Dinitrogen Fixation

Nitrogen fixation occurs in a diverse array of prokaryotic organisms including bacteria, actinomycetes, and cyanobacteria. These organisms include heterotrophic and autotrophic organisms that utilize different terminal electron acceptors for generation of energy. Table 18.2 identifies typical members of the various types of nitrogen-fixing groups. In general, the amount of nitrogen fixed by these free-living diazotrophs is small, in the neighborhood of 2–25 kg per hectare per year. This is due in part to the energy-intensive nature of the conversion, which is about 15 mg of nitrogen fixed per gram of carbon metabolized. Oxygen is an additional problem for many of the nitrogen fixers. The extent of the problem is maximal for strict aerobes, but even anaerobic microbes need to keep the nitrogenase enzyme free of oxygen. This can be done by a variety of mechanisms, including existence in the microaerophilic conditions found in the rhizosphere or production of extracellular polysaccharides to reduce free oxygen diffusion into the cell. Other organisms use high rates of respiration to reduce oxygen concentrations or undergo conformational changes of the nitrogenase enzyme when oxygen is present. During this conformational change the enzyme is protected but cannot fix nitrogen. Finally, some of the cyanobacteria produce thick-walled cells known as **heterocysts.** Fixation is also limited by the presence of free available nitrogen as nitrate or ammonium. Although the amounts of nitrogen fixed are small on an individual basis, almost all environments show some fixation. At a global level, the amount fixed is significant at about 50 Tg (10^{12} g) annually (Paul and Clark, 1989).

TABLE 18.2 Representative Genera of Free-Living Nitrogen Fixers

Status with respect to oxygen	Mode of energy generation	Genus
Aerobe	Heterotrophic	*Azotobacter* *Beijerinckia* *Acetobacter* *Pseudomonas*
Facultative anaerobe	Heterotrophic	*Klebsiella* *Bacillus*
Microaerophile	Heterotrophic	*Xanthobacter* *Azospirillum*
Strict anaerobe	Autotrophic	*Thiobacillus*
	Heterotrophic	*Clostridium* *Desulfovibrio*
Aerobe	Phototrophic (cyanobacteria)	*Anabaena* *Nostoc*
Facultative anaerobe	Phototrophic (bacteria)	*Rhodospirillum*
Strict anaerobe	Phototrophic (bacteria)	*Chlorobium* *Chromatium*

18.2.3 Associative Dinitrogen Fixation

Some of the free-living diazotrophs have developed the ability to form associations with plants in which the organisms are established on or in plant cells, where they have available carbon reserves supplied to them by the plant. In return they fix nitrogen, which is taken up by the plant. The casual or **associative symbioses** do not appear to require genetic interactions between the plant and the microbe, and no morphological modifications occur to either partner. Examples of associative symbioses include tropical grasses such as *Paspalum notatum* with *Azotobacter paspali,* in which the microbe exists within the grass root rhizosphere. *Azospirillum* spp. are also found associated with a diverse range of plant hosts including sugarcane, rye, and sorghum. Besides colonizing the rhizosphere, some diazotrophs occupy the outer root cell layers or even internal root tissues. Presumably, the more intimate the association, the more exudates are available for the microbe. *Acetobacter diazotrophicus* has been shown to fix nitrogen from within the internal root cells of sugarcane (Kennedy, 1997).

Attempts have been made to enhance crop production by inoculation with free-living diazotrophs such as *Azospirillum* or *Azotobacter,* but results have been inconsistent and many reports anecdotal. This may be due in part to the generally low rates of nitrogen that have been shown to be fixed within associative symbioses (Table 18.3). These have usually been reported as up to 20 kg per hectare per year. In addition, inoculant bacteria can have additional beneficial or detrimental effects on plants by mechanisms other than nitrogen fixation (see Section 18.3.4.2). The reasons for the low amounts of nitrogen fixed are likely to be similar to those limiting free-living fixation, namely high energy requirements, low oxygen requirement, and the inhibitory effect of nitrogen applied as fertilizer.

Overall, associative symbioses are unlikely to be significant for major crop production but may be highly significant for range and prairie grasses and a few tropical crops.

One example of an important association is that between tropical sugarcane and *A. diazotrophicus.* This organism exists inside the root cells and appears to be reasonably protected against oxygen inhibition of fixa-

TABLE 18.3 Estimated Average Rates of Biological N_2 Fixation for Specific Organisms and Associations

Organism or system	Dinitrogen fixed (kg/ha/yr)
Free-living microorganisms	
Cyanobacteria ("blue–green algae")	25
Azotobacter	0.3
Clostridium pasteurianum	0.1–0.5
Grass–bacteria associative symbioses	5–25
Plant–cyanobacterial associations	
Gunnera	12–21
Azolla	313
Lichens	39–84
Legumes	
Soybeans (*Glycine* max L. Merr.)	57–97
Cowpeas (*Vigna, Lespedeza, Phaseolus,* and others)	84
Clover (*Trifolium hybridum* L.)	104–160
Alfalfa (*Medicago sativa* L.)	128–300
Lupines (*Lupinus* sp.)	150–169
Nodulated nonlegumes	
Alnus (alders, e.g., red and black alders)	40–300
Hippophae (sea buckthorn)	2–179
Ceanothus (snow brush, New Jersey tea, California lilac	60
Coriaria ("tutu" in New Zealand)	60–150
Casuarina (Australian pine)	58

tion. Some sugarcane cultivars have been estimated to fix 100 to 150 kg N per hectare per year (Zuberer, 1997). The other significant example is rice production, which can be enhanced by free-living fixation as well as the associative symbiosis between the aquatic fern *Azolla* and the cyanobacterium *Anabaena*. In the flooded conditions required for rice production, algal growth is promoted while oxygen levels around the root systems are reduced. In this situation rice may gain up to 50 kg of fixed nitrogen per hectare per year from combined fixation activities.

18.2.4 The Legume–Rhizobia Symbioses

In contrast to the free-living nitrogen-fixing organisms, the legume-rhizobia association involves a formal symbiosis in which both partners benefit. Here, gram-negative heterotrophic bacteria originally classified within the genus *Rhizobium* interact with leguminous plants causing profound physiological changes in both organisms. These bacteria are known colloquially as rhizobia and are characterized as fixing nitrogen for a plant host in return for carbon sources supplied by the plant as photosynthates. The symbiosis occurs within newly formed root organs called root nodules that develop in response to the presence of specific soilborne rhizobia (Fig. 18.6). The rhizobia themselves undergo physiological changes, are known as **bacteroids,** and actually conduct the process of nitrogen fixation. As the plant host matures, ultimately the root nodules lyse and rhizobia are released back into the soil.

Many legumes such as peas, beans, and alfalfa are important agricultural crops, and many of them can be grown commercially with reduced inputs of fertilizer nitrogen because of the potential for symbiotically fixed nitrogen. Free-living nitrogen fixation associations often result in about 25 kg per hectare per year, but symbiotic fixation can be much more dramatic. Grain legumes such as peas, beans, and soybeans can fix about 50% of their total nitrogen requirements, with rates of fixation up to 100 kg per hectare per crop (Table 18.3). The remaining nitrogen required by the plant must be supplied from soil sources. Forage legumes such as alfalfa or clover can fix a greater fraction of their total nitrogen requirements, and fixation rates can be as high as 200 to 300 kg per hectare per year. Clearly, then, symbiotic nitrogen fixation has an economic impact globally, and environmental microbiologists have learned to maximize the efficiency of fixation (see Section 18.2.4.2).

Originally all rhizobia were classified within the genus *Rhizobium*, and species were identified on the basis of the legume host that each *Rhizobium* sp. nodulated. However, it became evident that some rhizobia could nodulate more than one host and different classification schemes evolved. The original genus *Rhizobium* is now divided into four genera and 16 species as illustrated in Information Box 1.

18.2.4.1 Root Nodule Initiation and Development

The formation of root nodules on the plant host root system is the result of subtle interactions between the host and the rhizobial endosymbiont. Here we describe the genetic exchanges that initiate nodule formation followed by the physiological manifestations of these interactions. Overall, rhizobia infect the growing nodules and ultimately inhabit the nodules as nitrogen-fixing bacteroids. The success of the symbiosis is due to both plant and bacterial genes that are turned on sequentially during nodule initiation and development. These interactions are highly specific and only specific genetic combinations lead to a successful symbiosis in which **effective nodules** result that can fix nitrogen. Other combinations can result in no nodule formation or nodules in which no nitrogen fixation occurs. Nodules that form but fix no nitrogen are termed **ineffective nodules.**

It has been shown that two different groups of microbial genes are necessary for infection. For *Rhizobium*

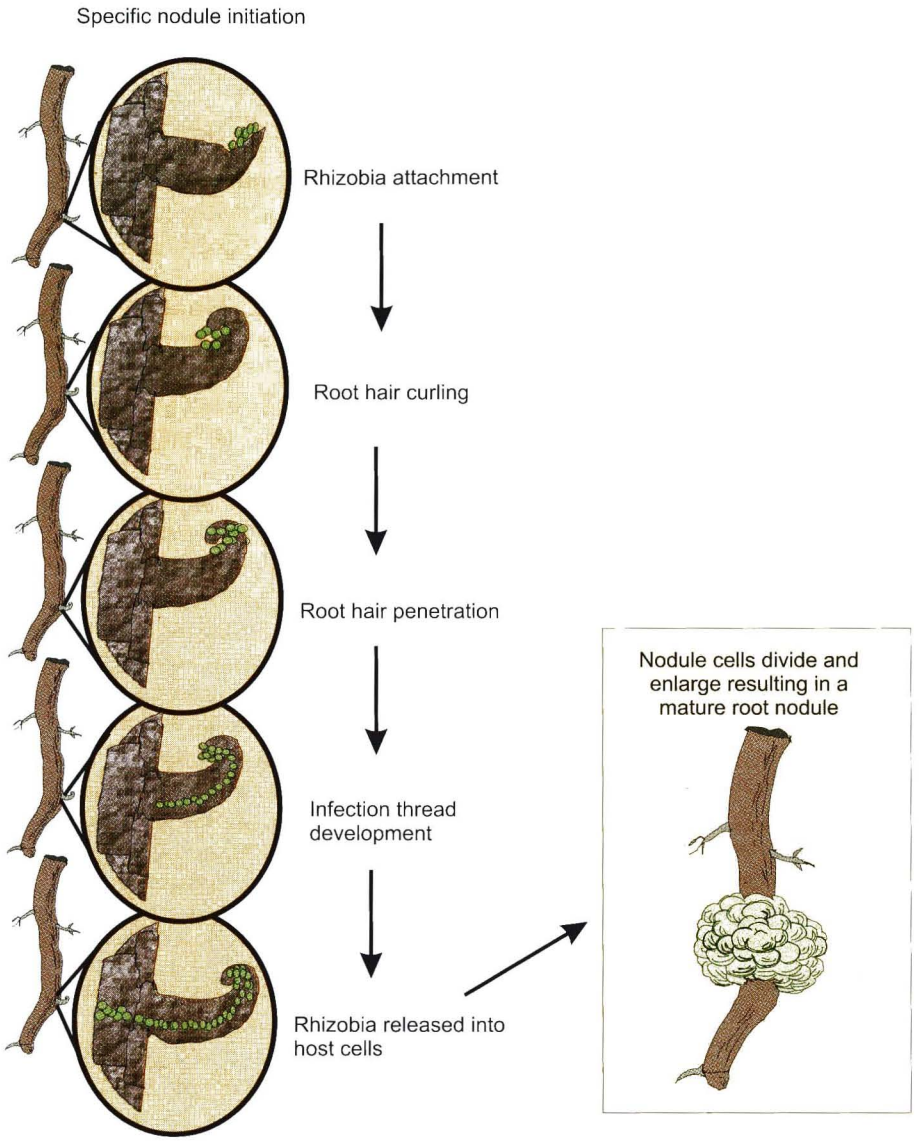


FIGURE 18.6 Nodule initiation and development.

spp., most of these genes are plasmid borne, whereas for *Bradyrhizobium* spp., *Azorhizobium* spp., and *R. loti* they are chromosomal. The **common nodulation genes** (*nodABC*) are found in all rhizobia. A fourth gene (*nodD*) is also common to all rhizobia but is the only *nod* gene expressed in the absence of a suitable host. Currently it is believed that the product of *nodD* interacts with the appropriate plant host and initiates nodule formation (Fig. 18.7). Specifically, **flavenoids,** which are complex phenolic compounds, are secreted by the plant host into the rhizosphere. If the correct rhizobial symbiont is present, the flavenoids interact with the *nodD* product and cause expression of the *nodABC* genes.

Host-specific nodulation genes differ depending on the type of rhizobia, and more than 50 genes have been defined in different rhizobia (Mergaert *et al.*, 1997). The roles of these different genes are now being researched vigorously as more information on the infection process becomes available. In essence, it is the expression of the common nodulation genes and the host-specific nodulation genes that controls successful nodule formation. Most of these nodulation genes are expressed from the initial encounter with the plant host until the release of rhizobia into the plant cells. Transcription is controlled by inducers from the plant, in particular flavenoids. One of the major host specificity determinants is the production of **lipo-**

Information Box 1
Genera and Species of the Root-Nodule Bacteria of Legumes

Genera in the square brackets refer to host legumes nodulated by each species of root-nodule bacteria. Common names are included for well-known legume genera. In several examples in this list, different species of root-nodule bacteria nodulate the same legume.*

Rhizobium†
- *R. leguminosarum* (three biovars: trifolii [*Trifolium*, clovers], viciae [*Pisum*, peas; *Vicia*, field beans; *Lathyrus*; and *Lens*, lentil], and phaseoli [*Phaseolus*, bean]
- *R. loti* [*Lotus*, trefoil]
- *R. tropici* [*Phaseolus*, bean; *Leucaena*, Ipil-Ipil, and *Macroptilium*]
- *R. etli* [*Phaseolus*]
- *R. galegae* [*Galega*, *Leucaena*]
- *R. huakuii* [*Astragalus*, milkvetch]
- *R. ciceri* [*Cicer*, chickpea]
- *R. mediterraneum* [*Cicer*, chickpea]

Sinorhizobium
- *S. meliloti* [*Melilotus*, sweetclover; *Medicago*, alfalfa; and *Trigonella*, fenugreek]
- *S. fredii* [*Glycine*, soybean]
- *S. saheli* [*Sesbania*]
- *S. teranga* [*Sesbania*, *Acacia*, wattle]

Bradyrhizobium
- *B. japonicum* [*Glycine*, soybean]
- *B. elkanii* [*Glycine*]
- *B. liaoningense* [*Glycine*]

Azorhizobium
- *A. caulinodans* [*Sesbania*]

Adapted from Sylvia *et al.* (1997).

* Other genus and species names exist in the literature. Some predate the present names. Others (e.g., *Photobacterium*) have not been accepted as valid.

† Strains of *Rhizobium* and *Bradyrhizobium* that do not belong in any named species are usually identified by the host from which they were isolated, e.g., *Rhizobium* spp. (*Acacia*) or *Bradyrhizobium* spp. (*Lupinus*).

chitooligosaccharide (LCO) molecules, which are also known as **Nod factors.** Nod factors from at least 13 rhizobial species have been characterized and their structures published (Dénarié *et al.*, 1996). All of these molecules have a similar basic structure composed of a chitooligosaccharide, which is a linear chain of β-1,4-linked *N*-acetylglucosamines, linked to an acyl chain (Fig. 18.8). Most rhizobial strains do not produce a single Nod factor but rather a population of factors with different combinations of features that may interact cooperatively to induce a specific nodulation response (Minami *et al.*, 1996). Originally it was thought that the host range of a specific strain of rhizobia was controlled by the number and diversity of the Nod factors, but it has been shown that even rhizobia with a narrow host range also produce mixtures of Nod factors (Mergaert *et al.*, 1997). However, even though total numbers of Nod factors are not correlated with the host range of a specific strain, it is clear that the Nod factors are the major determinants of host specificity, and that they are generated by transcription and translation of the *nodABC* genes plus host-specific genes. The common *nod* genes allow synthesis of the basic LCO structure, whereas the host-specific genes code for LCO-modifying transferases and enzymes for synthesis of precursors used by the transferases. Originally it was thought that the sequences of the common *nod* genes were functionally conserved, but it has been shown that different alleles of the *nodA* and *nodC* genes are present in different rhizobia and may also contribute to host range determination by specifying different degrees of polymerization of the LCOs (Kamst *et al.*, 1997).

The depth and range of information now available on the plant–microbe interactions involved in the legume–rhizobia symbiosis are remarkable and one of the triumphs of environmental microbiology in the

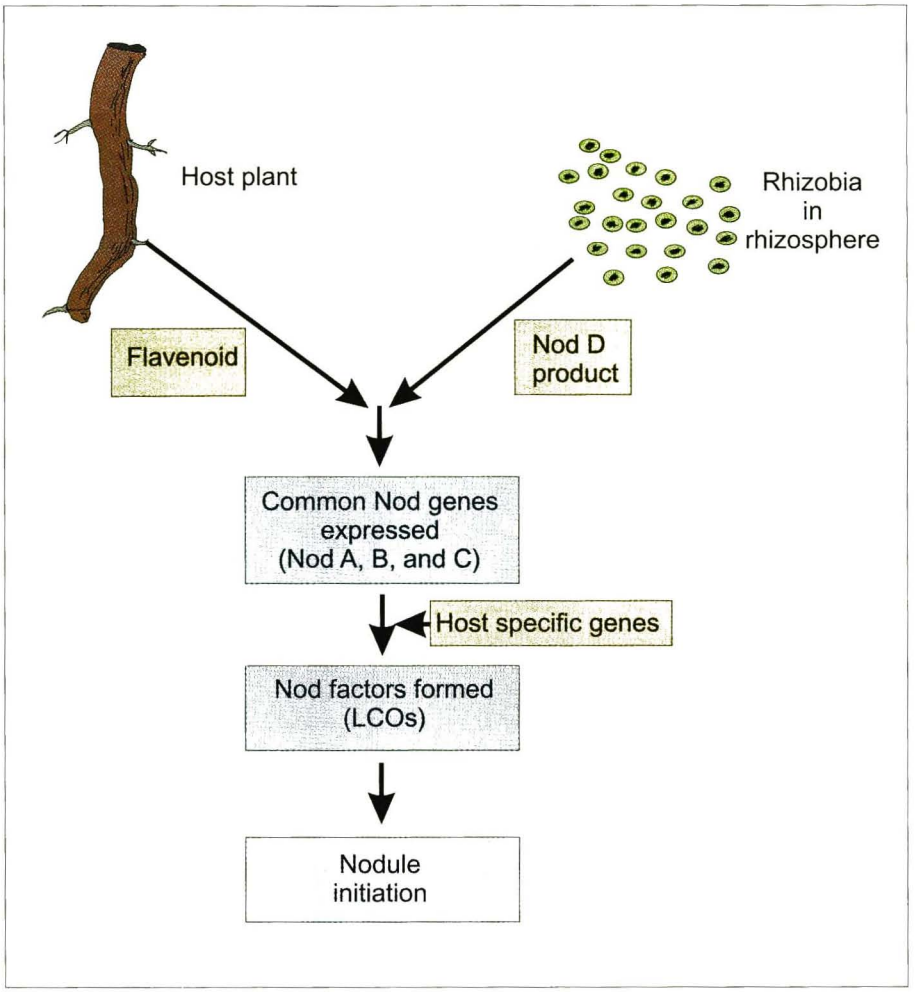


FIGURE 18.7 Plant–rhizobia genetic interactions that initiate nodule formation.

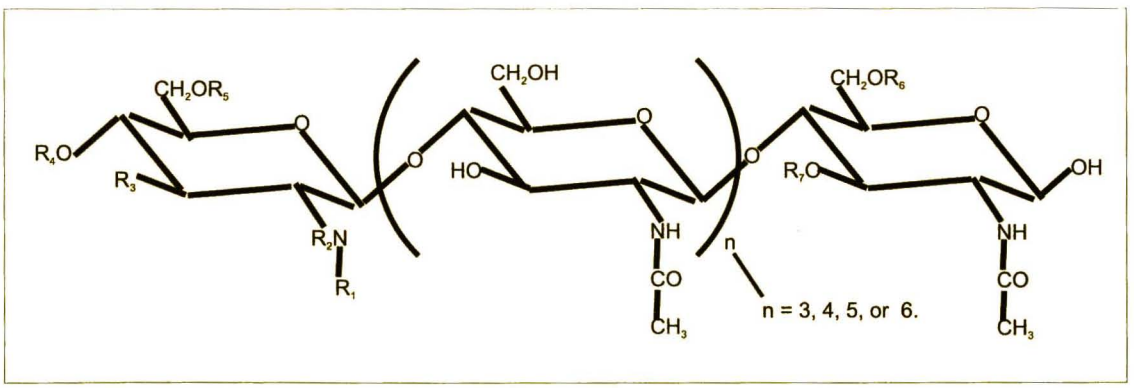


FIGURE 18.8 Overview of Nod factor structures. A chitooligosaccharide is shown. R_1 is a fatty acyl chain found in all Nod factors. R_2–R_7 are various functional groups that modify the basic structure. Nod factors of different rhizobia carry various combinations of these modifications and have different degrees of polymerization, resulting in a large diversity of structures.

molecular age. It is also apparent that it is an ongoing story, with more details still to be revealed. The end result of these genetic interactions is that nodule formation occurs (Fig. 18.6). Attachment of rhizobia to a root hair occurs very rapidly once plant and microbe are in close proximity. Rhizobia attach within minutes and after 4–5 hours, root hair curling begins. At this point, plant root cells are hydrolyzed, allowing rhizobia to enter the root hair. The rhizobia then travel along an **infection thread** manufactured by the plant, which separates the rhizobia from the rest of the root hair. As the infection thread penetrates the root cortex, the rhizobia are released, but remain enclosed by a plant-derived **peribacteroid membrane,** which again segregates the rhizobia from the plant host (Fig. 18.9).

18.2.4.2 Nodule Function and Fixation

As rhizobia are released from the infection thread, cell division occurs, and a visible nodule begins to be seen (1 to 2 weeks after infection). The size and shape of the nodules are in part determined by the plant host. **Determinate nodules** do not have continuous meristematic growth and are usually round in shape, as typified by soybean and common green bean root nodules (Fig. 18.10). In contrast, indeterminate nodules have continuous meristematic growth over the plant growing season. This results in multi-lobed nodules, as evidenced by nodules associated with peas and clovers.

Within the root nodule, the rhizobia enlarge and elongate to perhaps five times the normal size of rhizobia and change physiologically to forms known as **bacteroids.** The nodule also contains leghemoglobin, which protects the nitrogenase enzyme within the bacteroids from the presence of oxygen. The leghemoglobin imparts a pink color to the interior of the nodule and is indicative of active nitrogen fixation. Thus, examination of the interior of a nodule allows instant determination of whether the nodule is active. Prior to the end of the growing season, nodules begin to break down or senesce, at which point they appear white, green, or brown.

In **indeterminate nodules,** nitrogen is fixed as ammonia and ultimately exported to the plant shoot as asparagine. In **determinate nodules,** fixation again occurs as ammonia, but the fixed nitrogen is ultimately exported to the shoot as a purine. Fixation in either nodule type usually begins after about 15 days. During the maturation of a nodule, several proteins are produced within the nodule that are not found in the plant or rhizobia alone. These so-called **nodulins** are produced during all stages of the infection process. They include leghemoglobin and enzymes such as nitrogenase and glutamine synthetase.

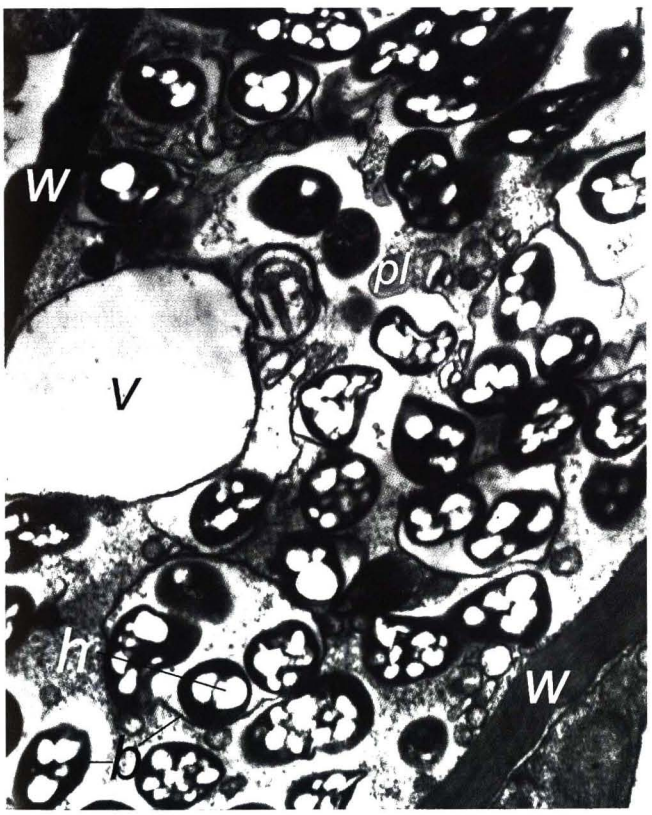


FIGURE 18.9 Transmission electron micrograph of bacteroids enclosed with the peribacteroid membrane. W; cell wall, V; vacuole, b; bacteroid (note that several bacteroids are enclosed within a membrane of plant origin) h; poly-β-hydroxy butyrate storage granule within a bacteroid, pl; plastid. (Photo courtesy I. L. Pepper)

FIGURE 18.10 Nodulated root system of *Phaseolus vulgaris* by strain KIM 5. Here nodules are determinate.

Overall, the amount of nitrogen fixed depends on both plant cultivar and rhizobial strain as well as a variety of environmental factors. In general, environmental factors that stress the plant such as extreme heat, cold, soil salinity, or lack of plant nutrients also limit nitrogen fixation, which is dependent on plant photosynthates (Graham, 1997).

18.2.4.3 Enhanced Fixation by the Legume–Rhizobia Symbiosis

Commercial legume crops are often aided in terms of nitrogen fixation through the application of rhizobial inoculants. This is particularly important when a new legume species is introduced into soils that are free of indigenous rhizobia. In this case, rhizobia introduced into the soil through the use of inoculants tend to establish themselves in the available ecological niche and are difficult to displace by any subsequent introduced rhizobia. Therefore, in such situations it is important that the originally introduced rhizobia are appropriate. The desired characteristics are outlined in Table 18.4. Usually rhizobia are impregnated into some kind of peat-based carrier with about 10^9 rhizobia per gram of peat. Production of commercial inoculants is now a big business in the United States, and internationally.

18.2.5 Mycorrhizal Associations

Clearly, mechanisms have evolved in soil microbial communities to enhance plant uptake of nitrogen as evidenced by the nitrogen-fixing bacteria. Of interest is the fact that microbial mechanisms have also evolved to enhance plant uptake of phosphates. The establishment and growth of most plants are enhanced by the presence of specialized fungi in soil that form close associations with their roots. These fungi are known as the **mycorrhizal fungi,** and they act as an extension of the plant root system. This aids in the uptake of almost all plant nutrients and is particularly important in the uptake of phosphates, which typically have low solubility in the soil solution and therefore exist at low concentrations. Such fungi assist in the plant uptake of nutrients from dilute solutions by scavenging soil nutrients and utilizing active transport mechanisms to concentrate nutrients against steep concentration gradients. When released from fungal hyphae, such nutrients can be taken up by plant roots. In addition, when nutrients are stored within the fungus, the fungus can act as a reservoir of nutrients for future plant utilization. The mechanisms that cause the fungus to release its nutrients are not well understood. The plant supplying the fungus with carbon compounds, mostly as hexose sugars, completes the mutualistic association. Thus, each symbiont aids the other in terms of required nutrients.

TABLE 18.4 Desired Characteristics for Rhizobia Utilized as Commercial Legume Inoculants

Characteristic	Definition
Infective	Capable of causing nodule initiation and development
Effective	Capable of efficient nitrogen fixation
Competitive	Capable of causing nodule initiation in the presence of other rhizobia
Persistent	Capable of surviving in soil between crops in successive years

Mycorrizal fungi become endemic in most soils and form extensive networks of fungal hyphae that can connect different plant species. In addition, on larger root systems, different fungi can infect the same root system. Mycorrhizal fungi naturally infect most plants, but in some commercial cropping systems such as the establishment of pine seedlings in pots, plants can be infected with known highly effective strains of fungi. There are several different types of mycorrhizal fungi, which are described next.

18.2.5.1 Vesicular–Arbuscular Mycorrhizas (VAMs)

These are the so-called **endomycorrhizal fungi,** which as the name implies are found mostly within the internal tissues of the root. This type of fungus is frequently found in fertile soils and is characterized by the presence of smooth **vesicles** and branched **arbuscules** that are involved in the storage and transfer of nutrients between the fungus and the plant (Fig. 18.11). About 90% of all vascular plants are associated with such fungal symbionts. The main group of fungi forming VAMs is within the order **Endoganales.** Six genera within this order are recognized, of which *Glomus* and *Gigaspora spp.* are typical.

18.2.5.2 Orchidaceous Mycorrhizas

These fungi are much more specific than other VAMs and infect only plants of the orchid family, which contains thousands of species, most of which are tropical. The physiological relationship between the orchid and the fungus is different because in this association it is the fungus that supplies the plant with a source of carbon. This is the only type of mycorrhizal association in which the carbon flow is into the plant from the fungus. In some cases, mature orchids can therefore live without conducting photosynthesis. It is also of interest that many orchids are associated with

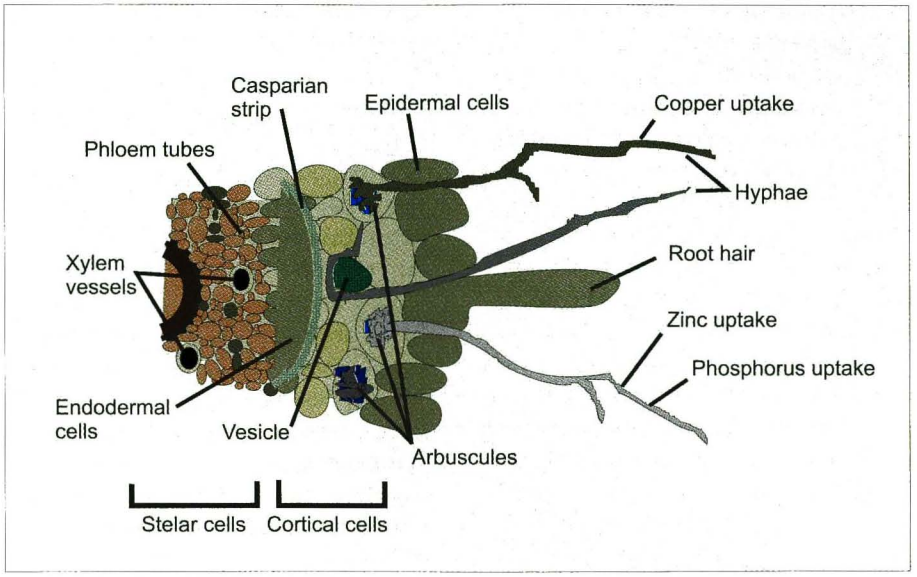


FIGURE 18.11 A typical endomycorrhiza showing hyphae extending beyond the root epidermis into the rhizosphere. Intracellular arbuscules and vesicles are also shown.

Rhizoctonia spp., including *R. solani,* which are common plant pathogens.

18.2.5.3 Ericaceous Mycorrhizas

These fungi are characterized by association with a specific group of plants known as the **Ericaceae,** which form important plant communities on moors, swamps, and peat. The plants involved include heathers, rhododendrons, and azaleas, which are often found on nutrient-poor, acid soil at high altitudes and at colder latitudes. The fungi involved are typical of the endomycorrhizal fungi in that they have intracellular hyphae. In this association, the fungus supplies the plant with nitrogen and the plant supplies the fungus with carbon substrate. The fungi also seem to be able to make the plants more tolerant of heavy metals and other soil contaminants. Most of the fungi involved seem to be members of the Ascomycetes or the Deuteromycetes.

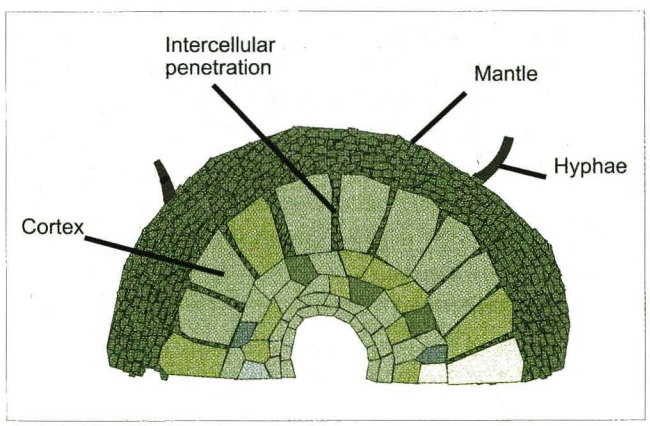


FIGURE 18.12 Cross section of ectomycorrhizal rootlet showing the exterior fungal sheath or mantle and intercellular penetration.

18.2.5.4 Ectomycorrhizas

These associations are characterized by intercellular hyphae as opposed to the intracellular penetration of the VAMs. These mycorrhizas are formed on the roots of woody plants, with a thick fungal sheath developing around the terminal lateral branches of roots (Fig. 18.12). This is also known as the **mantle** and is connected to the network of intercellular hyphae found in the root cortex known as the **Hartig net.** The plants involved with these mycorrhizas are all trees or shrubs, whereas the fungi involved are often Basidiomycetes or Ascomycetes. Carbon substrate is supplied by the plant to the fungus, and minerals, in particular phosphates, are supplied by the fungus to the plant. The ectomycorrhizal fungi have been utilized as inoculants for pine seedlings in containers prior to use in reforestation projects.

18.3 PATHOGENIC MICROBES IN AGRICULTURE

The major source of detrimental microorganisms that affect plant growth is, of course, plant pathogens. The importance of these pathogens has led to the

emergence of the discipline known as **plant pathology.** This can be defined as the study of the causes, mechanisms, environmental factors, and control of diseases of plants caused by microorganisms, many of which are soilborne. Clearly, a massive amount of literature exists on this subject including many excellent reference books such as "Plant Pathology" (Agrios, 1997). Therefore, the intent here is to present an overview of the important plant pathogens, which include viruses, bacteria, fungi, protozoa, and nematodes. The focus will be on *Agrobacterium tumefaciens,* a bacterial pathogen that has been extensively studied at the molecular level and exhibits a unique interaction between a prokaryotic microbe and the eukaryotic higher plants. Finally, important aspects of biological control of pathogens will be presented.

18.3.1 Plant Disease Caused by Fungi

Most plant pathogenic fungi have a filamentous structure known as a mycelium with individual branches known as hyphae (see Chapter 2). Almost all plant pathogenic fungi spend some of their time on the host plant and the remainder of their lives in soil or in plant debris within the soil. Thus, the survival and effects of the pathogen are controlled mainly by soil environmental factors including biotic (microbial) and abiotic factors such as temperature and moisture. The scope and diversity of plant fungal pathogens are extensive, and these organisms are responsible for billions of dollars of crop damage worldwide in all countries where agriculture is practiced. Some examples of important plant fungal pathogens and their plant hosts are shown in Table 18.5. Almost all commercial crops are subject to plant fungal attacks, which can result in diseases of seeds, roots, stems, leaves, fruit, or grain kernels.

18.3.2 Diseases Caused by Bacteria

Most plant pathogenic bacteria are rod shaped, with the exception of *Streptomyces,* which is a filamentous actinomycete. Almost all plant pathogenic bacteria occur within the host plant as parasites or on plant leaves as **epiphytes.** They also exist within plant debris or in soil as **saprophytes.** Some bacterial pathogens such as *Erwinia* predominate in the plant host, whereas others such as *Pseudomonas solanacearum* predominate in soil. However, most bacterial plant pathogens enter soil via the host tissue, and bacterial numbers often remain high only as long as the host tissue is still present within the soil. Important plant bacterial pathogens are shown in Table 18.6. Fruits and vegetables are particularly prone to pathogenic bacterial attack. Of all of the bacterial plant pathogens, *Agrobacterium tumefaciens* has perhaps been the best studied organism because of its mode of attack, which involves nucleic acids.

18.3.2.1 Crown Gall Disease–Agrobacterium tumefaciens

Crown gall disease is caused by the soilborne pathogen *Agrobacterium tumefaciens.* The disease manifests itself in uncontrolled cell division in the host plant, which results in the formation of a tumor or gall typically around the crown of the root. The disease is induced in a variety of dicotyledonous plants, particularly stone fruits, roses, and grapes. The majority of the bacterial genes necessary to induce the disease are plasmid borne. Specifically, a piece of the tumor-

TABLE 18.5 Examples of Important Fungal Plant Pathogens

Fungal pathogen	Plant host	Disease or symptom
Pythium	Almost all plants	Seed or root rot (damping off)
Phytophthora	Vegetables, fruit trees	Root rot
Plasmopara	Grapes	Downy mildew
Rhizopus	Fruits and vegetables	Soft rot of fruit or vegetables
Podosphaera	Fruit trees	Powdery mildewy growth
Alternaria	Vegetables	Leaf blight
Fusarium	Vegetables and field crops	Leaf wilting
Puccinia	Cereals and grains	Leaf and stem rusts
Ustilago	Cereals and grains	Corn smut
Rhizoctonia	Herbaceous plants	Root and stem rot
Armillaria	Fruit trees	Root rot

TABLE 18.6 Examples of Important Bacterial Plant Pathogens

Bacterial pathogen	Plant host	Disease or symptom
Pseudomonas syringae	Tobacco, vegetables	Leaf spots
Pseudomonas fluorescens	Potatoes	Soft rot
Xanthomonas campestris	Cereals, fruits	Several leaf spots (blights)
Xanthomonas campestris	Crucifers e.g., cabbage	Black rot
Erwinia tracheiphila	Cucumbers, melons	Vascular wilts
Erwinia carotovora	Fruits and vegetables	Soft rot
Agrobacterium tumefaciens	Fruit trees	Crown gall
Streptomyces scabies	Potatoes	Potato scab
Xylella fastidiosa	Grape	Pierce's disease

inducing **(Ti) plasmid** is transferred into the host plant cells, where it becomes integrated and functions within the plant. The overall features of the disease are shown in Figure 18.13. The transferred and integrated DNA (T-DNA) codes for the synthesis of two growth regulators, auxin and cytokinin, as well as for a group of amino acid derivatives known as "opines." The synthesis of these compounds, which is not regulated by the plant, gives rise to the symptoms of crown gall disease. The overall process also requires the expression of a variety of other genes on the Ti plasmid, which are termed virulence or ***vir* genes.** The *vir* genes are expressed in the presence of plant cell metabolites that are synthesized when a plant is wounded.

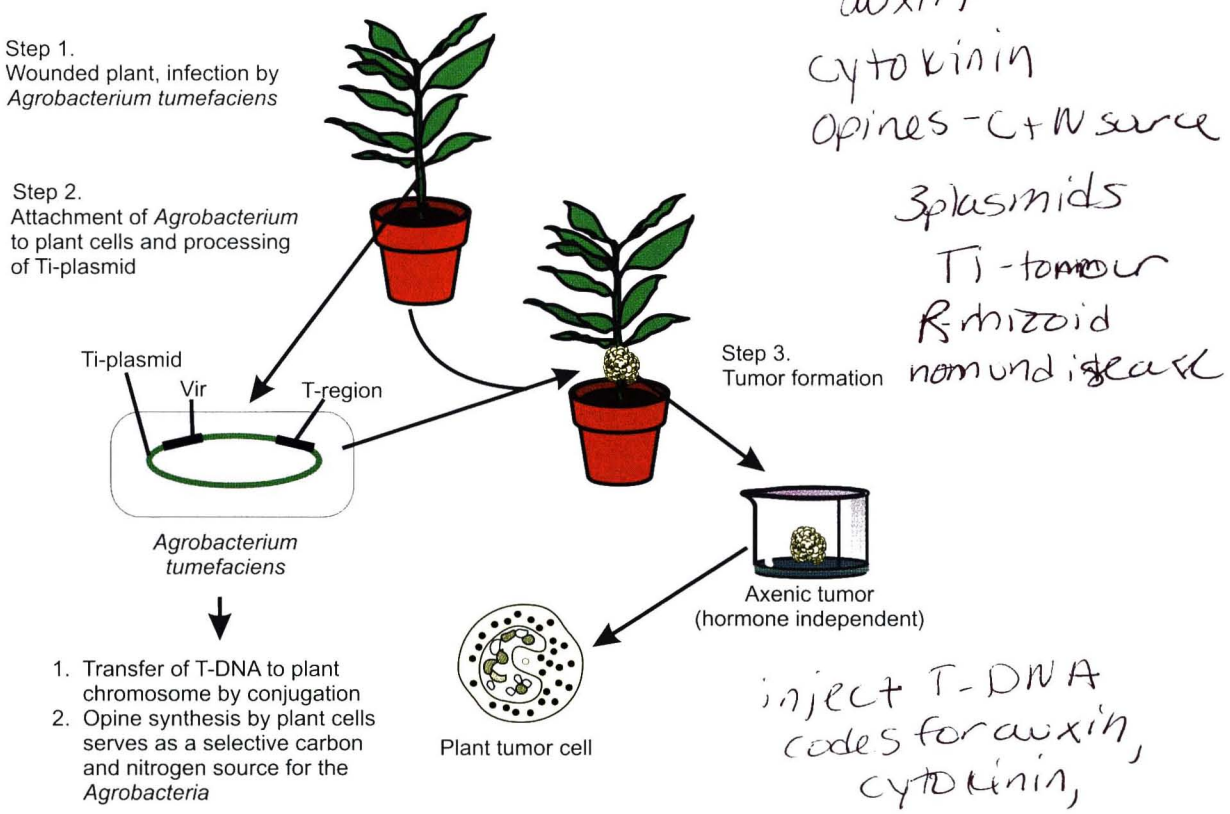


FIGURE 18.13 Overall features of crown gall tumor formation by *Agrobacterium*. It is possible to delete the T-DNA and insert useful genes under the control of plant promoters. The expression of these genes integrated into the plant DNA confers desired properties on the plant. This technology forms the basis of genetic engineering of plants using *Agrobacterium*.

Attachment of Bacteria to Plant Cells

Chromosomal genes are also known to be important in the transformation of plants by *Agrobacterium.* Several genes including the chromosomally encoded virulence genes ***chvA, chvB,*** and ***exoC*** are involved in the attachment of the bacterium to plant cells. Mutations in these genes have been shown to result in avirulent phenotypes that are unable to attach to plant cells (Marks *et al.,* 1987). All of these genes are involved in the synthesis of β-1,2-glucan, although it is not clear how this molecule functions in the process of attachment.

Ti Plasmid

Most of the genes necessary for tumor induction are located on the large 180-kb Ti plasmid. This plasmid contains the virulence (*vir*) genes which are required for the processing and transfer of specific plasmid DNA known as **T-DNA.** The *vir* genes consist of about 35 kb of DNA and are essential for tumor formation, although they are not transferred into the plant. The *vir* genes consist of eight operons (Table 18.7). The induction of the *vir* genes occurs following exposure to plant signal molecules, which are synthesized by the plant upon wounding. This explains why crops that rely on root cuttings are particularly susceptible to crown gall disease. One of the signal molecules has been identified as the phenolic **acetosyringone,** which appears to be a precursor for lignin biosynthesis (Nester *et al.,* 1996). This molecule plus sugar monomers, which are precursors of the plant cell wall, are sensed by *Agrobacterium* through the ***virA*** and ***virG*** genes, which control expression of all other *vir* genes. The *virA* gene produces a protein that appears to sense the phenolic compound directly. This protein becomes activated and in turn activates a *virG* protein, which subsequently results in the expression of all other *vir* genes. Specifically, the *virG* protein binds to a 12-bp conserved sequence known as the "vir box."

Following activation of the *vir* genes, the T-DNA is processed for transfer into plant cells. The ***virD*** operon plays an important role in the early events in the processing of the T-DNA. ***VirD1*** codes for a topoisomerase that allows the conversion of the supercoiled Ti plasmid into a relaxed form. Following this, *virD2* produces an endonuclease that results in site-specific cleavage of the bottom strand of two 25-bp direct repeats at the right and left borders of the T-DNA. The ***virD2*** protein remains attached to the single-stranded displaced DNA at the 5′ end and serves as a pilot protein. ***VirC*** also seem to be involved in the processing of T-DNA prior to its transfer into plant cells. Transport of the DNA is facilitated by products of the ***virB*** gene, which in conjunction with ***virD4,*** results in the formation of a pilus. Once inside the plant cell, the T strand is targeted to the plant cell nucleus, where both ***virE*** and ***D*** are necessary for optimal targeting.

Different T-DNAs code for different opines, which the bacterium can use as source of carbon, nitrogen, and energy, but the plant cannot. For example, two common opines are octopine and nopaline, and each is produced by different strains of *Agrobacterium.* Regardless of which opine is produced, the Ti plasmid of either strain contains *virA, G, B, C, D,* and *E* genes. However, there are differences in the *vir* gene composition. In addition, strains capable of octopine production contain genes concerned with the production of a cytochrome P-450 enzyme, which may detoxify inhibitory compounds in the plant cell environment. Octopine strains also contain the *virF* gene, which appears to be a host range determinant.

TABLE 18.7 Ti Plasmid-Encoded *vir* Genes of an Octopine Strain

vir	Inducibility	Size (kb)	Function
A	+	2.8	Plant signal sensor
G	+	1.0	Transcriptional activator
B	+	9.5	Transport of T-DNA
D	+	4.5	Processing of T-DNA, endonuclease, nuclear targeting
C	+	1.5	Processing of T-DNA
E	+	2.2	Single-stranded DNA binding protein, nuclear targeting
H	+	3.4	Cytochrome P-450 enzyme
F	+	0.6	Host range determinant

18.3.3 Plant Diseases Caused by Viruses

A variety of viruses can infect plants causing disease. Typically, plant viruses enter cells only through wounds made mechanically or perhaps via an infected pollen grain that is deposited in an ovule. Viruses which can contain RNA or DNA (see Chapter 2) typically result in leaf lesions. Although viral attacks can result in catastrophic crop losses, most virus diseases occur on crops year after year and cause small to moderate losses. Typical viral plant pathogens are shown in Table 18.8. Because of the small size of viral pathogens, their presence has been indicated primarily by the symptoms exhibited by the plant host. More recently, viruses are being identified by new molecular techniques including polymerase chain reaction (PCR) and reverse transcriptase (RT)-PCR analyses (see Chapter 13).

TABLE 18.8 Examples of Important Viral Plant Pathogens

Viral pathogen	Type of nucleic acid	Plant host	Disease or symptom
Tobamovirus (tobacco mosaic virus)	(ssRNA)[a]	Tobacco	Leaf chlorosis and distortion
Furovirus (wheat mosaic virus)	(ssRNA)	Wheat	Dwarf and mottled leaves
Potexvirus	(ssRNA)	Potato	Stunted plants
Potyvirus	(ssRNA)	Beans	Mottled, chlorotic leaves
Phytoreovirus	(dsRNA)	Rice	Galls or tumors
Caulimovirus	(dsDNA)	Cauliflower	General poor plant growth

[a]sS, single-stranded; ds, double-stranded.

18.3.4 Soil Biological Control of Plant Diseases

A relatively new approach to the control of plant pathogens is that of using microorganisms instead of chemicals. This can be done by either introducing microbes into a particular soil or manipulating the indigenous microflora. In either case, the objective is to reduce the numbers and activity of specific pathogens. **Biological control** can occur within the plant root itself, within the rhizosphere, or in the bulk soil in the vicinity of the root.

Antagonists are biological agents that reduce numbers or activities of pathogens through antibiosis, competition, or hyperparasitism. **Antibiosis** occurs when the pathogen is inhibited or lethally affected by metabolic products of the antagonist such as enzymes, acidic agents, or antibiotics. In contrast, **competition** can be for nutrients, growth factors, oxygen, or occasionally space. **Hyperparasitism** is due to the invasion of the parasite by the secretion of lytic enzymes. All of these mechanisms can result in decreased activities of pathogens, but because biological control acts by altering the biological equilibrium of the soil community, such control may take longer to act than chemical methods, and the efficacy of such methods may be more difficult to predict. On the other hand, when successful, biological control can last longer than chemical control. Finally, note that biological control methods are often most successful when used with integrated pest management strategies.

Pathogen-suppressive soils are soils in which a particular pathogen does not establish itself or persist, or if it does establish itself, it causes no damage or the disease becomes less severe with time (Cook and Baker, 1983). The main purpose of biological control is to maximize soil suppressiveness.

18.3.4.1 Maintenance of Suppressive Soils

Biological control through the manipulation of resident antagonists can be controlled by crop or soil management practices. **Soil management practices** that enhance suppressiveness include crop rotations and soil tillage, both of which reduce potential pathogen populations by reducing the incidence of specific crop residues that may harbor pathogens. Other practices include incorporation of organic amendments into soil, which apparently enhances the population of antagonists in the soil relative to pathogen populations. Crop management practices include the use of specific plant cultivars that select for specific rhizosphere antagonists. This may be due in part to the production of border cells that affect the ecology of the root system (Hawes *et al.*, 1998). In all of these cases, suppression of the activities of the pathogen occurs prior to the infection of the host root. In many cases, suppressive soils occur naturally (Agrios, 1997). However, in many instances, continuous cultivation of the same crop year after year results, at first, in a progressive increase of disease incidence, followed by years of reduced disease, presumably through a buildup of antagonists.

A well-documented example of soil suppression is the **"take-all disease"** of wheat, which is caused by the fungus *Gaeumannomyces graminis* var. *tritici*. General suppression of the pathogen is thought to be due to nonpathogenic, saprophytic *Fusarium* spp. In addition, specific suppression can occur due to antagonistic fluorescent pseudomonads. Specifically, *Pseudomonas fluorescens* has been shown to produce a phenazine antibiotic inhibitory to the pathogen. In an elegant piece of work, Thomashow and Weller (1988) showed that antibiotic-negative mutants produced by transposon mutagenesis were less suppressive to the fungus than the parent wild-type strain. Restoration of antibiotic production by complementation with a DNA fragment from the wild type restored the ability of the mutant to suppress take-all disease.

18.3.4.2 Introduced Biological Control Agents

A survey of the important plant pathogens shows that many act by attacking seeds or young root tis-

sues. Because of this, attempts have been made to control pathogens by controlling the organisms within the rhizosphere through the use of introduced biological control agents. Successful agents were originally believed to be intrinsically **rhizosphere competent,** in other words, capable of colonizing the expanding root surface. However, it is now clear that the root itself, in many instances, controls the microbial populations within the rhizosphere through the production of border cells (see Section 18.3.4.3). This has made the performance of many biological control agents unpredictable when used with different plant hosts.

The history of biological control agents originated in the 1960s, when many scientists, particularly from the then Soviet Union, utilized introduced bacteria to increase crop yields. The so-called **bacterial fertilizers** were usually *Azotobacter* and *Bacillus* spp., and yield increases were believed to be due to associative nitrogen fixation and phosphate solubilization, respectively. In reality, the actual mechanisms may have been far more subtle and complex than those proposed and may have been the result of antagonistic interactions within the rhizosphere. In addition, many of the studies in the 1960s were not replicated and thus were disputed. In the 1980s, a flurry of new studies and concepts were introduced. Terms such as **plant growth–promoting rhizobacteria (PGPR)** (Kloepper *et al.*, 1980) and **deleterious rhizosphere microorganisms (DRMOs)** (Schippers *et al.*, 1987) were introduced. The PGPR were thought to improve plant growth by colonizing the root system and preventing the colonization of the DRMOs. The DRMOs in turn were defined as bacteria that reduced plant growth but were not parasitic. Deleterious activities were thought to include alterations in the supply of water, nutrients, and plant growth substances which altered root functions (Graham and Mitchell, 1997). More recently, PGPR have been shown to induce biocontrol through a mechanism known as **systematic resistance.** One example of this is the precolonization of roots by a *Pseudomonas* sp. that induced systematic resistance in the stem against *Fusarium* wilt (Liu *et al.*, 1995). Although many different mechanisms for rhizosphere competence have now been proposed, clearly the ultimate success of an organism in the rhizosphere depends on the interaction of the organism not only with other rhizosphere organisms but also with the plant root itself. This perhaps explains why many organisms that appear to be antagonistic to other pathogens in pure culture studies in the laboratory are later seen to be ineffective or inconsistent when used in field studies with different hosts. This concept of the plant root iself controlling the ecology of the root system has now been formulated in terms of root border cells controlling plant health.

Biological control agents produce a number of chemical metabolites that can participate in controlling plant disease. Microbes that produce these metabolites can be inoculated into the rhizosphere as just discussed, or the metabolites themselves can be added. Perhaps the best known example of a metabolite used as a biological control agent is the crystal toxin produced by *Bacillus thuringiensis*, a naturally-occurring soil microbe. *B. thuringiensis* produces a paracrystalline body during its growth that is toxic to specific groups of insects. The toxic crystal Bt protein is only effective when eaten by the insects which have an alkaline pH in their gut (recall that animal stomachs have an acidic pH). In the insect gut, the toxin binds to specific receptors and eventually leads to gut paralysis. The insect stops feeding and dies from a combination of tissue damage and starvation. Bt toxin is marketed world-wide as a commercial microbial insecticide and is sold under various trade names. Even more interesting is that the toxin genes have been moved into plants to confer self-protection against insects. As an example, the genes have been inserted into potato plants to allow protection against the Colorado Potato Beetle. Other metabolites that are useful in biological control include microbially-produced HCN, antibiotics, and siderophores (see Chapter 17.6.5). Most recently it has been discovered that a rhamnolipid biosurfactant (Fig. 17.6) is a very effective biological control agent against a class of pathogens called the zoosporic plant pathogens (see Fig. 18.14) (Stanghellini and Miller, 1997). One infamous zoosporic plant pathogen is *Phytophthora infestans* the causative agent of late blight of potato, the disease that caused the Irish Potato Famine in the 1840's.

FIGURE 18.14 *Plasmopara latacae-radeculus*, a zoosporic plant pathogen, infecting a root hair. (Photo courtesy M. E. Stanghellini.)

18.3.4.3 Function of Root Border Cells as Biocontrol Agents

Plant roots must move into new soil areas in order to obtain nutrients, and to do this, new root tissue must be generated by the root meristem. Evidence has suggested that the direction of root growth, and in fact the rhizosphere ecosystem, is controlled by **root border cells** (Hawes *et al.*, 1998). These have been shown to be what used to be called root cap cells, and they are actually living plant cells that are designed to be released from the root surface into the external environment. Under controlled conditions, border cells and their associated products can contribute up to 98% of carbon released as root exudates. The propagation and release of border cells are under the direction of the root and directly affect the behavior of rhizosphere bacteria and fungi. Many of the functions of these cells are still being elucidated, but they are thought to include chemotactic signals to beneficial microbes as well as plant pathogens. In the latter case, it may well be that the border cells act as decoys to the pathogens, protecting newly formed intact root tissue from attack. Border cells have also been shown to be a source of flavenoid-based *nod* gene–inducing signals that may affect nodulation of legumes by rhizobia. The ultimate role and scope of border cells are still being evaluated, but it is attractive to think that these agents are a vital link in controlling rhizosphere populations and their associated activities.

18.3.4.4 Biological Control of Crown Gall Disease

One very successful example of biological control worthy of mention is the control of crown gall disease by *Agrobacterium tumefaciens* strain K84. This commercially important biological control method was developed in Australia (Kerr, 1980). Inoculation of planting stock with the nonpathogenic strain K84 is often successful in preventing the disease. The mechanism of control involves the production of a **bacteriocin** that inhibits closely related bacteria—in this case other virulent strains of *A. tumefaciens*. The bacteriocin is known as agrocin 84 and is a fraudulent adenine nucleotide that inhibits DNA synthesis. It is taken up by *Agrobacterium* strains that synthesize nopaline or agrocinopine. Part of the agrocin molecule is similar to the structure of agrocinopine; thus strains that contain agrocinopine permease are capable of taking up agrocin 84. It is believed that bacterial colonization of the root surface may also be involved in the control mechanism. However, virulent bacterial mutant strains that do not take up agrocinopine are not sensitive to the bacteriocin.

QUESTIONS AND PROBLEMS

1. Discuss the usefulness and also the limitations of using R:S ratios as a means to evaluate the rhizosphere effect?
2. How does the rhizosphere differ physically, chemically and biologically from bulk soil?
3. How important is free living nitrogen fixation in i) production crop agriculture; and ii) natural ecosystems?
4. How important is symbiotic nitrogen fixation in crop production?
5. Describe your understanding of the genetic interactions between rhizobia and leguminous plants.
6. Compare and contrast the major mycorrhizal fungi/plant interactions.
7. Compare and contrast rhizobia and agrobacteria.

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