Microorganisms and Biotic Interactions

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Abstract

Most ecosystems are populated by a large number of diversified microorganisms, which interact with one another and form complex interaction networks. In addition, some of these microorganisms may colonize the surface or internal parts of plants and animals, thereby providing an additional level of interaction complexity. These microbial relations range from intraspecific to interspecific interactions, and from simple short-term interactions to intricate long-term ones. They have played a key role in the formation of plant and animal kingdoms, often resulting in coevolution; they control the size, activity level, and diversity patterns of microbial communities. Therefore, they modulate trophic networks and biogeochemical cycles, regulate ecosystem productivity, and determine the ecology and health of plant and animal partners. A better understanding of these interactions is needed to develop microbe-based ecological engineering strategies for environmental sustainability and conservation, to improve environment-friendly approaches for feed and food production, and to address health challenges posed by infectious diseases. The main types of biotic interactions are presented: interactions between microorganisms, interactions between microorganisms and plants, and interactions between microorganisms and animals.

Keywords

Agronomy • Antagonism • Biogeochemical cycles • Commensalism • Competition • Ecosystem functioning • Infectious diseases • Parasitism • Predation • Symbiosis • Trophic networks

11.1 Main Types of Interactions

11.1.1 Interaction: A Key Aspect of Living

Since the end of the era known as prebiotic era, which happened about 3.8 billion years ago, life has existed on earth in the form of cells, more or less complex in structure and operation, but still built on the same basic pattern (membrane + cytoplasm + nucleic acids). Conversely, if the pattern has remained fundamentally the same throughout evolution, diversification of genetic information – for all kinds of processes and not only by point mutations – has given birth to a biosphere composed of myriads of different organisms, from bacteria to the more organized multicellular eukaryotes.

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A striking feature of the biosphere, repeatedly stressed among others by Stephen Jay Gould (Gould 1989), is the appearance of new body plans, which has not consistently resulted in the elimination of body plans that had formed previously. Thus, even though individuals have constantly disappeared and the species succeeded each other, the main types of living beings continue to exist to this day, whether they appeared 3 billion years ago or recently. The most striking example is provided by prokaryotes, which have remained ubiquitous and diversified in all areas of the world, despite the emergence of all kinds of unicellular or multicellular organisms. The evolution of life has led to the emergence of multiple interactions between all levels of organization, the first species to appear also taking advantage of the emergence of new ones, as shown, for example, by the colonization of eukaryotes by prokaryotes.

Interactions are so much the rule that we may even wonder if, in a given ecosystem – inasmuch as there is unity of place and time – there are living species not interacting directly or indirectly with other species. Interaction thus appears as one of the fundamental features of life, alongside metabolism and reproduction. We should not be surprised that interactions take many forms and those involving microorganisms are both diverse and particularly important for the exchange of matter and energy.

A fundamental character of interactions of living beings is that any individual can interact with any other individual. For example, viruses interact with prokaryotes, unicellular eukaryotes, or humans; prokaryotes interact with multicellular eukaryotes; individuals of a given species interact with others of the same species; etc. Even individual cells of the same multicellular organism interact with other cells of the same organism, through continuous exchange of electrical (nerve impulses) or chemical (hormones) signals between cells that are genetically identical but express different genes.

Curiously, this universality of interactions is a strong argument in favor of the unity of life. This concept of unity, of which Geoffroy Saint-Hilaire was the defender in the middle of the nineteenth century, is evidenced, for example, by the construction of proteins from a list determined once and for all of 20 amino acids, by the similarities of metabolic pathways and biophysical processes, and of course by the universal genetic code. The ability of a transposon to insert foreign DNA, the ability for bacteria to invade animal tissues, the ability of *Toxoplasma* (protozoan) to manipulate the behavior of a rodent, and the possibility of limitless pairwise interactions are all further evidence that life probably arose only once, or at least only the descendants of a particular life form survived (*cf.* Sect. 2.1). For example, any pathogen and its host share a common ancestor. The result is that signals can be exchanged between organisms whose common ancestor existed billions of years ago, and this might explain why a pathogen can use and manipulate to his advantage the biochemical processes of its host. What has changed throughout evolution is the complexity of organisms, but not the basis for their functioning or the nature of the molecular signaling pathways: this could only facilitate the establishment of multiple interactions (Table 11.1).

Table 11.1 Different types of interactions and their effects on partners

	Effect on partner #1	Effect on partner #2
Mutualism (symbiosis)	+	+
Commensalism	+	0
Parasitism, predation	+	–
Neutralism	0	0

0 means absence of effect, + means a positive effect, – means a negative effect

11.1.2 Conflictual Interactions

2.7 billion years ago, cyanobacteria developed molecular tools allowing photosynthesis, a feature that allows us to divide most living beings into two major groups, photosynthetic organisms such as algae and green plants that get their energy from the sun and those who, directly or indirectly, derive their energy from the former. Food chains and webs in ecosystems are essentially based on these two types of organisms, typically referred to as primary producers (comprising also chemoautotrophic bacteria such as nitrifiers) and consumers, respectively, as well as on microbial decomposers. These food webs involve several types of conflictual interactions.

Conflictual interactions within ecosystems are of multiple types. They can be classified according to trophic level, distinguishing, e.g., primary consumers (such as herbivores), secondary consumers (e.g., carnivores that eat herbivores), etc. We can distinguish the interactions between organisms from different trophic levels from interactions between individuals at the same trophic level, which may lead to intra- or interspecific competition. **Competition*** is an interaction in which partners use the same resource, whether nutrients, water, or even space.

Intraspecific competition can be particularly intense, when the partners involved have needs that are very close and use comparable if not identical means for the resource acquisition. Competition can also occur between individuals belonging to different trophic levels, and even different kingdoms, such as the competition for nitrate between plant roots and microorganisms in soil.

Competition is sometimes assisted by the production of toxic compounds in the environment, such as bacteriocins or antibiotics. It corresponds to **interference competition***, which relies on chemical warfare mechanisms, to be distinguished from competition by exploitation. The distinction between the two types of competition is sometimes difficult to establish. Another difficulty lies in the distinction between interference competition and another conflictual interaction, **amensalism***. Amensalism (synonym of antagonism) is an interaction that has a negative effect on one partner but no effect on the other. Amensalism assumes that the two species do not compete significantly with each other. Amensalism is based on a physical or chemical modification of the environment and, in the latter case, often involves the release of toxic compounds. Thus, the production of antibiotics (i.e., antibiosis) may correspond to interference competition or antagonism, depending on the nature of the partner affected and on the energy invested in the synthesis of these secondary metabolites. Allelopathy, i.e., the synthesis by a higher organism of compounds bioactive on another higher organism, is conceptually close to antibiosis and deserves to be mentioned in this chapter because microorganisms can metabolize some of these compounds and thus interfere with the interaction.

Finally, there are two types of conflictual interactions for which the interaction is negative for one partner but beneficial for the other, i.e., predation and parasitism. **Predation*** is of short duration (a cat that eats a mouse or a protozoan that feeds on a bacterium) and usually leads to destruction of the genetic information of the prey (in this case the mouse or the bacterium). **Parasitism*** is sustained in time (the parasite in its host) and the genetic informations of the partners remain in intimate contact over time; here we recognize the traditional distinction between predator–prey and parasite–host relationships. The predator is free, while the parasite is physically associated with its host, at least for part of its life cycle. It should be noted that the importance of signals is very different in the two types of interactions. When a cat chases a mouse, signals are visual, auditory, or olfactory, and they last only the duration of the hunt. When a virus or bacterium settles in a host, the exchange of molecular signals can persist for months or years, for example, involving the immune system of the host and processes by which pathogens circumvent these defenses. One of the most amazing aspects of these sustainable interactions is manipulation: *Toxoplasma* are parasitic protozoa whose complex life cycle requires them, when they are in a rodent, to pass into a cat to perform their sexual reproduction; *Toxoplasma* settles in the nerve cells of the rodent and induces in them a suicidal attraction to cats, as well as altered social behavior. Although we do not know the precise mechanisms involved, it is clear that this manipulation of rodent behavior must rely on biochemical compounds produced by *Toxoplasma*. The more research is done on it, the more manipulation appears to be widespread – the result of natural selection – in pathogen–host interactions.

11.1.3 Beneficial Interactions

Some interactions, unlike the previous ones in which conflict predominates, provide benefits to the different partners. Among beneficial interactions, **commensalism*** is the only one for which the positive effects are exerted on only one of the two partners. This interaction is therefore the counterpart of amensalism. **Cooperation*** is a mutually beneficial, facultative relationship, this facultative aspect distinguishing it from symbiosis.

In the case of **mutualism*** or symbiosis (Box 11.1), both species benefit from the interaction. It is interesting to note that, as in conflictual interactions, genetic distance is not a barrier to partnership: one can observe mutualistic interactions between plants and animals, bacteria and vertebrates, and fungi and plants; a complete list would be endless. Mutualistic interactions have played a very important role in evolution (Fig. 11.1). Mitochondria and chloroplasts – and certainly other structures – of eukaryotic cells are nothing more than ancient bacteria. In fact, the modern eukaryotic cell, the cornerstone of all multicellular organisms, can be seen as a collection of bacteria. Such interactions are also reversible: for example, the agent of malaria, the *Plasmodium* protozoan, has lost its chloroplasts (which its ancestors had, as indicated by recently discovered remnants of these chloroplasts), the same way tapeworms lost their gut.

Box 11.1: Story of a Word, Symbiosis

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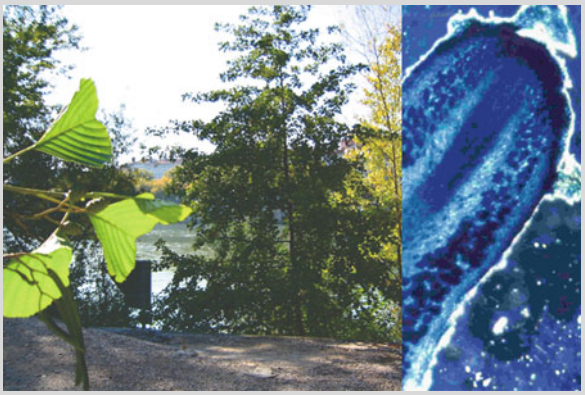
Symbiosis, from the Greek "syn" (with) and "biosis" (life), illustrates the ongoing interaction (obligatory) of two or more organisms, their lack of autonomy. It has been defined, at least initially, as the intimate and sustained interaction of two organisms belonging to different species. Currently, the symbiosis is generally regarded, especially in Microbial Ecology, as a mutually beneficial obligatory interaction.

The paternity of the term has been attributed to Heinrich Anton de Bary (de Bary 1879), a German botanist (1831–1888) who described in his book *Die Erscheinung der Symbiose* (*The Phenomenon of Symbiosis*) life in association of different organisms, definition thus including the parasitism of fungi, on which de Bary worked all his life. This term has quickly gained popularity and has been applied in 1885 to mycorrhizae and in 1889 to nodules induced by *Rhizobium* on peas by the German botanist Albert Bernhard Frank (1885, 1889) who would prove to be an effective proselytizer of the word. Root nodules form following sophisticated molecular dialogues and are inhabited by nitrogen-fixing Actinobacteria (*Frankia*; Box Fig. 11.1) or Proteobacteria (*Rhizobium* and many other taxa), depending on the type of plant partner.

The term has been modified by different prefixes or adjectives. We thus speak of endosymbiosis and ectosymbiosis whether the host is penetrated or not, of obligatory or facultative symbiosis, depending on whether or not the partners can live without the other. In the latter case, which corresponds to cooperation, partners are sometimes termed aposymbiotic, asymbiotic, or pre- or post-symbiotic. The word protosymbiosis is also used, when the relationship is with little mutual benefit as in the case of yogurt where *Lactobacillus bulgaricus* and *Streptococcus thermophilus* coexist without strong metabolic complementarity. The term associative symbiosis is also used as a synonym for cooperation, especially in the case of saprophytic microorganisms in the rhizosphere that enhance plant growth. Parasymbiosis is a symbiosis where an additional organism secondarily joins an already formed symbiosis.

The meaning of symbiosis has evolved over time, and today the term is rarely used anymore in biology to talk of parasitism. On the contrary, some confusion has emerged about the distinction between mutualism (sustainable, mutually beneficial interaction) and symbiosis. According to Odum (1971), true symbiosis (obligatory) and mutualism are equivalent, which also corresponds to the definition given in the manual of Microbial Ecology of Atlas and Bartha (1981). However, others, such as Barbault (1997), distinguish mutualism (not obligatory) from symbiosis (mandatory).

A Google search permitted to identify in June 2014 more than 10 million sites with "symbiosis" in the fields of biology, management, politics, and culture.



Box Fig. 11.1 Nitrogen-fixing symbiosis between the nodulating actinobacterium *Frankia* and the actinorhizal plant alder. On the left, a black alder growing along the bank of river Rhône in Lyon; on the right, a longitudinal section of an alder nodule stained with toluidine blue showing the central stele and, on both sides, the enlarged cortical cells (*deep blue*) filled with nitrogen-fixing *Frankia* cells (Photographs: Ph. Normand).

It should be added that the distinction between parasitism and mutualism is not always easy to make. In some cases it is difficult to measure mutual benefits; in other cases we can question the "honesty" of the interaction. To cite just one example, many organisms living in deep ocean hydrothermal ecosystems harbor sulfate-reducing bacteria that provide the bulk of their energy resources. This interaction can be seen as a simple exchange of protection against food, but if we take into account that the host regularly consumes part of the bacterial population, one can also see true parasitism. The length of the interaction is not an absolute guarantee of peace between the partners. For instance, "mitochondrial bacteria," which in sexually reproducing organisms are usually transmitted to zygotes by females, "endeavor" to skew the sex ratio at the expense of males. This is a well-known strategy in the interaction between *Wolbachia* bacteria and their arthropod hosts (*cf.* Sect. 11.4.3).

The mutualistic interaction reaches perhaps its perfection in multicellular organisms. While in a population of unicellular organisms, competition for resources is the rule, it is logically excluded between members of multicellular organism that constitute a clone: there is a real change in target

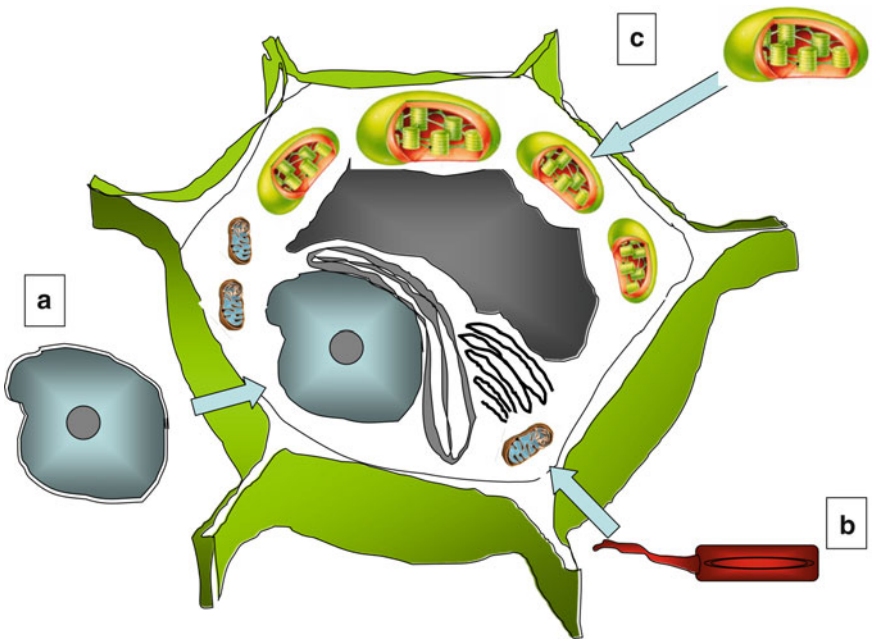


Fig. 11.1 Plant cell comprising a nucleus (*gray*) originating from an ancestor such as *Thermoplasma* (**a**), mitochondria (*red*) originating from an ancestor such as an alphaproteobacterium (**b**), chloroplasts (*green*) originating from an ancestor such as cyanobacteria (**c**) (Redrawn from John H. Miller (jhmiller@uh.edu))

selection; it does not oppose cellular individuals but rather groups of cells. Of course, this totally peaceful interaction is made possible by the perfect genetic similarity of the cells constituting the group. Evidence of the importance of this similarity is provided by instances where mutations lead to the emergence of rival groups within the same population.

11.1.4 Dynamics of Interactions

Interactions, whether involving predator–prey relationships in a forest or more complex relationships between bacterial populations in the soil, can only be understood in a perspective that is both dynamic and Darwinian. Interaction dynamics are important to consider because the terms of the interactions change over time, even if sometimes they oscillate momentarily around an equilibrium position. A Darwinian perspective is also needed because biotic interactions are the result of selective pressures that organisms exert on each other, even if other factors such as genetic drift can also affect the reproductive success of individuals.

When interactions are conflictual, they usually give rise to an arms race. Arms races represent one of the fundamental drivers of evolution, because selective pressures that organisms exert on each other remain the *sine qua non* of evolutionary change. In a 1973 paper, the American evolutionist Leigh Van Valen gave for this driver of evolution an explanation known as the Red Queen hypothesis, which has become a classic (Fig. 11.2). He suggested that changes constantly generate new adaptations to meet adaptations in the competing species; if species share, even partially, the same spatial or energy resources, and if one of them increases its fitness, the other species have then to compensate for this adaptive advantage or else will disappear. Species that adapt will in turn modify the fitness of the others; and so forth, says Van Valen, life is in a self-sustaining perpetual movement.

Van Valen's hypothesis leads to the concept that humans and bacteria, although differing profoundly in their complexity, may be equally adapted to their environment. Indeed, microorganisms can thrive in environments where vertebrate cannot survive and adapt quickly to drastic changes in their environment.

In mutualistic interactions, one can have the impression that the arms race concept does not apply. However, as we have seen, even an interaction as ancient as that of the eukaryotic cell with mitochondria is not without discordant notes. If we add that, throughout the history of the association, the majority of genes in mitochondria (it is the same for chloroplasts) was transferred to the nucleus, this interaction – capital for evolution – can be seen as a trusteeship followed by genetic looting.

11.1.5 Weapons Specific to Microorganisms

In Red Queen-type clashes, infectious disease agents have their own weapons. One of them, particularly formidable, is their very short generation time, inherited from the early ages of life, which can lead to high production of individuals with mutated genes. In contrast, hosts of microbial parasites must compose with one major constraint that evolution has imposed onto multicellular organisms, i.e., their long generation time. Indeed, the advent of multicellularity was accompanied by increased body size, which resulted also, inevitably, in longer generation time. While the time between two divisions, thus opportunities for mutations and selection, is sometimes counted in minutes in bacteria, many multicellular organisms only acquire the ability to reproduce, and thus to mutate, after several years (somatic

Fig. 11.2 Alice and the Red Queen, illustration by John Tenniel for "Through the Looking-Glass" by Lewis Carroll. "... Alice never could quite make out, in thinking it over afterward, how it was that they began: all she remembers is, that they were running hand in hand, and the Queen went so fast that it was all she could do to keep up with her: and still the Queen kept crying 'Faster! Faster!' but Alice felt she could not go faster, though she had no breath left to say so. The most curious part of the thing was, that the trees and the other things round them never changed their places at all: however fast they went, they never seemed to pass anything. 'I wonder if all the things move along with us?'". (http://www.victorianweb.org/art/illustration/tenniel/lookingglass/2.4.html)

mutations that arise during development and postembryonic life are not transmitted and are thus useless for evolution). This issue is discussed by Ochman and Wilson (1987). They argue that replication is not the only time when mutations occur; instead these may occur at any time over the lifespan of organisms. They conclude that mutations generally take place at constant rates, independently of generation time, which of course is not always true, as, for instance, stressful situations lead to the emergence of hypermutator clones (Foster 2007).

In addition to their short generation time, another advantage enjoyed by infectious agents is their capacity to change their genetic information. Mutation rates in microorganisms can be high. In addition, viruses and bacteria are capable of altering their genomes by exchanging and incorporating foreign nucleic acid sequences, while the nuclear membrane has made such exchanges rarer in eukaryotes; evolutionists even think that one of the reasons for genetic recombination in meiosis is to maintain gene flow.

Living organisms have developed many types of interaction during evolution; the interactions of microorganisms with each other, with plants, and finally with animals will be detailed in the following text.

11.2 Interactions Between Microorganisms

Most biotopes contain many microbial taxa that coexist and interact in many ways, of which few details are known or even considered. These interactions are in most cases not specialized, based mainly on trophic aspects because resources are always limiting, but in some cases there are instances of mutualistic or parasitic relations.

11.2.1 Conflictual Interactions

Martin (2002) has classified into a few categories the known strategies of predation or parasitism: pack predation, epibiotic attachment, direct cytoplasmic invasion, and periplasmic invasion. These strategies constitute a continuum toward more and more specialized forms.

11.2.1.1 Parasitism

The best-known example of conflictual interaction involving two bacterial taxa concerns *Bdellovibrio bacteriovorus* (Fig. 11.3). This interaction is often described as predation, but corresponds rather to parasitism because it is a lasting interaction, with several cell doublings of *Bdellovibrio* while inside the periplasm of the target cell. In fact, this bacterium should more accurately be called parasitoid since it leads to the death of the host. The small deltaproteobacterium *B. bacteriovorus* consumes motile cells belonging to many Gram-negative taxa. It starts by entering the periplasm and sealing the pore entrance and then replicates in the periplasm, yielding daughter cells without flagella that will invade all the cell space. Thereafter, *Bdellovibrio* hydrolyzes cell constituents, forms filamentous cells that become septate, lyses the host cell, and releases flagellated offsprings.

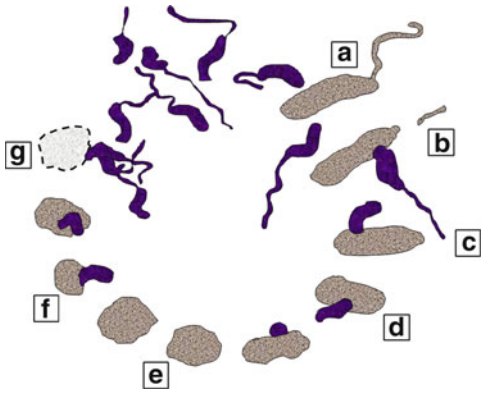


Fig. 11.3 Clockwise starting at the top, two cells of *Bdellovibrio* (in *blue*) approaching their bacterial prey (in *gray*) (**a**), one *Bdellovibrio* cell attaches to the prey (**b**) and loses its flagellum (**c**), penetrates the periplasm (**d**), multiplies in the cell (**e**) before the daughter cells emerge (**f**), leaving a lysed prey (**g**). (Redrawn from Stephan Schuster, Tübingen, Germany (scs@bx.psu.edu))

Fig. 11.4 Paris mushrooms (*Agaricus bisporus*) attacked by *Pseudomonas tolaasi* causing bacterial brown rot (bacterial blotch disease) (Photo Ph. Normand)

This way of life and the lack of attack on mammalian cells have even led some to consider the use of *Bdellovibrio* for the treatment of infections in humans (Stolp and Starr 1963). The genus was divided to yield *Bacteriovorax* to accommodate marine strains physiologically distinct and thereafter *Peredibacter starrii*, but all these genera are phylogenetically very close to each other.

In addition, there are many bacteriophages that attack bacteria and act as parasites. Finally, the mushroom *Agaricus bisporus* is parasited by *Pseudomonas tolaasi*, forming brown spots on carpophores, i.e., brown blotch disease (Fig. 11.4), and by various other parasitic bacteria and fungi.

11.2.1.2 Predation

The main microbial predators are protozoa, which regulate bacterial populations in various ecosystems. In soil, however, many bacterial taxa belonging to Actinobacteria and Proteobacteria were described as predators of other bacteria and fungi (Zeph and Casida 1986), but few studies have been carried out subsequently, except on the actinobacterium species *Agromyces ramosus* and the proteobacterial genera *Ensifer* (reclassified recently in genus *Sinorhizobium*) and *Pseudomonas*.

Soils and many other biotopes contain bacterivorous organisms belonging to different taxa. This is the case of *Caenorhabditis* nematodes and *Brachionus* rotifers.

Another type of predation, quite dramatic, involves fungi such as *Hirsutella minnesotensis* or *Arthrobotrys robusta* that catch and metabolize nematodes. Nematodes are captured using a constrictor ring or adhesive structures such as nets or buttons (http://www.edslides.nematologists.com/fungal.html), after which lytic enzymes attack the nematode (Fig. 11.5). This interaction is used to fight against nematode pests in mushroom farms, intestinal nematodes parasiting sheep (Waller and Larsen 1993), or even phytoparasitic nematodes in soil.

11.2.1.3 Antibiosis

Antibiosis is generally negative for some of the taxa sharing the same habitat as the antibiotic producer. Therefore, it is often a case of interference competition, i.e., an interaction between two species in which one of the two inhibits the development of the other and thus gains greater access to food resources in the biotope. This strategy is widespread in prokaryotes and eukaryotes and has been extensively studied since Fleming (Fleming 1922) (*cf.* Sect. 9.5), mainly because of its developments in public health. Compounds involved belong to several chemical classes ranging from simple molecules such as aminoglycosides to complex compounds such as macrolides or polypeptides, targeting several cellular functions such as protein synthesis (kanamycin) or RNA synthesis (rifampin).

Taxa known to produce antibiotics are bacteria especially soil actinobacteria and fungi, and the types of compounds produced and their mechanisms of action are described in Sect. 9.5. Many discussions have been held to determine if antibiosis was positive for the organism that synthesizes antibiotics, if not we should speak of antagonism. It is difficult to determine the cost of antibiotic synthesis, which includes the genetic burden of maintaining dozens of

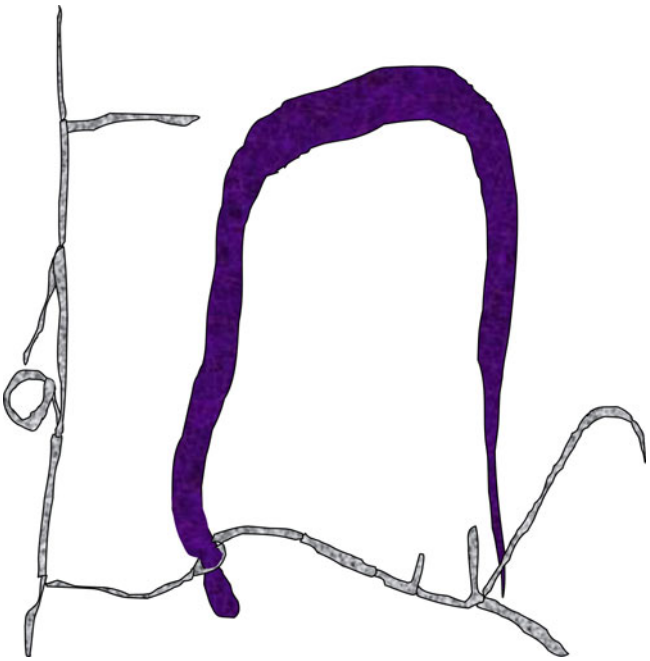


Fig. 11.5 Nematode (*in purple*) captured using a constrictor ring by the fungus *Arthrobotrys dactyloides* (*in gray*). Another ring at the left is ready to catch another nematode (Redrawn from B.A. Jaffee (plpnemweb.ucdavis.edu))

biosynthetic genes, resistance genes, and genes necessary for their transport out of the cell. The presence of an antibiotic is not necessarily detrimental for a given taxon, either because it has acquired genes permitting its degradation, and thus to feed on it, or a mutation has occurred in the genes whose product is the antibiotic target (Birge and Kurland 1969). This kind of phenomenon could in principle occur with any antibiotic.

There has been a debate on the relationship between antibiotics and bacteriocins. In contrast to antibiotics, bacteriocins affect bacteria closely related to the producing organism, often belonging to the same species. They are often of a proteinaceous nature. The mode of action is varied and includes pore formation in the membrane inducing leakage of cellular constituents, DNA or RNA degradation in the target strain, and inhibition of the production of murein in cell walls. They have been described in various bacteria especially *Escherichia coli, Vibrio, Lactococcus*, and *Pediococcus* and have a high potential in biotechnology, for example, in yogurt manufacturing. The distinction between antibiotics and bacteriocins is not always clear, because some bacteriocins have targets in distant taxa, such as *Lactobacillus* spp. against *E. coli* or *Listeria* (Millette et al. 2007), and also because antibiotics and bacteriocins are less and less defined by their modes of action.

Antibiotics have been given several definitions, initially by Waksman (1969) who used this term for any natural compound active on other organisms, to distinguish from synthetic compounds such as sulfonamides. Given the progress of synthetic chemistry that helped change natural compounds to modify their properties, they are now designated as synthetic–natural compounds. The word antibiotic now refers to compounds, natural or otherwise, with antimicrobial activity and that are used in human and veterinary medicine or in biotechnology.

Genes for antibiotic synthesis, carried on plasmids, are often exchanged by conjugation or transformation. They are especially abundant in soil organisms with large genomes such as *Streptomyces* (Bentley et al. 2002), particularly in telomeric regions subject to high recombination rates. Bacterial isolates are generally resistant rather than sensitive to antibiotics produced by isolates from the same soil (Davelos et al. 2004), which probably comes from the phenomenon of coadaptation.

It has long been argued that the synthesis of antibiotics belongs to the same type of evolutionary event as the emergence of wings, called exaptation (spandrel) by Gould and Vrba (1982) to indicate that their primary function could be quite different from the final one. The genes encoding the synthesis of antibiotics belong to secondary metabolism; that is to say, they are expressed mainly at the end of the exponential phase and later, and furthermore they are not always essential. They would therefore be genes resulting from random recombination as ORFans, initially without detectable function but that have gained one due to reactivity toward various cell constituents, and their selective advantage would have resulted in their fixation in the genome and their subsequent dispersion by lateral transfer (Daubin and Ochman 2004).

11.2.1.4 Competition

Competition* is an interaction defined as a simultaneous demand by two or more organisms for a limited environmental resource, such as a nutrient, water, living space, or light. This is of course the rule in the microbial world where many habitats include several taxa with closely related metabolic capabilities. For example, the addition to the soil of a complex carbon source like deciduous tree litter stimulates many fungi and bacteria capable of metabolizing polymers such as cellulose, hemicelluloses, and pectin. Microbial populations that grow in the soil thus vary according to the carbon sources added (Hery et al. 2005). A wastewater treatment plant is another biotope (anthropized), in which is found a rich mixture of carbon molecules inducing fluctuations of the microbial community present (Snaidr et al. 1997).

11.2.2 Beneficial Interactions

When comparing various beneficial interactions between microorganisms, it appears that the terms cooperation,

mutualism, and syntrophy have been used for conceptually similar phenomena. The term cooperation is sometimes used to designate situations encompassing the other two. Mutualism is little used to describe microbe–microbe relations, but rather is reserved for relationships between microbes and higher organisms. The term **syntrophy*** would cover just cases where microorganisms have complementary metabolisms and are in mutualistic situations, each partner providing substrate(s) to the other(s). At one extreme of the spectrum, there are situations of loose or non-exclusive relationships, based on trophic exchanges thus designated syntrophic; at the other extreme are situations of mutualism or symbiosis involving potentially more than trophic aspects of detoxification and entailing toxin synthesis, signaling, etc. It has been argued that the origin of eukaryotic cells was a syntrophy that has evolved into mutualism and finally into an obligatory interaction (López-García and Moreira 1999).

11.2.2.1 Cometabolism

Cometabolism is a prominent case of beneficial interaction taking place between different microorganisms. It corresponds to cooperation, syntrophy, or even symbiosis when metabolisms are different and complementary and when the interaction is sustained. Microorganisms that live in complex habitats such as soils, sediments, or digestive tracts are typically in contact with complex trophic resources they are unable to catabolize alone. For a single organism, cometabolism is the transformation of a compound that cannot serve as sole carbon and energy source (i.e., a non-growth substrate), which is made possible by parallel degradation of a growth substrate. We also speak of cometabolism when several microorganisms must cooperate as a consortium to synthesize all the enzymes necessary for a catabolic pathway. This is the case of soil isolates individually unable to metabolize polycyclic aromatic compounds, but which are able to do so when they are grown in a consortium (Bouchez et al. 1999). It is likely that this consortium includes strains able to compensate for the inhibition caused by a metabolic intermediate by degrading it or when the product of a strain is used as a substrate by a different strain.

11.2.2.2 Mutualism

The deltaproteobacterium *Syntrophus aciditrophicus* can metabolize various saturated or unsaturated fatty acids, hexanoate and butyrate esters, or benzoate when in coculture with microorganisms capable of metabolizing hydrogen or formate (Mouttaki et al. 2007). Anaerobic degradation of saturated fatty acids and aromatic acids in the absence of a terminal electron acceptor necessitates the presence of an organism capable of maintaining a hydrogen partial pressure low enough so that these reactions are thermodynamically possible. We therefore find it associated with hydrogenotrophic microorganisms such as *Desulfovibrio* or the archeon *Methanospirillum hungatei* (*cf.* Chap. 3 and Sect. 14.2.5). Mutualism is also found during methanogenesis which is a complex process described in detail in Chaps. 3 and 14. It involves many taxa that perform various steps in the complex pathway that transforms organic compounds into acetate and eventually into methane (*cf.* Sects. 3.3 and 14.2.5).

Sometimes the metabolic basis of the association is unknown, as in the case of the two archaea *Nanoarchaeum equitans* (Nanoarchaeota; *cf.* Chap. 6) and *Ignicoccus hospitalis* (Crenarchaeota) present in hydrothermal environments, which are in close interaction in a relationship described as symbiosis or parasitism (Jahn et al. 2008). These two organisms are closely nested into one another and cannot be cultured separately. Another interesting case concerns *Symbiobacterium thermophilum* (Firmicute) (Watsuji et al. 2006), which cannot be cultivated in the absence of a *Bacillus* sp. Both thermophilic bacteria are found in composts. *Bacillus* sp. provides *S. thermophilum* with CO_2 from its respiration, which allows *S. thermophilum* to compensate for the absence of a carbonic anhydrase, an enzyme that allows different processes such as photosynthesis, respiration, pH homeostasis, and ion transport. It also catabolizes indolic compounds, which are self-inhibitors to *S. thermophilum*. The advantage for the *Bacillus* sp. is not clear; it may simply be a situation of commensalism or catabolism of recalcitrant compounds found in the compost.

Mutualism is not restricted to catabolic functions. Sometimes, diseases of plants and animals are caused by consortia or teams consisting of very different partners. This is the case of seedling blight of rice, caused by the fungus *Rhizopus microsporus*, which contains in its cytoplasm endosymbiotic *Burkholderia* bacteria necessary for production of the virulence factor rhizotoxin and plant disease (Partida-Martinez and Hertweck 2005). The presence of *Burkholderia* within fungal tissues has been reported in many fungi.

Biofilms (*cf.* Sect. 9.7.3) found in soils, sediments, higher organisms, and man-made environments are complex assemblies that can include many taxa, some more active than others for the synthesis of exopolysaccharides. These polymers, which trap heavy metals or antibiotics, allow the development of a three-dimensional structure and protect all cells present, those synthesizing the polymers as well as the others. Microbial mats (*cf.* Sect. 9.7.3) include many microbial taxa with complementary physiological properties such as sulfate reduction and sulfate oxidation, photosynthesis, and heterotrophy. Despite the synthesis in these mats of compounds with antibiotic activity (Socha et al. 2007), the taxa present complement and protect each other during the development of microbial mats (Fourcans et al. 2006) and are therefore in situations of mutualism.

11.2.2.3 Cooperation

Trophic relations such as the ones above would correspond to cooperation if facultative. One of the main cases of cooperation between microorganisms is quorum sensing. Bacteria have mechanisms that allow them to control certain physiological functions (such as conjugation) according to cell density, which is useful to ensure success. This quorum sensing mechanism is based on synthesis and perception of signals such as *N*-acyl homoserine lactone (described in details in Sect. 9.3) and is sometimes disturbed by higher plants or algae (Teplitski et al. 2000; Bauer and Robinson 2002). Thus, it has been shown that gamma-amino butyric acid (GABA) (synthesized by plants upon wounding or infection by *Agrobacterium*) induces the catabolism of *N*-acyl homoserine lactone and thus may modulate quorum sensing (Chevrot et al. 2006). Quorum sensing may also be affected by bacteria that synthesize lactonases, especially *Bacillus thuringiensis* and *Agrobacterium tumefaciens*, a mechanism that is being evaluated for biological control of various infectious agents.

11.2.2.4 Commensalism

It is difficult to identify situations of true commensalism between microorganisms, i.e., where a taxon derives benefits from an interaction but the other derives none. This could be the case of bacteria involved in nitrification, a process that occurs in two steps. Chemolithotrophic ammonia-oxidizing archaea and bacteria convert ammonium to nitrite, while nitrite-oxidizing bacteria *Nitrobacter* and *Nitrospira* convert nitrite into nitrate. Nitrite oxidizers obviously depend on the provision of nitrite by ammonia oxidizers, while the benefit for the latter is less obvious. However, nitrite is toxic for many taxa, including certain ammonia oxidizers, which would then benefit from nitrite removal. The interaction involved would rather be a cooperation in this case.

In many habitats, an element is limiting for bacterial growth and taxa with access to this element will be at the base of a food chain. This is the case of cyanobacteria capable of fixing carbon dioxide through photosynthesis, which are trophic partners of marine bacteria particularly those of the microbial loop (*cf.* Chap. 13). This is also the case for nitrogen-fixing bacteria in the rhizosphere and soils.

There are other examples of commensalism, in particular, *E. coli* that is optionally aerobic, consumes oxygen, and renders the intestinal tract anaerobic and therefore suitable for *Bacteroides*, a strict anaerobe. Similarly, bacteria involved in milk souring have a fermentative metabolism and release acidic compounds that acidify the medium and provide favorable growth conditions for acid-tolerant lactic acid bacteria.

Plants and microorganisms also synthesize and secrete compounds that are toxic to many microorganisms. In the same vein, many toxic compounds from human activity are found in the environment, where they selectively inhibit microbial taxa. Various microorganisms may degrade them, as illustrated with olive wastes metabolized by composting (Zenjari et al. 2006) or pentachlorophenol used as a wood treatment that is degraded by *Sphingobium* (Dams et al. 2007). These biodegrading microorganisms enable or enhance the growth of others.

11.2.2.5 Horizontal Gene Transfer

Horizontal gene transfer (*cf.* Chap. 12) may be mutually beneficial and would fall within the framework of cooperation but is not easy to qualify in terms of ecological interaction. The best-known case is that of antibiotic treatment in a medium comprising more than one microbial taxon, where initially most bacteria are sensitive to a compound to which they have never been in contact. Mutants resistant to this antibiotic will proliferate. Finally, the DNA fragment conferring resistance to the antibiotic may be transferred to sensitive cells of the same taxon or belonging to a remote taxon. One of the first described cases of transfer of resistance genes took place in Birmingham, England, in 1960. A care unit for burn victims found waves of nosocomial infections starting initially with *Klebsiella aerogenes* resistant to carbenicillin and carrying a resistance plasmid (RK2), followed by a second wave of *Pseudomonas aeruginosa* also carrying a closely related resistance plasmid. It has been shown that this phenomenon involved conjugative plasmid transfer (Ingram et al. 1973). Such cases of transfer of antibiotic resistance genes are common nowadays; they threaten our ability to curb infections and are one of the public health problems of greatest concern (Fig. 11.6).

Another case of gene transfer following a massive chemical selection pressure was found regarding mercury, a metal present in the bedrock and abundant in many natural or man-made habitats such as hydroelectric reservoirs or gold panning workshops. It was shown that *mer* genes carried by conjugative plasmids or transposons and conferring the ability to volatilize mercury were transferred between phylogenetically distant bacteria by conjugation (Mindlin et al. 2002). A similar phenomenon has also been observed with man-made compounds such as atrazine, currently the most widely used herbicide in the USA (*cf.* Sect. 16.7.2). When the herbicide is added to a field and ends up in the soil, it induces massive transfer to the soil microbiota of a plasmid carrying genes *atz* and *trz* for atrazine degradation (Devers et al. 2005). *atz* and *trz* are often on plasmids, but also on the chromosome near insertion sequences, suggesting that transposition plays an important role in the dispersion of this metabolic competence (*cf.* Sect. 16.7.2).

Maintaining large plasmids is not neutral in terms of fitness if they do not carry essential genes, which is reflected in the existence of strains having lost their plasmids in the soil, as is the case for many bacteria in particular

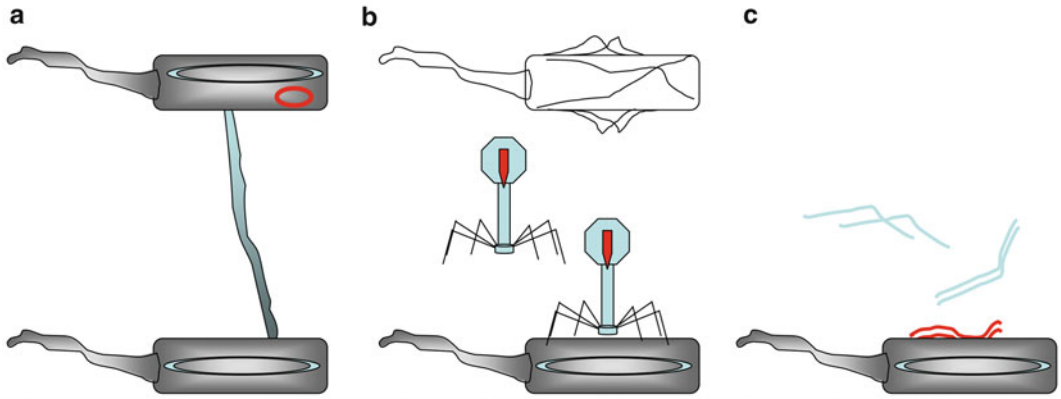


Fig. 11.6 (**a**) Bacterial conjugation between a donor cell (*upper*) and a recipient cell (*below*) by means of a conjugative pilus (*medium*) within which DNA (*red*) is transferred is transferred and will remain independent (plasmid) or integrate by recombination into the chromosome of the recipient cell. (**b**) Transduction between lysed bacterial cell (*above*), which has released a phage (*medium*) containing a bacterial gene (*red*) that will be integrated into the genome of the infected cell by the phage (*low*). (**c**) Transformation of a bacterial cell (*bottom*) by the naked DNA (*top*); bacteria that can be transformed with naked DNA are called competent. Two other processes of horizontal gene transfer have been proposed, i.e., via intercellular nanotubes and phage-like gene transfer agents (GTA) (Redrawn from Popa and Dagan 2011)

Agrobacterium (Krimi et al. 2002). It was shown that the presence of a host plant may increase the rate of conjugative transfer, as in *Sinorhizobium loti*, and give rise to many strains able to nodulate (Sullivan et al. 1995).

11.3 Interactions Between Plants and Microorganisms

11.3.1 Introduction

Plants play a major ecological role as the main primary producers of organic matter in terrestrial ecosystems. Part of this organic material is available for plant-associated microorganisms. The latter are present within the plant, at the surface of the plant (roots or shoots), or in the immediate vicinity of the roots, i.e., in rhizosphere (the soil under the direct influence of living roots) (Fig. 11.7). Indeed, plants release a fraction of photosynthates in the form of exudates or more generally (in the case of roots) rhizodeposits (Nguyen 2003). The availability of these organic nutrients stimulates plant-associated microorganisms, resulting in increased population size and physiological activity (Garbeva et al. 2004; Bais et al. 2006). In aquatic ecosystems, algae and other photosynthetic eukaryotes play a similar role in terms of primary production, but the relationship between algae and microorganisms is poorly documented, and these interactions are not considered in this chapter.

In return, the microorganisms associated with the plant will have a significant impact on the nutrition, development, growth, and health of the plant partner. Some of these microorganisms especially pathogens (parasites) have a negative impact on the plant. Conversely, others will have direct phytobeneficial effects, noticeably those in symbiosis (Mathesius 2003; Brundrett 2009) or cooperation with the plant (Dobbelaere et al. 2003; Mantelin et al. 2006). Indirect phytobeneficial effects are also possible, in particular, for microorganisms competing with or antagonistic toward phytoparasites (Alabouvette et al. 2006; Raaijmakers et al. 2009). Therefore, microorganisms associated with plants have considerable practical significance, which explains why historically they have often been studied to address concerns in the fields of agronomy and phytopathology. In this context, newer issues pertaining to food quality, environmental health, and global climate change are receiving increased research attention, along with long-standing issues about plant nutrition and health and soil fertility (van Elsas et al. 2007).

11.3.2 Location and Population Levels of Microorganisms Associated with Plants

Microorganisms associated with plants are mainly bacteria and fungi, as well as protozoa to a lesser extent. Depending on their location on/in plants, these microorganisms will be exposed to contrasting environmental conditions. A difference can be made between the root system and aerial parts. Microorganisms at the surface of aerial parts are confronted to fluctuating microclimatic situations, and they may be exposed to stressful conditions in particular with regard to desiccation and ultraviolet radiations (Kinkel et al. 2000). In addition, organic exudates are released in smaller quantities by leaves than by roots (Lucas and Sarniguet 1998; Leveau

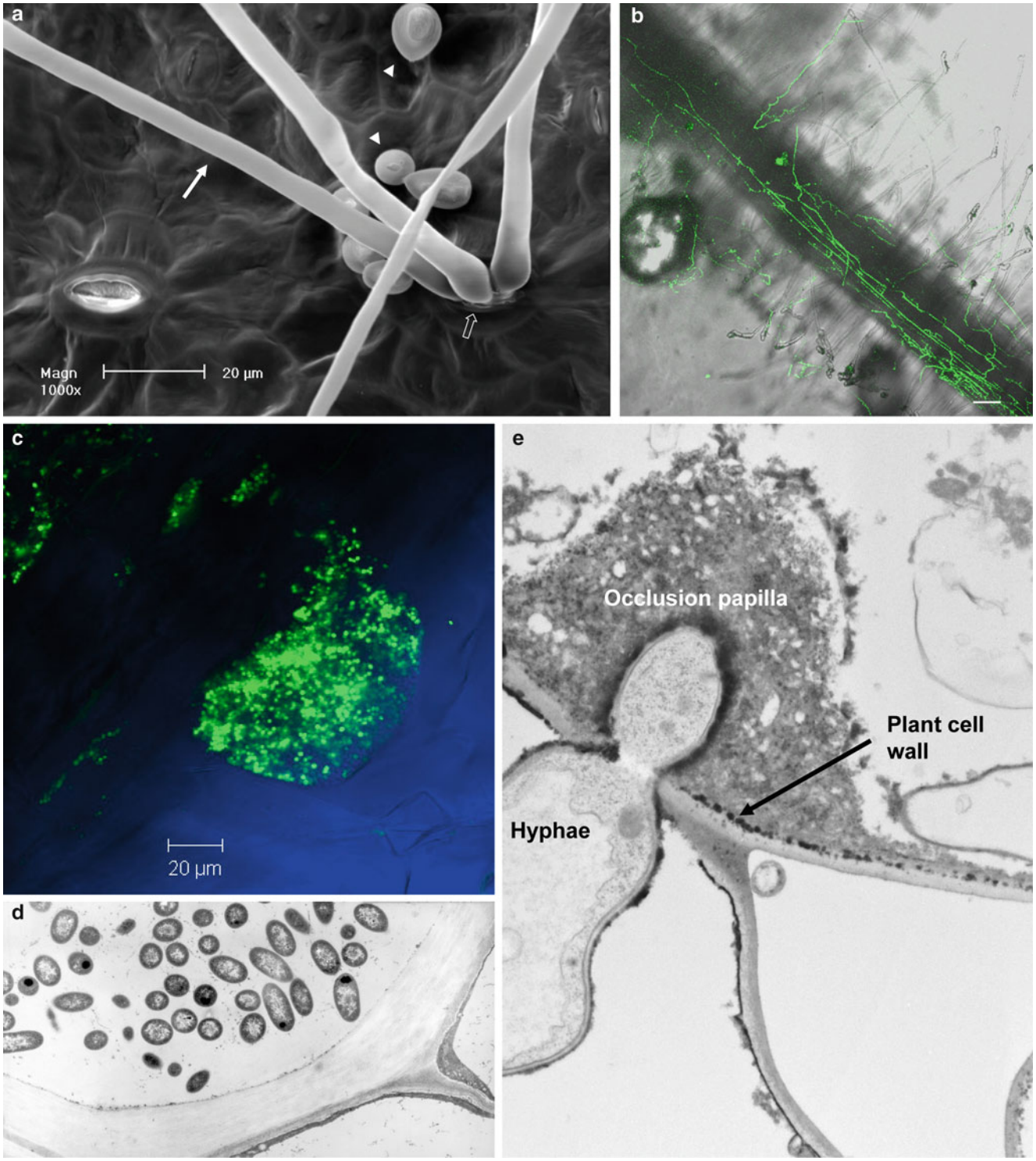


Fig. 11.7 Microorganisms associated with plants (Photos by electron microscopy). (**a**) The downy mildew agent *Plasmopara viticola* on the surface of a grapevine leaf. The oomycete developed within the leaf tissues and initiated its sporulation phase by generating sporangiophores (*solid arrow*) emerging from stomata (*open arrow*). These sporangiophores produce many sporangia (*arrowheads*), which contribute to parasite spread and buildup of a secondary inoculum source (Photo S. Trouvelot, UMR INRA 1347 Agroécologie, Dijon, France). (**b**) Nonpathogenic *Fusarium oxysporum* strain Fo47 in the root hair zone of a tomato root in a cultivated soil previously inoculated with the fungus. The strain was labeled by transformation, using a gene encoding a green fluorescent protein (GFP), and the sample was observed by confocal laser microscopy. Scale bar = 100 µm (Photo C. Humbert, UMR INRA 1347 Agroécologie, Dijon, France). (**c**) Microcolonies formed by the PGPR bacterium *Azospirillum brasilense* Sp245 on wheat root. The strain was labeled using the *egfp* gene, and the sample was observed by confocal laser microscopy (Photo C. Prigent-Combaret, UMR CNRS 5557 Microbial Ecology, Villeurbanne, France). (**d**) Pathogenic bacterium *Xanthomonas campestris* pv. *manihotis* in a cassava stem (×22,000) (Photo B. Boher, UR IRD 075 Resistance des Plantes, Montpellier, France). (**e**) Pathogenic fungus *Fusarium oxysporum* f. sp. *lycopersici* in tomato root tissues. The growth of the fungus is inhibited by the defense reactions of the plant. This involves parietal deposits of callose and phenolics, which form an occlusion papilla. Scale bar = 1 µm (Photo C. Olivain, UMR INRA 1347 Agroécologie, Dijon, France)

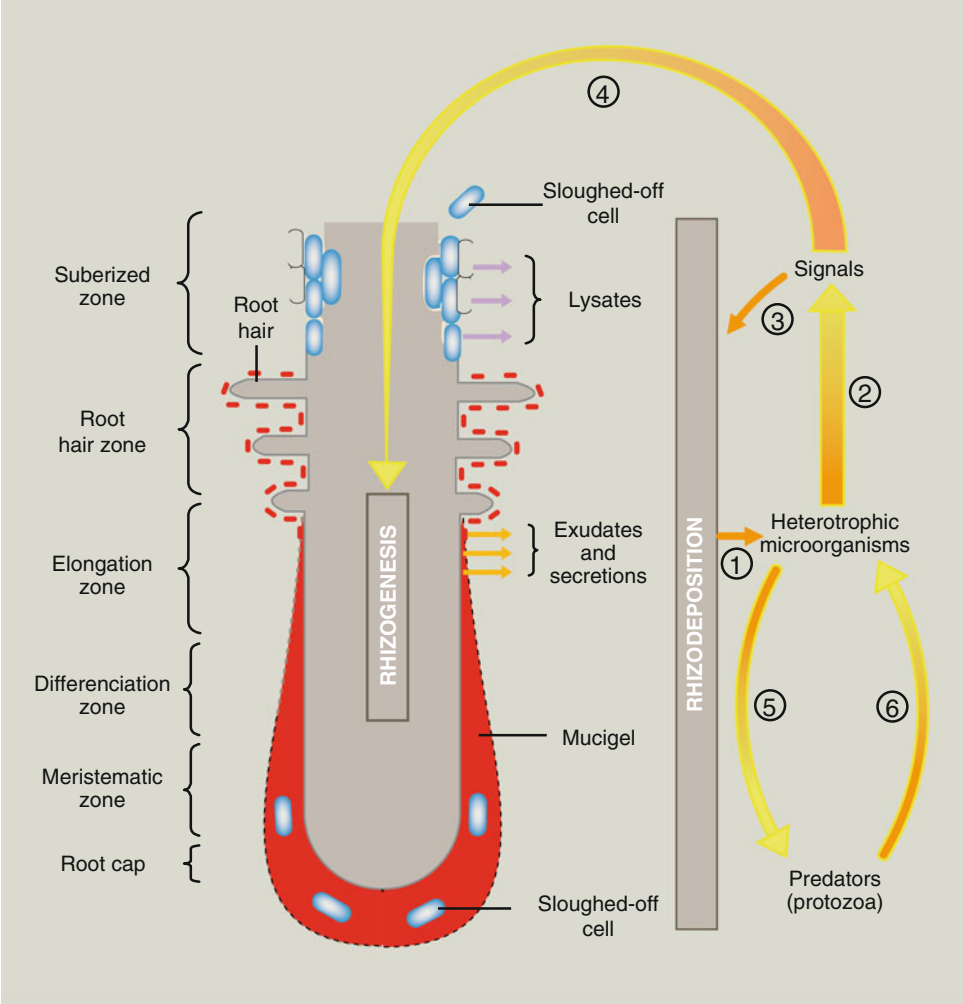


Fig. 11.8 Rhizodeposition and role of multitrophic interactions in the rhizosphere. Rhizodeposits correspond to exudates/diffusates, secretions, lysates, and the fraction of the mucigel that is of plant origin. They stimulate the growth of heterotrophic microorganisms in the rhizosphere (*1*) and their production of molecular signals (*2*), such as indole-3-acetic acid (IAA) and 2,4-diacetylphloroglucinol. These microbial metabolites stimulate rhizodeposition (*3*), as well as root system branching and root growth (*4*), and the latter effects on rhizogenesis stimulate rhizodeposition even further. The proliferation of rhizosphere microorganisms also stimulates predators, including protozoa (*5*). Selective predation and ammonia excretion by protozoa favor some of the rhizosphere microorganisms (*6*), including IAA producers and nitrifying bacteria, further stimulating rhizogenesis and rhizodeposition and so on (Inspired from Bonkowski 2004. Drawing: M.-J. Bodiou)

and Lindow 2001). Microorganisms colonizing aboveground plant surfaces are called epiphytes. Microbial numbers on leaves depend on the plant species, with densities in the order of $10^{5–7}$ bacteria per cm^2 (i.e., $10^{5–8}$ per g fresh weight) and 10^4 fungal propagules per cm^2 (Kinkel et al. 2000; Lindow and Brandl 2003). The stems can also be colonized by microorganisms, but at lower levels. It is especially documented for nitrogen-fixing bacteria, such as photosynthetic *Bradyrhizobium* that induce stem nodules on *Aeschynomene* (Wong et al. 1994) and *Frankia* on *Casuarina equisetifolia* (Prin et al. 1991).

In contrast, microorganisms present on the surface of roots (i.e., at the rhizoplane) and in rhizosphere soil have access to significant amounts of organic rhizodeposits (Nguyen 2003) (Fig. 11.8), and their habitat is buffered (Lucas and Sarniguet 1998) by the presence of the soil (porous solid phase) and the root mucigel (a gelatinous layer on the root surface that is composed of plant and microbial polysaccharides). They can reach high numbers, in the order of $10^{8–9}$ bacteria per g and 100–150 cm fungal hyphae per cm^2 root surface (Cavagnaro et al. 2005). These numbers are modulated by the amount of available organic substrates, which are mainly released by growing roots during the vegetative growth of the plant (Nguyen 2003) and by deleterious interactions (including competition between microorganisms and predation by protozoa). Microbial populations well adapted to the rhizosphere produce compounds (such as phytohormones, nitric oxide, or

2,4-diacetylphloroglucinol) that stimulate root growth and/or exudation, thereby amplifying the rhizosphere effect (Bonkowski 2004) (Fig. 11.8).

There are also differences according to the microbial habitat within the phytosphere, in particular at the scale of the root system. The quantitative importance of root systems varies depending on pedoclimatic and plant community properties, with root densities up to 100–200 cm roots per cm^3 of soil and roots occupying typically 1–5 % of the soil volume. However, most exchanges between the plant and the soil, both in terms of nutrients/water acquisition and rhizodeposition, occur at root tips (Cardon and Whitbeck 2007). Rhizosphere microbial populations are thus higher in the root elongation and root hair zones compared with the root cap (Cardon and Whitbeck 2007). Cell differentiation is accompanied by a decrease in the permeability of plant cell walls, and therefore older root parts are rather involved in sap transport. In the case of aerial parts, microbial numbers depend on leaf position and age, and they are often higher on the lower side of leaves (Kinkel et al. 2000). Whether on roots or leaves, microbial growth is stimulated following lysis of plant cells resulting from attacks by phytoparasites.

Microorganisms on plant surfaces are often found as microcolonies (sometimes with a cell density of over 10^4 bacteria per microcolony) and biofilms (Wimpenny and Colasanti 1997; Ramey et al. 2004). This may concern up to 80 % of the bacterial populations on the surface of leaves (Morris and Monier 2003). Biofilms occur particularly at the junction of epidermal cells, at wounds, and (for aerial parts) at stomata, hydathodes, and along veins (Beattie and Lindow 1999). Biofilm distribution becomes more erratic on older leaves (Leben 1988) and root tissues (Lübeck et al. 2000). Biofilms, which include the mucigel matrix in the case of roots, can be several tens of micrometers thick (Fig. 11.7c). Their buildup reflects the availability of nutrients (Leveau and Lindow 2001), and by comparison with other types of environments, their development level is intermediate (phyllosphere) to high (rhizosphere) (Wimpenny and Colasanti 1997).

Finally, a difference can be made between the plant surface and the interior of the plant, which can also be colonized by microorganisms (Gamboa et al. 2003). Microorganisms present in the plant are found in the cortex, more rarely in the stele (vascular cylinder). They often have intercellular localization, but some (often parasites or symbionts) may be within plant cells (Fig. 11.7). In the case of fungi, the mycelium is either entirely in the plant or only partly within the plant, as in the case of ectomycorrhizal fungi (Brundrett 2009). It should be noted that certain mycorrhizal fungi harbor bacteria, forming multitrophic associations (Bertaux et al. 2003). Microorganisms present in the plant but that do not lead to physiological dysfunctions (as pathogens do) or host differentiation (as bacterial symbionts nodulating roots do) are termed endophytes. The plant tissues provide particular environmental conditions, inasmuch as microorganisms have direct access to available substrates in the plant, while being directly exposed to defense mechanisms of the host. The microbial numbers in the plant are generally low ($10^{2–5}$ bacteria per g), except for some endophytic bacteria (up to 10^8 per g) and especially symbionts and parasites, when they are engaged in a successful interaction with the host (McInroy and Kloepper 1995; Gamboa et al. 2003).

11.3.3 Sources of Microorganisms Associated with Plants

Microorganisms interacting with plants have various provenances. First, many originate from soil. They can find themselves in contact with plants if they are present in an area of the soil that is penetrated by a root. However, many terrestrial microorganisms have the ability to actively come into contact with seeds or roots, through a process of chemotaxis (for bacteria) or chemotropism (for fungi) triggered by the perception of plant exudates (Bais et al. 2006). Terrestrial microorganisms in contact with plants will mostly be associated to the root system, but some may colonize plant shoots or fruits. This situation is mainly documented for pathogens, including fungi such as *Fusarium* spp. (Deuteromycetes) that produce mycotoxins (Leplat et al. 2013).

Second, certain microorganisms that interact with plants come in contact with the plant through precipitation and irrigation water, the fall of atmospheric dust, or wind (Agrios 1997; Morris et al. 2010). Precipitations also lead to runoff events along the aerial parts of the plants and the soil surface. This runoff will lead to a redistribution of some phyllospheric microorganisms. The presence of microorganisms in the atmosphere can be explained by the formation of aerosols, i.e., microdroplets, which originate from liquid phases that are turbulent or that face convection, e.g., by wind. This presence can also be explained by the transportation, caused by wind or air currents, of microorganisms or microparticles colonized by microorganisms, including for resting stages such as many fungal spores (Savage et al. 2012).

Third, animals can act as carriers of microorganisms. This will particularly involve phytophagous arthropods (especially insects) and herbivorous mammals, which will inoculate the plant with their mouthparts, as well as parasitic animals through their stylet or spear (nematodes) or rostrum (biting insects) (Villate et al. 2012). However, the mere contact between the animal (or its feces) and the plant can also allow plant contamination by microorganisms from the surface of the animal (or its digestive system), whether microarthropods or livestock.

Fourth, importing seeds, seedlings, and plants from remote areas brings microorganisms that interact with plants. Unintentional deliveries can also occur through the use of agricultural tools or machinery (farming practices), while some phytobeneficial microorganisms are deliberately introduced to inoculate crops (Agrios 1997; Dobbelaere et al. 2001; Alabouvette et al. 2006).

Fifth, the plant itself can be a source of microorganisms. Indeed, the microorganisms present on living plants or plant remnants (litter, crop residues) are a source of inoculum for plants that will later develop in the same place. For example, mycotoxin-producing *Fusarium* can survive in overwintered corn stalk residues and may colonize wheat in the following growing season (Leplat et al. 2013). In addition, certain microorganisms are present in the seed, whether as internal or external contamination. These microorganisms are in a favorable position to colonize plant seedlings emerging from these seed. Finally, endophytic microorganisms can be found in plants of the next generation if these plants are propagated vegetatively, as with sugarcane cuttings.

In the end, microorganisms associated with plants have contrasting sources, and they have undergone dissemination phenomena at different spatial scales. In terms of biogeography, even though many of them have an endemic distribution, some microorganisms appear to be transported over long distances (Ramette and Tiedje 2007). This is particularly the case of microorganisms associated with cultivated plants, which colonize plant shoots and/or are easily spread by wind, and some of them are plant pathogens.

11.3.4 Diversity and Activity of Microorganisms Associated with Plants

11.3.4.1 Ecological Factors

Due to high microbial numbers and the importance of microbial interactions in the rhizosphere, the ecological specificities of this microbial habitat are summarized below. In comparison with non-rhizosphere soil, the rhizosphere has specific physical and (bio) chemical features. From a physical point of view, the root surface is not homogeneous in terms of topography and rhizodeposit flux, noticeably with the occurrence of a groove and the preferential release of exudates at the junction of epidermal cells (Beattie and Lindow 1999). The level of hydrophobicity of the root surface (and thus its contact properties) may vary depending on the plant considered. Root growth results in the creation of a root-based soil macroporosity, which leads to compaction of the rhizospheric soil. It is also in the rhizosphere that diffusion of soluble rhizodeposits occurs, resulting in stimulation of microbial populations. Insoluble rhizodeposits render the mucigel gelatinous, which facilitates adhesion between root and soil particles. The mucigel also affects water movement in the rhizosphere, thereby modulating the availability of water to roots.

From a chemical point of view, the properties of the rhizosphere are largely related to the presence of rhizodeposits (formerly referred to as root exudates). Depending on the mode of release of rhizodeposits in the soil (Fig. 11.8), we distinguish exudates (in the modern sense of the term) or diffusates (soluble or gaseous low-molecular-weight compounds released by passive transport), secretions (compounds actively transported out of the root cells), cell lysates (compounds released during the lysis of epidermal cells, cortical cells, or senescent sloughed-off cells detached from the root cap), and the mucigel (plant polysaccharides, to which microbial exopolysaccharides can be added) (Nguyen 2003). Quantitatively, the rhizodeposits represent 15–20 % of the net organic carbon from photosynthesis, i.e., about half the organic carbon translocated to roots (Nguyen 2003). The organic rhizodeposits have a high functional and chemical diversity. They include the following:

1. Simple substrates, namely, a very large number of amino acids, organic acids, sugars, fatty acids, sterols, nucleic acid derivatives, etc.
2. Insoluble polymers (cellulose and other carbohydrates)
3. Vitamins and other growth factors (biotin, inositol, thiamine, etc.)
4. Phytohormones (auxins, cytokinins, etc.)
5. Enzymatic proteins (amylases, phosphatases, proteases, etc.)
6. Toxins (e.g., calystegines) and defense compounds (such as phytoalexins and glucosinolates)
7. Signals acting on microorganisms as chemoattractants (sugars, organic acids, etc.) and/or transcription inducers (e.g., flavonoids), etc. (Bais et al. 2006).

The rhizosphere exhibits other chemical particularities, which are also related to root functioning. Plant nutrition requires selective uptake of mineral nutrients by roots, which leads to accumulation and sometimes also precipitation (e.g., calcium carbonate or iron oxide) of excess constituents and especially depletion for metabolized nutrients (to a larger extent for soluble anions such as nitrate than for poorly soluble anions such as phosphate or for cations) in the rhizosphere. Cations and anions uptake involves parallel excretion of proton or hydroxide anion, respectively, which can lead to local pH change in the rhizosphere (Hinsinger et al. 2003). Finally, root (and microbial) respiration may lead to a decrease in oxygen concentration, with a parallel increase in carbon dioxide content, in particular, when soil is very wet. However, these processes can be offset by the ease of gas exchange in the root macroporosity. This is particularly the case in soils containing swelling clays, which retract during drying. It can be noted that certain plants that thrive in submerged

conditions (e.g., rice) can release oxygen through their roots (Liesack et al. 2000). Depending on these factors, the availability of oxygen in the root zone will be lower (most often) or higher than in non-rhizosphere soil.

11.3.4.2 Microbial Diversity

To colonize plants, a microorganism must not only use the trophic resources they provide (in a context of competition with the rest of the microbial community) but also tolerate abiotic stress conditions (including desiccation and UV in the phyllosphere) as well as toxins and other host defense compounds. The best adapted microorganisms will be thus favored over the others. This selection process is amplified by the molecular dialogues with the plant, including microbial chemotaxis/chemotropism phenomena (*cf.* Sect. 9.2) and the ability of certain plants to interfere with bacterial quorum sensing (Bauer and Robinson 2002). Finally, all terrestrial microorganisms are not affected the same way by the antimicrobial compounds produced by the other microorganisms associated with plant (Gilbert et al. 1993; Raaijmakers et al. 2009) and by predation by protozoa (Bonkowski 2004) and nematodes (Jousset et al. 2009). In total, the trophic stimulation of microbial growth by the plant is therefore selective. This usually results in a lower microbial diversity when comparing bulk soil with rhizosphere soil or the surface with the inside of the plant. However, the species diversity of plant-associated microorganisms is important, and many bacterial and fungal taxa have been identified on leaves or roots (Lindow and Brandl 2003; Morris and Monier 2003; Garbeva et al. 2004).

Selection of microorganisms by plants can be observed at different taxonomic levels, from the family to the strain (Mavingui et al. 1992; Lindow and Brandl 2003; Garbeva et al. 2004; Cardon and Whitbeck 2007). At the lowest taxonomic levels, especially the intraspecies level, horizontal gene transfer can influence microbial selection. Indeed, plants favor horizontal gene transfer events, including the exchange of conjugative plasmids between bacteria (Bjorklof et al. 2000). Many abiotic and biotic factors modulate microbial selection by the plant, mainly through an effect on plant photosynthetic activity (and hence on exudation). This can be reflected by the occurrence of microbial successions in the rhizosphere, which can be related to host phenology (Garbeva et al. 2004; Mougel et al. 2006). Plant selection processes are also scale dependent. Since both the soil habitat and plant organization/physiology display spatiotemporal heterogeneity, different parts of a same individual plant may display different microbial communities, e.g., from one root microsite to another (Garbeva et al. 2004).

In the rhizosphere, the reduction of microbial diversity resulting from plant selection may be partly compensated by other processes that enhance genetic variability of microorganisms. First, it is believed that certain constituents released by the plant, particularly the seed, may have mutagenic effects (Miché et al. 2003). Second, certain bacteria display phenotypic variation (also termed phase variation), a process often reversible that generates at high frequency several different cell types (e.g., with different mobility patterns) from a single strain (Vial et al. 2006). Phenotypic variation facilitates root colonization of symbiotic and pathogenic bacteria that have this property (Achouak et al. 2004).

11.3.4.3 Microbial Activities

For microorganisms adapted to plants, trophic stimulation by exudates leads to an increase in the rates of cell physiological activities, the synthesis of many metabolites (including microbial exopolysaccharides of root mucigel), the rate of proliferation, and (for bacteria with a viable nonculturable state) the culturability level of cells (Bjorklof et al. 2000; Lübeck et al. 2000; Bais et al. 2006; Troxler et al. 2012). This trophic stimulation results in an increase in overall physiological activity of the microbial community (van Elsas et al. 2007), for example, in terms of carbon dioxide production (Griffiths et al. 2004). When considering a particular microbial function, the plant may have an impact on the size of the corresponding functional group (by a factor of 100 or more for rhizosphere ammonifiers) and/or the implementation level of the function (Cardon and Whitbeck 2007; Patra et al. 2007). This impact varies according to the stage of development of the plant, the age of the root or leaf, environmental factors influencing photosynthesis (which also impacts on microbial numbers and diversity), and the microbial function considered.

Generally, microbial transformations involving heterotrophic/saprophytic microorganisms are strongly stimulated by plants (Cardon and Whitbeck 2007). This is the case for many biodegradation functions for which the substrate comes exclusively/predominantly from plant exudates (Griffiths et al. 2004), as in the case of calystegines (Guntli et al. 1999) and opines, or both from exudates and soil organic matter (organic nitrogen compounds, cellulose, etc.). Substrates not of plant origin such as exogenous aromatic compounds degraded by cometabolism are transformed faster in the rhizosphere (Cardon and Whitbeck 2007). Plants also favor nitrogen fixation (whose energy cost is high) by nonsymbiotic aerobic bacteria (Patra et al. 2007). Conversely, transformations carried out by autotrophic microorganisms (e.g., nitrification) are little affected by the presence of the plant (Patra et al. 2006). Finally, there are transformations that are either stimulated or inhibited by the plant depending on the availability of oxygen and overall redox conditions in the rhizosphere. This is mainly the case of fermentations and anaerobic respirations, e.g., denitrification (Patra et al. 2006), which are favored by the presence of organic rhizodeposits but also require specific redox conditions partly determined by soil structure and soil

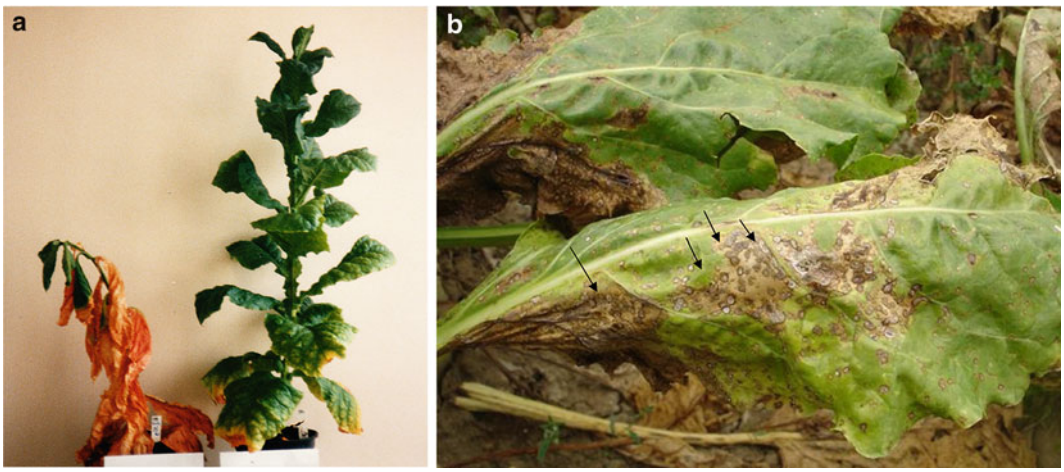


Fig. 11.9 Symptoms of plant disease. (**a**) Bacterial wilt of tobacco plant caused by the bacterium *Ralstonia solanacearum* (*left*). The bacterium colonizes spiral protoxylem vessels, which leads to vessel clogging and stops sap flow (Photo R. Pépin, UMR CNRS 5557 Microbial Ecology, Villeurbanne, France). (**b**) Leaf coalescent necrosis (*arrows*) on beet due to the epiphytic fungus *Cercospora beticola* (Deuteromycete) (Photo C. Steinberg)

moisture (Liesack et al. 2000). Redox conditions also modulate aerobic transformations including nitrification (Bohrerova et al. 2004).

Besides microbial transformations, most functions related to biotic interactions are also stimulated in the presence of plants. Most of them involve the plant directly, whether they are favorable (cooperation and symbiosis), unfavorable (competition, antagonism, and parasitism), or without effect on the plant (commensalism). Interactions not directly involving the plant can also be stimulated. These are positive interactions such as gene transfer (Bjorklof et al. 2000) or quorum sensing (a mechanism involving signals such as *N*-acyl homoserine lactones and used to perceive microenvironmental changes; Hense et al. 2007) and negative interactions such as predation of bacteria by protozoa (Bonkowski 2004). It also applies to interactions between microorganisms and soil fauna, for instance, the antagonism of certain bacteria toward plant-parasitic nematodes (Dabiré et al. 2005). Overall, the stimulation of biotic interactions by the plant often has a positive impact on the latter, except when phytoparasites are favored.

11.3.5 Biotic Interactions of Microorganisms Associated with Plants

11.3.5.1 Overview

The plant is exposed to high numbers of microorganisms, but most of them probably have little effects on the plant if any. Phytobeneficial microorganisms are much less prevalent comparatively. Cooperating microorganisms predominate over symbiotic ones on plant surfaces or in the rhizosphere, but this is less significant or even the opposite within the plant. Plant-parasitic microorganisms are a minority, except sometimes in infected plants, but their impact on the latter can be high. It is important to note that there is a continuum of plant–microbe interactions, ranging from mutualism to parasitism, and that the implementation of many plant–microbe interactions is highly dependent on plant genotype, interactions with other members of the microbial community, and environmental conditions.

11.3.5.2 Parasitism

Plant Parasites

Most parasitic diseases of plants (Fig. 11.9) are caused by fungi or oomycetes with the remainder due to bacteria or viruses (Agrios 1997) (Box 11.2). Phytopathogenic fungi and oomycetes are often facultative parasites called necrotrophic, whose saprophytic and parasitic phases alternate. They are responsible for penetrating injuries and necroses on a broad host range. Others are obligate parasites (biotrophic) developing only in living host plant. They require specialized bodies intrusion (**appressorium*** and **haustorium***), their host range is narrow, and often they cause different types of symptoms. The majority of phytopathogenic fungi and oomycetes affect the aerial parts of plants, causing diseases such as downy mildew (caused by oomycetes *Phytophthora parasitica* on tomato or *Plasmopara viticola* on vines), powdery mildew (*Erysiphe graminis* on herbaceous or *Uncinula necator* on vines), corn smut (*Ustilago maydis* on corn), rust (*Puccinia* spp. on cereals and *Uromyces* spp. on Fabaceae), or rot (*Botrytis cinerea* on grapevine) (Agrios 1997; Heitman 2011). In some cases, the disease symptoms are subtle, like ergot (*Claviceps purpurea*) or head blight of cereals (*Fusarium* spp.) because the phytoparasite produces mycotoxins such as

lysergic acid [*C. purpurea* (Lorenz et al. 2007)]; trichothecenes, fumonisins, and zearalenone [*Fusarium* spp. (Osborne and Stein 2007)]; and ochratoxins and aflatoxins [(*Penicillium* spp. and *Aspergillus* spp. (Berthiller et al. 2013)]. These mycotoxigenic fungi are present all around the world and cause great losses to the world's agriculture. The disease caused by these molds reduces seed vigor, crop yield, and grain quality, making them poisonous to animals and humans. Mycotoxins are produced in the cereals, fruits, as well as in vegetables, in the field, and during transportation, storage, and processing. The problem of mycotoxins fluctuates from year to year, due to changes in the environmental conditions favorable for the production of mycotoxins and the development of the producing fungi. The Food and Agriculture Organization (FAO) of the United Nations and the European Union have set limits for the most important mycotoxins in different crops, to avoid the adverse effects toward animals and humans.

Box 11.2: Impact of Parasitic Microorganisms on Crop Plants

Christian Steinberg

Diseases caused by parasitic microorganisms on crops can result in food shortages and famines, and historically some of them have had a major impact on human populations (Rosenzweig et al. 2000). Late blight of potato, caused by the oomycete *Phytophthora infestans*, was a major factor in the great famine in Ireland (more than one million deaths out of a population of eight million inhabitants) and Irish emigration to the USA (two million emigrants) in the years 1845–1849. In more recent times (1943), the fungus *Helminthosporium oryzae* (Deuteromycete) resulted in the death of more than two million people in India and Bangladesh due to malnutrition caused by the destruction of rice crops.

The importance of crop losses due to diseases varies according to the type of pathogen, pathogen inoculum size, the development stage, and the genotype of the plant, as well as soil properties, climatic factors, and agronomic conditions (Agrios 1997). For the present time, the estimation of Oerke et al. (1995) based on eight major crops is 42 % of the yield (before harvest) is lost due to biotic factors across the globe (31 % for North America). Plant-parasitic microorganisms are responsible for a third of these losses, the rest being caused by insects and weeds. Loss due to diseases ranges from 28 % (for corn, soybean, and tobacco) to 40 % (potato) of all losses of biotic origin. Each year, yield loss due to diseases (before harvest) exceeds $15 billion for potato, $20 billion for wheat, and $50 billion for rice for the whole world (2008 estimate).

Currently, the extent of these damages increases mainly due to the development of trade in seeds and agricultural products and changes in farming methods, such as agriculture intensification and a switch from crop rotation to crop monoculture (Rosenzweig et al. 2000). The ongoing climate change will likely have major repercussions on crops and diseases affecting them.

Soil-borne fungi and oomycetes that infect the seed or roots cause important diseases. Damping-off is generally caused by oomycetes such as *Aphanomyces cochlioides* on sugar beet and more particularly *Pythium* spp. on most crops, but fungi such as *Rhizoctonia solani* can also cause damage in vegetables or forest nurseries. Conversely, necroses and root and crown rots are mainly due to fungi (*Thielaviopsis basicola, R. solani*), although oomycetes such as *A. euteiches* are a real obstacle to grow legumes in temperate countries (Persson et al. 1999). White mold of many vegetable crops is caused by *Sclerotinia sclerotiorium* and leads to general wilting symptoms associated with water-soaked lesions on stems, while sclerotia are formed inside stems. Other fungi such as *Fusarium oxysporum* and *Verticillium dahlia* also cause partial or general wilting, but they are tracheomycoses because the fungi are able to penetrate the roots to rapidly reach and colonize the vascular tissues (Olivain et al. 2006).

Many foliar bacteria are phytopathogenic, such as *Erwinia amylovora* (fire blight pathogen of pear and apple), *Xanthomonas campestris* (which causes stalk lesions and necrosis), or *Pseudomonas syringae* (causing cankers in many fruit trees or brown necrotic spots on the glumes of wheat) (Agrios 1997). Part of *P. syringae* epiphytes produce IceC membrane protein, which has an ice nucleation activity and promotes frost damage on strawberry (Lindow and Brandl 2003). Some are soil-borne phytopathogenic bacteria and are responsible for vascular disease (*Ralstonia solanacearum*), soft rot (*Pectobacterium carotovorum*), or gall (*Agrobacterium tumefaciens*, *Streptomyces scabies*). Certain *Agrobacterium tumefaciens* carry the Ti plasmid that confers them the ability to transfer genes to the plant (Krimi et al. 2002; Tzfira and Citovsky 2006).

Phytopathogenic bacteria are often facultative parasites, and an inoculum threshold is generally required for infection (10^4 cells of *P. syringae* per g of bean leaf). Some protozoa including *Phytomonas* are phytopathogens. These trypanosomes are responsible for heart-rot disease of coconut palms, and they can be transmitted back and forth from host plants to insects. Finally, some viruses such as poxvirus cause significant damages, but most of the time, they need vectors to infect their host plants. For instance, parasitic nematodes (e.g., *Xiphinema* spp.) serve as vectors of viruses such as the grapevine fanleaf virus (GFLV). Moreover, the wound caused by stylet penetration of the parasitic nematodes may also facilitate the plant infection by fungal (*Verticillium*), oomycete (*Phytophthora*), or bacterial (*Clavibacter michiganense*) pathogens. Besides nematodes, the main virus vectors are Hemiptera insects, but some viruses can also be transmitted to the plant by fungi (*Olpidium* spp.) or protists (*Polymyxa betae*) or between plants through parasitic plants such as dodder (Agrios 1997).

Infection Processes

Infection of the plant is done in several steps. The contact of the plant with spores (constituting the primary inoculum) of fungal pathogens involves, as presented above, several environmental factors including wind, water, insects, and microfauna that vectorize the pathogens, but also the biochemical and mechanical infectious potential of these pathogens. Production of fungal mucilage consisting of polysaccharides and/or glycoproteins ensures the adhesion of spores and germ tubes to the plant tissues. Enzymatic hydrolysis of the cuticle surface (*U. necator* on grapevine) or production of small proteins such as hydrophobins Mgp1 (*Magnaporthe grisea* on rice) enhances the adhesion of the propagules to the plant (Ebbole 2007). In most cases, there is no specific site of infection on the surface of the plant. Fungi penetrate the aerial part of the plant by piercing the cuticle, growing through the anthesis, exploiting wounds (opportunistic fungi), or growing between the stomata guard cells. The fungal germ tube generally forms an appressorium that is simply a swelling of the tip of the hypha (*B. cinerea*) or results from cell differentiation (case of rusts) to puncture through plant cell wall using high physical pressure, cell wall degrading enzymes, or both. In the case of pathogenic bacteria too, penetration occurs most often through natural openings, such as nectaries of flowers (*E. amylovora*), stomata or hydathodes (*X. campestris*), or wounds (*P. syringae*). Their lytic enzymes play an important role in virulence (Kazemi-Pour et al. 2004). Quorum sensing is sometimes involved in the infectious process (Smadja et al. 2004). Different types of trophic relationships can then be established. Some fungi such as *Venturia inaequalis*, responsible for apple scab, establish under the leaf cuticle and use epidermal walls as trophic base thanks to pectinases they produce. Others, such as powdery mildews, exploit the cytoplasm of epidermal cells thanks to a sucker they develop from the surface of the leaf. In rust or mildew agents, colonization of a first cell is followed by the development of secondary hyphae that gradually colonize other cells. Similar strategies are observed among soil-borne fungi and oomycetes when penetrating the plant root and crown. For instance, no specific site of infection was found for *F. oxysporum* attacking tomato roots (Olivain et al. 2006), and a set of extracellular enzymes is produced by the pathogens to degrade cellulose, hemicelluloses, and pectins of the plant epidermis and parenchyma leading to necroses, generalized rot, or tracheomycoses.

Interplay with Plant Defense Systems

As we saw previously, plants interact with many microorganisms, some of which being neutral or beneficial, while others are deleterious. It is important for plants to recognize and discriminate between them and to be able to respond accordingly. Conversely, it is important for beneficial and pathogenic microbes to modulate the host immune system to establish an intimate relationship or to prevent defense reactions. The plant, for instance, can respond to the presence of pathogens by the production of volatile compounds that will either promote or block microbial development, as for *B. cinerea* on strawberry (Abanda-Nkpwatt et al. 2006). It now appears that plants rely on the innate immunity of each cell and on systemic signals emanating from the infection site. This immune system allows them to recognize and respond specifically to invading pathogens (Jones and Dangl 2006).

Upon infection, compounds such as lipopolysaccharides, flagellin, peptidoglycans, and chitin are released from the pathogens. These microbial elicitors called microbe-associated molecular patterns (MAMPs), also referred to as pathogen-associated molecular patterns (PAMPs), are recognized at the surface of the host cell by receptor proteins called pattern recognition receptors (PRRs). MAMPs are basic components of all classes of pathogens. They may be constituents of the wall of the pathogen (exogenous elicitors) that are recognized by receptors. They may also correspond to the constituents of the plant cell wall that are released after degradation by microbial enzymes and recognized by receptors. A number of these common MAMPs and their role in the elicitation process have been recently described (Pel and Pieterse 2013). Recognition by the plant of these non-self compounds is a first step toward an effective immune response. PRRs generally consist of an extracellular leucine-rich repeat (LRR) domain and an intracellular kinase domain. Stimulation of PRRs leads to PAMP-triggered immunity (PTI), which provides a first line of defense against most nonspecific pathogens (Jones and Dangl 2006).

The second line of defense involves recognition by intracellular receptors of pathogen virulence molecules called effectors. The pathogens deliver effectors into the host cell by type III secretion (for bacteria) or using haustoria or other intracellular structures by an unknown mechanism (for fungi

Table 11.2 Nature of the interaction (compatible or incompatible) between pathogen and host plant in relation to the gene-for-gene concept (Based on Flor 1956)

	Host plant genotype[a]	
	*R*1, *r*2	*r*1, *R*2
Pathogen genotype[b]		
*Avr*1, *avr*2	Incompatible	Compatible
*avr*1, *Avr*2	Compatible	Incompatible

Recognition of the microbial elicitor (encoded by the avirulence gene *avr*) by the plant receptor (encoded by the resistance gene *R*) gives an incompatible interaction leading to the absence of infection (avirulence), while the absence of one or the other leads to a compatible interaction and the infection of the host plant, which reacts most often by a hypersensitive reaction

[a]Functional resistance allele *R*, non-functional resistance allele *r*

[b]Functional avirulence allele *Avr*, non-functional avirulence allele *avr*

and oomycetes) (Dodds and Rathjen 2010). These intracellular effectors often act to suppress PTI. However, many effectors are recognized by intracellular nucleotide-binding (NB)-LRR receptors; these sentry proteins are present at various levels in the plant (wall, cytoplasm, nucleus, cell membrane), and they allow a rapid response upon pathogen intrusion by inducing effector-triggered immunity (ETI). The gene-for-gene interaction takes place in that context (Table 11.2). The pathogen avirulence genes encode the elicitor. Host plant resistance genes corresponding to avirulence gene in the pathogen encode plant receptors recognizing this elicitor. This recognition leads to cascades of defense reactions involving essential metabolic pathways such as the salicylic acid and jasmonic acid pathways, as well as a massive influx of calcium ions into the cell together with potassium and chlorine efflux. Then, alarm signals are transmitted to the interior of the cell inducing the production of oxidizing radicals capable of inhibiting pathogen development. They lead to the activation of defense genes, synthesis of phytoalexins (plant antibiotics), and defense proteins called PR proteins (pathogenesis-related proteins), whose spectrum of activity is broad or narrow (van Loon et al. 2006). Some correspond to chitinases (PR3) and glucanases (PR2), whereas others are poorly known (PR17). To reduce the spread of the pathogen, the plant cell wall thickens (Fig. 11.7e) or, in the case of the hypersensitive response (HR), the infected cell transmits alarm signals to the neighboring cells that then destroy them (**apoptosis***). It has to be noted that NB-LRR-mediated disease resistance is effective against pathogens that grow only (obligate biotrophs) or during part of the life cycle (hemibiotrophs) on living host tissue, but not against pathogens that kill host tissues during colonization (necrotrophs) (Jones and Dangl 2006).

According to Dodds and Rathjen (2010), ETI and PTI reveal different coevolution dynamics between the plant and the pathogen, as, in contrast to PAMPs, effectors are variable and unessential. Similarly, the diversity of ETI receptors and pathogen effectors both within and between species is frequent, whereas PRR functions are widely conserved across families. Usually, PTI and ETI provide similar responses, but ETI is qualitatively stronger and faster and often it is responsible for the hypersensitive response (HR). PTI is generally effective against nonspecific pathogens, whereas ETI is active against specific pathogens. However, these relationships are not exclusive and depend on the elicitor molecules present in each infection (Dodds and Rathjen 2010).

Jones and Dangl (2006) proposed a simple zigzag scheme including four steps to illustrate the functioning of the plant immune system: First, PRRs recognize PAMPs, which results in PTI and may stop further pathogenic invasion. Then, successful pathogens produce effectors that contribute to pathogen virulence. They negatively interfere with PTI. In a third step, a given effector is "specifically recognized" by one of the NB-LRR proteins, which leads to ETI. ETI is an accelerated and amplified PTI response, resulting in disease resistance and, usually, a hypersensitive cell death response at the infection site. At last, on one side, natural selection drives pathogens to avoid ETI by diversifying the recognized effector gene or by acquiring additional effectors that suppress ETI. On the other side, natural selection favors new plant NB-LRR alleles that can recognize one of the newly acquired effectors, resulting again in ETI. Therefore, the interplay between microbial pathogens and plants presents several similarities with the case of animal diseases (*cf.* Sect. 11.4).

Host Range and Pathogen Diversity

Some pathogens such as *Phoma betae* are restricted to a plant species (sugar beet), while others like *Rhizoctonia solani* have a wide range of host plants belonging to different families. Plant-pathogenic fungi are drawing specific attention not only because of their negative impact on plant growth, but also because studying the interactions between the fungi and their host plant has revealed the potential of fungi to adapt to changing external constraints such as the ones we saw before. For instance, the *Fusarium* genus includes many soil-borne species that can infect roots and crowns as well as shoots, thanks to their ability to adapt infection mechanisms to the host plant, the plant phenology, and the plant's organs. In *F. oxysporum*, the large intraspecific diversity allows the fungus to infest a broad spectrum of host plants through very narrow fungus–host plant interactions. Indeed, more than 80 formae speciales have been described so far, each gathering one or more *F. oxysporum* populations that are able to infest one and only one plant species. Diversity studies revealed that different and probably independent genetic events within the species have led to the "speciation" of *F. oxysporum* into different formae speciales (Rep et al. 2005). In some cases, we can assume that the fungus–host plant interaction was such that the plant set up defense reactions that were circumvented by the fungus. This process

Box 11.3: Resistance and Host Specificity in Plant–Pathogen Relationships

Christian Steinberg

In fungi and bacteria, isolates of the same species causing the same symptoms on a particular plant genotype, whether a cultivated (cultivar) or wild variety, are a race.

The concepts of race and variety result from a pathogen–plant coevolution. Indeed, the emergence of a resistance gene in the host plant gives rise to a new variety, which will no longer be infested by the original pathogen (race 0), as is the initial variety. The appearance by mutation of a gene for virulence in the pathogen, allowing it to circumvent this resistance, gives birth to race 1, capable of infecting both varieties, while race 0 is limited to the first variety, and so on.

The nature of the resistance of the host plant can be divided into two types. In horizontal resistance, many genes are involved (polygenic resistance). Vertical resistance in a single gene gives the plant (cultivar or variety) total resistance to a very specific biotype (race) of the parasite, but the variety concerned is sensitive to other biotypes of the pathogen. Any biotype able to overcome a vertical resistance factor is said to be virulent.

led to the definition of resistant plant genotypes and adapted pathogenic fungal races (Takken and Rep, 2010) (*cf.* Box 11.3). The gene-for-gene relationship is well known and exists in many host–pathogen interactions, but in *F. oxysporum*, such interactions occur within a formae specialis (Rep et al. 2005). In rare cases, the formae speciales are quite homogenous. Finally, the same *F. oxysporum* species also includes nonpathogenic strains (Edel et al. 2001).

Phytopathogen Control

Control of microbial pathogens of crops relies on a combination of various methods. Fungicides, which represent about 20 % of the pesticides used in agriculture, are ineffective against soil-borne bio-aggressors, and for human health and environmental quality, their use must be reduced. Prophylaxis should be preferred. This involves the regular cleaning of farm machinery and irrigation systems, but also the removal of dead plant matter from fields as well as the management (burial) of crop residues to minimize potential reservoirs for the plant pathogens. Similarly, many pathogens can live on and in seeds. Therefore, seed companies must ensure the quality of plant seeds. Innovative farming practices should be promoted. This includes new rotation schemes (longer and more diverse than the present ones), the use of intermediate crops having sanitizing impact, and appropriate tillage systems to preserve both soil structure and biological activity (multitrophic interactions), so that pathogen populations decline between host plantings. These practices should be combined with the use of resistant cultivars when possible as well as biological control agents if any in given conditions. Based on what was shown previously, these control methods (when used) can only limit the extent of damage (Box 11.2).

11.3.5.3 Symbiosis

Certain bacteria and fungi form a mutualistic symbiosis with plants (Cardon and Whitbeck 2007). Those between bacteria and plants consist mainly of nitrogen-fixing symbioses involving the floating fern *Azolla* (with the cyanobacterium *Anabaena*), phylogenetically diverse plants called actinorhizal (with the actinobacterium *Frankia*), and Fabaceae (with selected alphaproteobacteria, such as *Rhizobium*, and selected betaproteobacteria) (Mathesius 2003; Huguet et al. 2005). The *Rhizobium*–Fabaceae dialogue involves molecular signals produced by the plant (including flavonoids) and leads to bacterial synthesis of a lipo-chito-oligosaccharide termed Nod factor, which is a signal inducing root nodulation (Mathesius 2003) (Fig. 11.10a). Host specificity depends largely on the chemical specificity of these two types of signals. In the case of *Frankia* and of photosynthetic *Bradyrhizobium*, the bacterial signal involved is different, and indeed their genomes are devoid of canonical *nod* genes (Giraud et al. 2007; Normand et al. 2007). The Fabaceae nodule is formed by proliferation of cells from the cortex and the pericycle. The bacteria usually enter the plant through root hairs, which are deformed during the interaction, and find themselves in an infection thread of plant origin. This thread enables proliferation and delivery of bacteria to plant cells in the nodule. Once endocytosed by nodule plant cells, the bacteria differentiate into bacteroids but remain separated from the cytoplasm of the plant cell by the peribacteroid membrane. In *Medicago truncatula*, this transformation into bacteroids is triggered by nodule-specific cysteine-rich peptides resembling antimicrobials from the plant innate immune response (Van de Velde et al. 2010). The peribacteroid membrane and the bacteroid(s) it contains constitute the symbiosome. The nitrogen fixed by the bacteroids will serve as nitrogen source for the plant, which in return provides a source of carbon and energy (25–35 ATP necessary for each N_2 reduced) in the form of dicarboxylic acids (mainly malate). The nitrogenase is sensitive to oxygen but is protected by low oxygen diffusion within

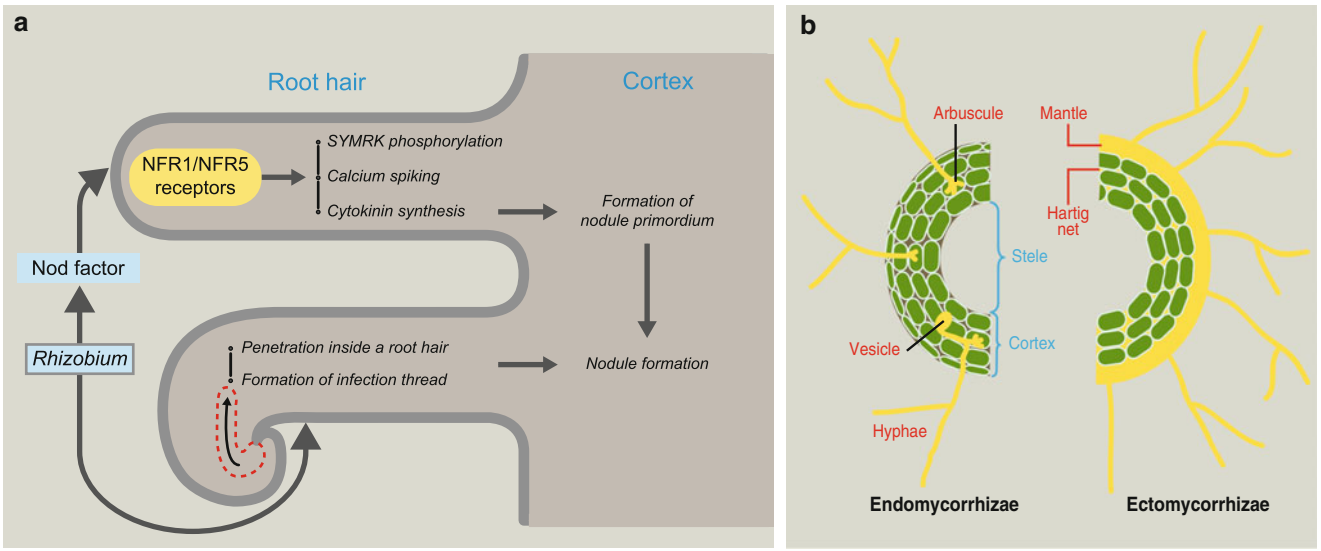


Fig. 11.10 Symbiotic interactions between microorganisms and plants. (**a**) Root nodulation process. Nod factors released by rhizobia are perceived by receptors of the epidermal cells of the root, which alters calcium flux and activates a signaling pathway leading to the production of cytokinins. The local increase in cytokinin concentration activates division of cortical cells and induces the formation of the nodule primordium. *Rhizobium* infects and invades the nodule via the infection thread, initiated in the root hair (From Oldroyd 2007). (**b**) Structural comparison of endomycorrhizae and ectomycorrhizae. In the case of endomycorrhizal fungi, the fungal filaments colonize the intercellular spaces of the cortex without reaching the central cylinder. Most endomycorrhizae are characterized by the differentiation of arbuscules (intracellular sites of exchange with the plant partner) and vesicles (intra or intercellular storage structures). In the case of ectomycorrhizae, the surface of the root tips is covered by a mantle made up of fungal mycelium that also colonizes the intercellular spaces of the cortex (forming the Hartig net) (Drawing: M.-J. Bodiou)

the nodule. The oxygen supply to the bacteroid is provided by leghemoglobin, a carrier of both bacterial (heme) and plant origin (globin). The leghemoglobin allows a high flux of oxygen without the latter reaching a concentration deleterious for the nitrogenase. The nitrogen fixed by the bacteroid is transferred to the plant in the form of ammonia and more rarely alanine. It will be transported in the phloem sap as amides (for temperate Fabaceae, with indeterminate nodules) or ureides (for tropical Fabaceae, with determinate nodules). In the bacterial partner, key genes (over fifty) involved in symbiosis are generally clustered as islands of genes, often plasmid-borne. They may be transferred horizontally, which probably explains their presence in both alphaproteobacteria and betaproteobacteria (Moulin et al. 2001).

The mycorrhizal symbioses implicate plant roots (Fig. 11.10b) (Mathesius 2003; Bailly et al. 2007). The fungus provides the plant with minerals taken from the soil (phosphorus and to a lesser extent nitrogen), whereas the plant provides carbon substrates derived from photosynthesis (Brundrett 2009). The vast majority of plants are mycorrhized in nature, the few plant species not forming mycorrhizae belong mainly to the family Chenopodiaceae (beets) and Brassicaceae (rapeseed, *Arabidopsis*). There are several types of mycorrhizae, particularly ectomycorrhizae, arbuscular endomycorrhizae, and ectendomycorrhizae (Mukerji et al. 2000; Brundrett 2009).

Ectomycorrhizal fungi (Ascomycetes and Basidiomycetes) develop extensively at root tips, forming fungal sheaths over short roots, which is very characteristic and easily visible to the naked eye (Brundrett 2009). Less than 5 % of plant species are concerned, among which mainly forest species growing in temperate areas. The mycelium grows between root cortical cells but does not penetrate living cells, thus forming an intercellular network called the Hartig net, which is involved in nutrient exchanges between the two partners. The functioning of ectomycorrhizae is under the metabolic and genetic control of both partners (Bailly et al. 2007).

Arbuscular endomycorrhizal fungi (Glomeromycota division) do not develop a mantle around the root (Brundrett 2009). The endomycorrhizae are widespread and affect about 90 % of plant species. They are found mainly in herbaceous plants and in some woody species. The fungus penetrates through plant cell walls and develops arbuscules and vesicles in cortical cells. Ectendomycorrhizae are intermediate between ectomycorrhizae and endomycorrhizae; an external mantle may be produced, but the fungus penetrates into root cells, as coils (arbutoïd mycorrhizae; among Ericaceae) or very short hyphae (monotropoid mycorrhizae; among Pyrolaceae). The establishment of mycorrhizal symbiosis shows common features with that of bacterial

nitrogen-fixing symbioses (Mathesius 2003). Before contact, the mycorrhizal fungus stimulates the production of fine roots to increase the contact sites, secreting hormones including the auxin indole-3-acetic acid (IAA). After contact, the fungus enters the root and must overcome defense mechanisms. It switches from a saprophytic to a biotrophic phase, which recalls the case of biotrophic parasites.

Signaling mechanisms between partners in the establishment of a nitrogen-fixing symbiosis or a mycorrhizal symbiosis involve common symbiosis genes (*SYM*) in the plant, including *SYMRK*/*NORK*/*DMI2* and *DMI3* that code for receptor-like leucine-rich kinases, *CASTOR*/*DMI1* and *POLLUX* encoding ion channels ensuring transmembrane calcium flux, and (in the case of nitrogen-fixing symbiosis in Fabaceae) *ENOD40* (early nodulin 40) (Oldroyd and Downie 2006; Reinhardt 2007; Gherbi et al. 2008). The structure of the Myc factors, i.e., sulfated and non-sulfated lipo-chito-oligosaccharides consisting of four substituted *N*-acetylglucosamines, is very close to that of the Nod factors, which reinforces the view that the two types of symbioses are functionally very close (Maillet et al. 2011). In terms of evolution, the bacterial symbioses (relatively young; about 65 million years) and the ectomycorrhizal symbiosis (also recent; about 180 million years) probably recruited *SYM* genes already involved in the arbuscular mycorrhizal symbiosis, which is older (probably 400 million years) (Simon et al. 1993).

11.3.5.4 Cooperation

Several bacteria and fungi actively cooperate (syn. associative symbiosis) with the plant. In the case of bacteria, this ability is mainly found in **plant growth-promoting rhizobacteria*** (PGPR) (Fig. 11.11a). PGPR are documented mainly in Proteobacteria and Firmicutes and to a lesser extent in Actinobacteria. Growth stimulation usually results from a combination of direct and indirect phytobeneficial effects (Dobbelaere et al. 2001). Direct phytobeneficial effects may entail improved mineral nutrition of the plant, e.g., via free-living nitrogen fixation or phosphate solubilization (Dobbelaere et al. 2003) and/or improved water uptake through rhizosphere soil structuration by bacterial exopolysaccharides (Amellal et al. 1998) or aquaporin stimulation (Groppa et al. 2012). Interference with plant hormonal metabolism may also be involved, via bacterial production of phytohormones (auxins and cytokinins) and/or bacterial deamination of 1-aminocyclopropane-1-carboxylate (ACC), the ethylene precursor in the plant (Dobbelaere et al. 2003). The auxin indole-3-acetic produced by PGPR helps getting around plant defense mechanisms, thereby facilitating PGPR colonization of the plant (Remans et al. 2006). Finally, phytobeneficial effects also include triggering of systemic resistance in the plant, in particular, induced systemic resistance (ISR) pathways relying on jasmonate and ethylene (van Loon et al. 2006).

Indirect phytobeneficial effects of PGPR entail inhibition of parasitic bacteria, oomycetes, fungi, nematodes, and even parasitic plants (e.g., *Striga*), mainly through competition or antagonism (Chapon et al. 2002; Raaijmakers et al. 2009). Competition can take place for macronutrients (such as organic carbon), micronutrients (such as soluble ferric iron via high-affinity siderophores), and/or infection sites. Antagonism (syn. amensalism) may involve extracellular lytic enzymes (e.g., cellulases, chitinases, proteases), which act on the cell wall of pathogenic microorganisms, their virulence factors (such as fusaric acid from *Fusarium oxysporum*, degraded by some *Burkholderia*), or their intercellular signals (such as *N*-acyl homoserine lactone in *Pectobacterium carotovorum*) (Raaijmakers et al. 2009). Antagonism may also involve type III secretion system effectors (*cf.* Sect. 11.4.3) with the ability to reduce the virulence of certain pathogens (Rezzonico et al. 2005). Finally, antagonism can rely on production of antimicrobial secondary metabolites (antibiosis), such as 2,4-diacetylphloroglucinol, and a single antagonistic strain often produces several of these antimicrobial metabolites (Raaijmakers et al. 2009). Some pathogens can defend themselves by suppressing the production of these metabolites (case of 2,4-diacetylphloroglucinol, via fusaric acid from *Fusarium*) or the expression of genes involved in root colonization by the antagonistic bacterium (Fedi et al. 1997; Raaijmakers et al. 2009).

The metabolites released by PGPR are important for their interactions with the plant and plant pathogens, and many of them play multiple roles. Indeed, 2,4-diacetylphloroglucinol (enabling antagonism toward phytoparasites) and some siderophores such as pyoverdine (involved in iron competition) can also induce plant resistance (Raaijmakers et al. 2009). Phenylacetic acid, synthesized by *Azospirillum brasilense* Sp245 and others, is both an auxinic phytohormone and an antimicrobial compound. Therefore, certain PGPR are multifunctional in that they can act both on plant (via induction of resistance and ACC deamination) and phytopathogens (antagonism or competition).

Fungi in cooperation with the plant remain poorly documented compared with PGPR. Their direct phytobeneficial effects include solubilization of mineral nutrients (*Trichoderma* and *Gliocladium*) and the induction of systemic resistance in plants (*Trichoderma*, *Gliocladium*, and nonpathogenic *F. oxysporum*) (Harman et al. 2004). However, indirect phytobeneficial mechanisms are more important. Hyperparasitism toward different phytoparasitic oomycetes and fungi is one of the modes of action of *Trichoderma*. The fungus coils around its target, e.g., *R. solani*, and secretes lytic enzymes (chitinases and cellulases) that alter phytoparasite cell wall. The degradation

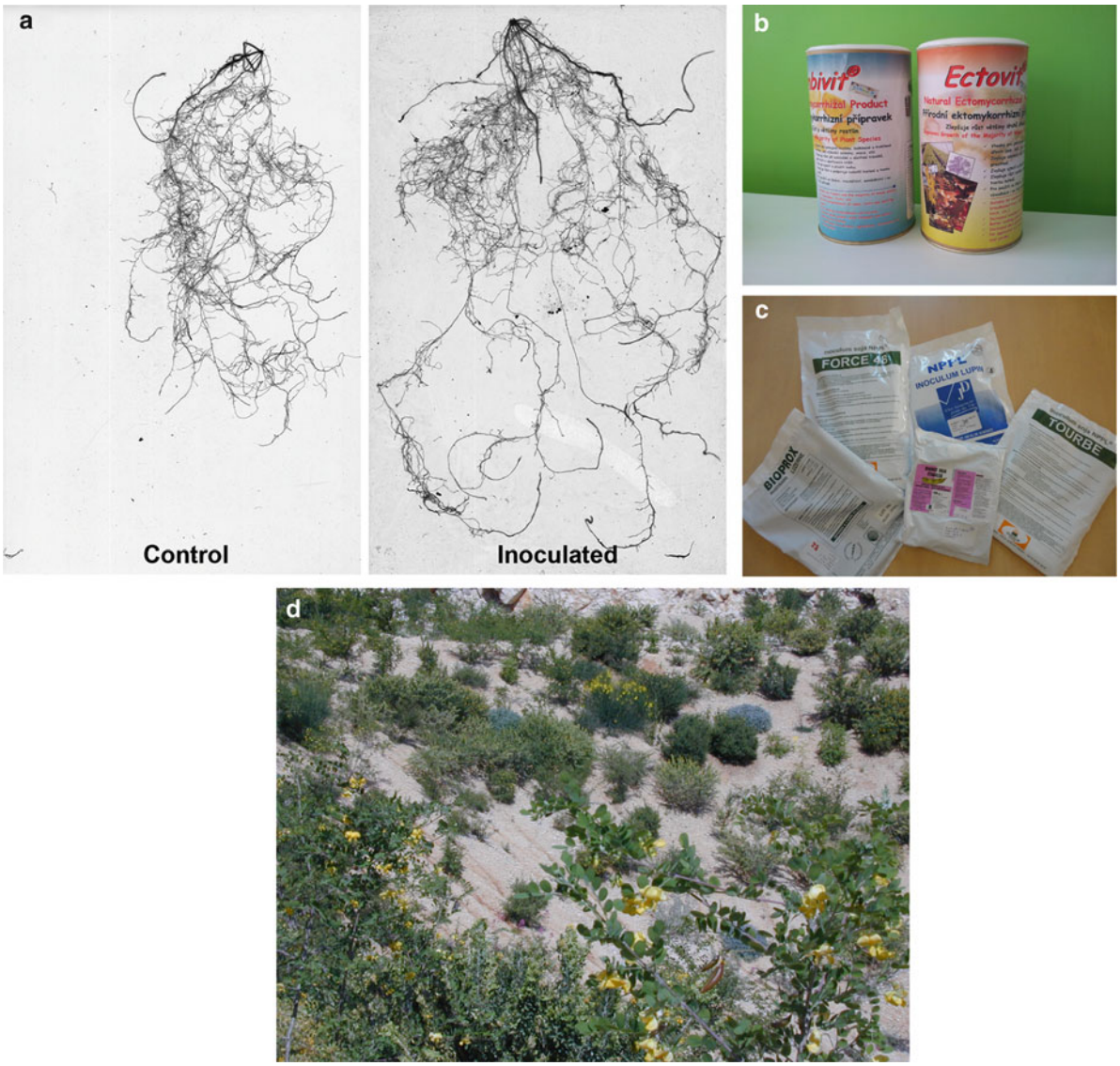


Fig. 11.11 Biotechnological use of beneficial plant–microorganism interactions. (**a**) Effect of a PGPR bacterium on the development of the root system of wheat seedlings (Photo C. Prigent-Combaret, UMR CNRS 5557 Microbial Ecology, Villeurbanne, France). (**b**) Commercial inoculum preparations based on endo- or ectomycorrhizal fungi (Photo Y. Moënne-Loccoz). (**c**) Commercial inoculum preparations based on nitrogen-fixing bacteria for Fabaceae crops (Photo C. Steinberg). (**d**) Rehabilitation of bare slopes in an abandoned limestone quarry, based on revegetation using the Fabaceae *Medicago arborea*, *Coronilla glauca* (yellow flowers in the foreground), and *Dorycnium hirsutum* associated with symbiotic nitrogen-fixing bacteria. The photo is taken after 3 years of plant growth (Photo J.C. Cleyet-Marel, UMR 113 IRD/CIRAD/SupAgro/UM2 Laboratoire des Symbioses Tropicales et Méditerranéennes, Montpellier, France)

products will allow chemotropism toward the phytoparasite. Physical contact between fungi will trigger external colonization and then penetration of the phytoparasite by *Trichoderma*. *Trichoderma* and *Gliocladium* may also antagonize phytoparasites through the production of antimicrobial secondary compounds (Harman et al. 2004). Finally, competition for trophic resources can be a major mode of action, as in nonpathogenic *F. oxysporum* (Alabouvette et al. 2006). Overall, as for PGPR, phytobeneficial fungi often display a combination of different modes of action.

11.3.6 Significance for the Plant

Plant–microbe interactions have a major impact on plant functioning and plant community ecology. At the scale of a given plant, negative effects of parasitic microorganisms are usually easy to identify, based on observation of disease symptoms. Many other plant-associated microorganisms have positive effects on plant growth or development, but these effects are often more difficult to visualize. Phytobeneficial microorganisms promote plant development

primarily by altering plant hormonal balance, while their impact on plant growth may also involve trophic microbial effects. For example, the potential for symbiotic nitrogen fixation is typically in the order of 60–200 kg N/ha/year, for which the plant will invest 3–25 % of net photosynthates, versus only 5–25 kg N/ha/year for free nitrogen fixation. Nevertheless, the symbiotic nitrogen fixation potential is often poorly exploited in modern agriculture, which relies on massive use of chemical nitrogen fertilizers. Mycorrhization improves plant nutrition in phosphorus and nitrogen, and to a lesser extent in potassium and iron, especially in low-fertility soils (Brundrett 2009). Phosphorus can be recovered by mycorrhizal hyphae at more than 7 cm from the root. Mycorrhizal fungi also improve plant resistance to biotic stress (disease, herbivory; Bennett and Bever 2007) and abiotic stress (drought, metal pollution; Auge 2004).

Regarding plant health, the role of plant-protecting bacteria and fungi is important in the rhizosphere. In soils that are suppressive to disease, their interactions are sufficient to limit disease severity despite the presence of the pathogen, plant susceptibility to disease, and environmental conditions favorable for infection (Alabouvette et al. 1996; Garbeva et al. 2004). These disease-suppressive soils are documented for root diseases caused by fungi and to a lesser extent by bacteria or nematodes. In some pathosystems, suppressiveness gradually develops during crop monoculture in soils that were initially conducive to the disease. This is the case of wheat take-all caused by *Gaeumannomyces tritici*; take-all severity increases over the early years of wheat monoculture, reaches a peak (usually in years 4–8), and decreases thereafter to minor levels in the following years (Lucas and Sarniguet 1998; Raaijmakers and Weller 1998). In other pathosystems, disease suppressiveness is a natural property of the soil and does not require monoculture, as for soils suppressive to Fusarium wilt (caused by *F. oxysporum*) (Alabouvette et al. 1996) or to Thielaviopsis black root rot (caused by *Thielaviopsis basicola*) (Kyselková et al. 2009). Soil suppressiveness is often attributed to *Pseudomonas* PGPR subpopulations producing 2,4-diacetylphloroglucinol, although other phytobeneficial microorganisms might also play an important role (Kyselková et al. 2009).

Plant–microbe interactions have a significant impact on natural plant communities (Cardon and Whitbeck 2007). Nitrogen-fixing and mycorrhizal symbioses may be important to promote the establishment of pioneer plant groups. This is the case for plant recolonization of ground surfaces following deglaciation (at the end of ice ages or with current global warming), volcanic eruptions, or forest fires (van der Maarel 2005). Pioneer plant species will modify soil microbial community composition/functioning which, in turn, may then influence plant succession and the composition of intermediate groups, both by favoring certain plant species and counterselecting others. Finally, when the climax stage is reached, pathogens adapted to the main plant species may decrease their dominance or eliminate enough individuals to allow re-installation of pioneer species (within forest clearings) and reinitiate plant succession (van der Maarel 2005). In the particular case of invasive plant species, the soil microbial community may facilitate their establishment at the expense of native plants. Plant–microbe interactions are also important for the functioning of plant communities, particularly in terms of recycling of plant litter by microbial decomposers and nitrogen fixation (van der Maarel 2005). Organic matter transformations contribute to pedogenesis and to soil functioning, and as such they impact both on plant growing conditions and plant community successions.

11.3.7 Biotechnological Uses of Plant–Microbe Interactions

Certain microorganisms are used as inoculum in agriculture (Fig. 11.11b), for biofertilization, phytostimulation, or biocontrol purposes (Dobbelaere et al. 2001; Alabouvette et al. 2006). The goal is to improve crop yield (productivity) and/or reduce chemical inputs (environmental quality). Biofertilizers aim at improving plant mineral nutrition, e.g., symbiotic nitrogen-fixing bacteria (*Rhizobium* and other genera) and to a lesser extent mycorrhizal fungi. For PGPR having mainly hormonal effects on the plant, the term phytostimulation is used instead of biofertilization. The main phytostimulators used belong to the genus *Azospirillum*, especially for cereals (0.5–1 million ha of corn inoculated each year). *Azospirillum* inoculation resulted in increased cereal yield (by 10–30 %) in two-thirds of the cases (Dobbelaere et al. 2001). Biological control is most often implemented in commercial greenhouse, using bacteria or fungal inoculants. The objective is to protect crops against phytopathogens (often fungi or oomycetes, sometimes bacteria), parasitic nematodes or plants, or weeds. The microorganisms used correspond to Firmicutes (*Bacillus*), Proteobacteria (*Pseudomonas*), and Actinobacteria (*Streptomyces*) for bacteria as well as Deuteromycetes (*Trichoderma*, *Gliocladium*, *Coniothyrium minitans*) for fungi (Gilbert et al. 1993; Harman et al. 2004; Rezzonico et al. 2005). Inoculum formulation is a key issue determining inoculant performance, and spore-forming microorganisms have better storage capacities. The inoculum is often applied to the seed before sowing or in the furrow, more rarely on the soil surface or on the plant during growth. Current regulations on the use of inoculants in agriculture vary greatly from one country to the next (regarding the need for toxicological assessment, proof of effectiveness, guarantee for inoculum level in the product), which can make it expensive in certain countries to develop new microbial products, especially for biological control purposes.

In a broader sense, the biotechnological use of plant–microbe interactions in agriculture also includes the use of plants benefiting from symbiotic nitrogen fixation (Moulin et al. 2001). This is particularly the case of Fabaceae, as forage or cash crops, traditionally used to improve soil fertility, particularly in crop rotations. Some of the Fabaceae are used as green manure, i.e., once grown they are incorporated into soil rather than being harvested. In agroforestry, Fabaceae shrub species and actinorhizal plants (e.g., *Casuarina*) can be used for alley cropping with herbaceous companion crops. Two other types of nitrogen-fixing symbioses, which do not lead to the formation of nodules, are also important from a biotechnological point of view. One involves aquatic ferns and cyanobacteria, such as the *Azolla–Anabaena* symbiosis, which can provide in the order of 30–60 kg N/ha/year for submerged rice fertilization (Roger et al. 1993). The other is formed by sugarcane and endophytic Proteobacteria such as *Gluconacetobacter diazotrophicus*. These nitrogen-fixing symbioses may provide up to 100–150 kg N/ha/year, making sugarcane particularly attractive for biofuel production. This crop contributes to atmospheric flux of the greenhouse gas N_2O, but much less than other crops (Crutzen et al. 2007). Finally, crop plants are often involved in cooperative interactions with PGPR and/or endophytic fungi. This capacity is of agronomic interest, but it varies depending on plant genotype (Picard and Bosco 2006), and so far it has been largely ignored in crop breeding schemes.

In nonagricultural soils, plant–microbe interactions are of interest for the establishment of windbreaks and landscaping of roadside and other anthropogenic sites. Nitrogen-fixing symbioses based on Fabaceae or actinorhizal shrubs are often used in this context, all the more as these species also benefit from mycorrhizal symbioses. These symbioses are particularly useful for revegetation of land low in organic matter (abandoned quarries or eroded slopes; Fig. 11.11c), degraded, highly acidic, and/or rich in heavy metals (mining areas, etc.) (Roy et al. 2007). In the latter case, revegetation (*cf.* Sect. 16.2.1) can stabilize the contaminated soil (phytostabilization), and interaction with PGPR displaying ACC deaminase activity and/or with mycorrhizal fungi is important for improving plant tolerance to the metals (Belimov et al. 2005).

In the case of contaminated soils, interactions between plants and microorganisms are also of interest for two phytoremediation techniques, i.e., phytoextraction and rhizoremediation (Cardon and Whitbeck 2007; Roy et al. 2007). Regarding metal pollution, some of the rhizosphere bacteria and fungi can solubilize metals such as chromium, lead, and arsenic (Khan 2005), while promoting their absorption by the roots (phytoextraction). For some organic pollutants, rhizodeposit availability can promote their biodegradation by the rhizosphere microbial community, whose action may be supplemented by enzymes released by the root. Organic pollutants that can be treated through such rhizoremediation include pesticides, simple hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) (Rentz et al. 2005). Microbial biodegradation by rhizospheric microorganisms also helps protect the plant from phytotoxic effects of organic pollutants, at the same time promoting site revegetation.

Plant–microbe interactions also have an interest for purification of effluents. This is the case of rhizoremediation systems, including those based on reeds or bamboo, used to decompose organics in certain water treatment operations (*cf.* Chap. 16). However, the role of plant–microbe interactions in these systems is poorly documented. A simplified system based on unicellular microalgae (*Chlorella*) and bacteria (*Azospirillum*) co-immobilized in alginate beads was also developed for tertiary treatment of wastewater, allowing removal of nitrogen (ammonium mainly) and phosphorus (de-Bashan et al. 2004).

Finally, the ability of *Agrobacterium* to transfer genes into higher plants is one of the means used to obtain transgenic plants (Tzfira and Citovsky 2006). This ability relies on the pTi plasmid, which allows the transfer of a particular DNA segment (the T-DNA) located between 25-bp flanking repeats and that includes genes involved in the synthesis of phytohormones (auxins and cytokinins) and opines. The T-DNA is transferred in single-stranded form, combined with VirD2 and VirE2 proteins, and is randomly integrated into the nuclear genome of dicotyledonous plants. To obtain transgenic plants, the genes of interest replace genes between the flanking sequences. This technology has been enhanced to allow genetic modification of monocots, a transfer that does not occur under natural conditions.

11.3.8 Epilogue

Interactions between microorganisms and plants have played a fundamental role in the evolution of plants, allowing the emergence of the first plant cells (thanks to cyanobacteria) and, later, land colonization by plants thanks noticeably to the endomycorrhizal symbiosis (Simon et al. 1993). Plant–microbe interactions are very diverse, ranging from facultative relationships with minor consequences to obligatory partnership with major evolutionary consequences. The microbial community contributes significantly to the functioning and ecology of the plant partner and to soil quality. Indeed, microorganisms modulate the growth, nutrition, and health of plants. Conversely, plants grow in biotopes where thousands of microbial taxa lead an existence whose focal point is the plant as a source of exudates or litter or as a partner to attack or collaborate with. It is therefore inevitable that plants have evolved and will continue to do so according to the microorganisms interacting with them. Mankind must

improve plant growth to meet the global challenge of feeding billions of people in a changing environment and to do so must better integrate the constraints and benefits of plant–microbe interactions. In the field of agronomy, this concerns in particular the principles of crop breeding schemes, cultivation techniques, and the development of ecological engineering approaches in cropping systems.

11.4 Interactions Between Microorganisms and Animals

11.4.1 Introduction

Man and animals, whether vertebrate or invertebrate, live in environments (air, water, soil) constantly populated by microorganisms consisting mainly of bacteria, fungi, protozoa, and viruses. Such cohabitation usually leads to more or less intimate associations between microorganisms and animals. Spatial proximity, contact time, and the degree of dependence between the interacting partners influence the type and evolution of these associations. Microorganisms use animals as habitats in which they live and from which they retrieved nutrients necessary for their multiplication. They colonize the outlying areas, such as the surface of the skin or mucous membranes, and can penetrate the tissues and organs of host animals. Some microorganisms can colonize the cytoplasm of germ cells, oocytes, and sperm and are thus subject to possible vertical transmission (from parents to offsprings), which can allow a greater ability to spread among host population. The presence and activities of microorganisms will affect development, reproduction, and survival of host animals. The nature of the effects caused by microorganisms and the animal responses to these infections are very diverse and are part of a continuum from parasitism to mutualism (Buchner 1965). Whatever the outcome of the interaction (neutral, beneficial, or deleterious), animals respond to infection by implementing defense systems, either by inducing immune responses or setting up physical barriers to prevent or limit the spread of microorganisms considered as potentially infectious agents. In parallel, microorganisms develop strategies to circumvent the defenses of animals by manipulation host traits or molecular mimicry. It is the trade-off between cost and benefits, that is to say, the balance of the association in terms of adaptive value, which defines the type of interaction (Moran 2007). Knowledge and understanding of the interactions between microorganisms and animals represent major challenges in various areas of biology. In the fundamental domain, microorganisms and their animal hosts entail a great diversity of species potentially giving rise to a variety of interactions. These interactions are of great interest in cell biology, molecular biology, evolutionary biology, and ecology. In human and animal health, the interactions between microorganisms and their vertebrate or invertebrate hosts are important because they are the cause of many diseases involving emerging human and animal pathogens.

11.4.2 Diversity, Distribution, and Abundance of Microorganisms Associated with Animals

Since the first microscopic observations of microbes by Dutchman Antonie van Leeuwenhoek, followed by successful cultivation of some of them by Louis Pasteur and other scientists, and, more recently, the development of molecular methods for the detection and identification without prior culturing (culture-independent methods), the list of microbes documented in animals is increasing. It remains that, for most microbes, animals are hostile habitats due to the physico-chemical conditions for growth and survival. One can mention, in healthy individuals, the often constant temperature inside the body but variable at the surface exposed to environmental stress (radiation), the pH that can be either neutral or acidic in the stomach, the osmotic pressure (variation of ion concentration), the partial pressure of oxygen (aerobic and anaerobic), the presence of various microbial inhibitors, and limited energy resources both in quality and quantity.

Compared to the great diversity of microbial phyla (DeLong and Pace 2001) present in areas where animals live, only certain groups or certain strains or variants of a group have the capacity for colonization (adhesion, secretion systems, lytic enzymes, metabolism) and are able to adapt and associate transiently or permanently with animals.

11.4.2.1 Microbial Diversity

Bacteria are the group most frequently encountered both at the surface and in the internal parts of animal bodies (*cf.* Sect. 8.1.5). Bacteria associated with humans belong to the mesophiles and have their growth optimum around 37 °C. The culturable bacteria from the skin mostly belong to two groups (Noble 1993). One is the Gram-positive cocci family *Micrococcaceae*, with a dominance of staphylococci, and the other consists of corynebacteria, in particular, bacteria belonging to genus *Corynebacterium*, which are found on all parts of the human body surface. Bacteria belonging to genera *Propionibacterium* and *Brevibacillus* occupy more limited areas with high production of lipids such as the sebaceous glands, the face, and the scalp. Microbial flora of the skin of pets (cats, dogs) and farm animals (pigs, cows) is also dominated by staphylococci and some fungi (Nagase et al. 2002).

Review of various studies on the microbiota (cultivable or unculturable microbes associated to an individual) of human or other vertebrate species confirmed previous dominance of bacteria, but it has also expanded the repertoire of microbial taxa (fungi, protozoa, viruses) residing in external

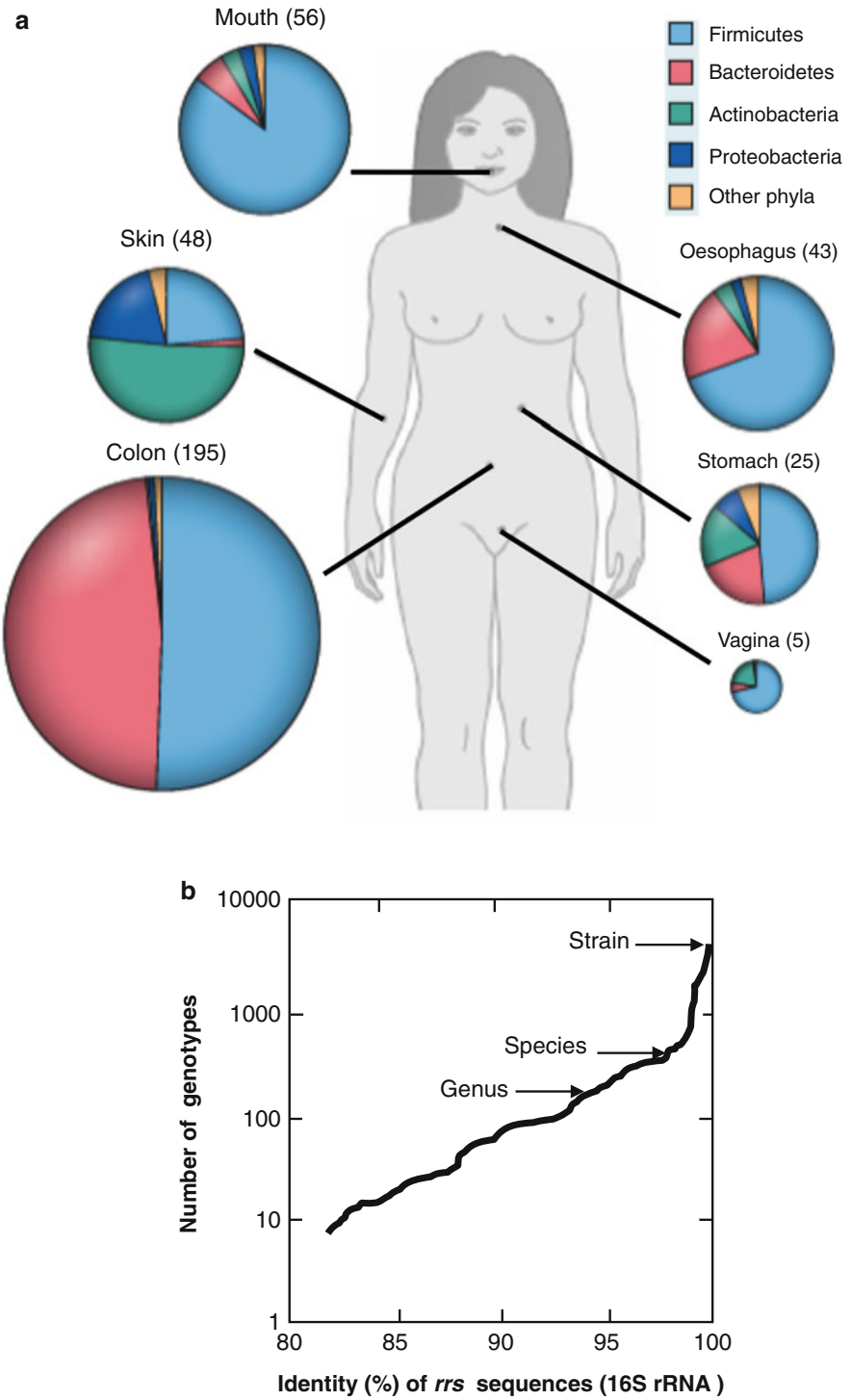


Fig. 11.12 (**a**) Distribution of bacterial phyla in healthy individuals. The area of each part is an average number of distinct phylotypes based on *rrs* gene sequences (16S rRNA). The average number of phylotypes per individual is given in brackets (Adapted from Dethlefsen et al. 2007). (**b**) Richness in taxonomic units of bacteria in the human gut. Estimated percentage of *rrs* sequence identity to the genus level (95 % identity), species (98 % identity), and strain (unique sequence) (Adapted from Bäckhed et al. 2005)

parts or internal organs (Nardon and Charles 2001; Dethlefsen et al. 2007). Extraction of DNA from human tissues and large-scale sequencing of the gene encoding 16S ribosomal RNA have revealed bacterial phyla of the skin, mouth, intestines, and genitals (Fig. 11.12). Among more than 50 bacterial phyla described, only four phyla are commonly found in humans: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (Dethlefsen et al. 2007). These four phyla coexist with nine other secondary phyla (*Chlamydiaceae, Cyanobacteria, Deferribacteria, Deinococcus-Thermus, Fusobacteria, Spirochaeta, Verrucomicrobiota*, and two new phyla TM7 and SR1) in humans as well as in other vertebrate hosts (Ley et al. 2006). The low diversity in bacterial phyla (13 of 50) contrasts with a wide variety of genera, species, and strains (Fig. 11.12b) identified in both humans and animals (Bäckhed et al.

2005; Ley et al. 2006). Commensal bacteria, symbiotic bacteria (*Bacteroides*, *Lactococcus*), or pathogens such as enteropathogenic *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus*, or *Chlamydia* are those most often found (Bäckhed et al. 2005). Diversity and structure of the microbiota reflect contrasting types of evolutionary selection pressures and the nature of interacting organisms (Ley et al. 2006).

Invertebrates (arthropods and nematodes) consist of phyla including a large number of species, 1 million species described for 4–5 million estimated for the single group of insects (Novotny et al. 2002). Invertebrates harbor diverse microbial communities, some of which contribute to their development and sometimes play an important role in their ability to adapt to extreme environments (Buchner 1965; Nardon and Charles 2001). Most wood-feeding termites depend on microbial partners (bacteria, fungi, protozoan flagellates) to degrade cellulose and lignified compounds in their intestinal tract (Brune 2003). More than 700 species of bacteria belonging to different phyla (Spirochetes, Bacteroidetes, Proteobacteria, Actinobacteria, Mycoplasma) have been described in the gut of termites *Reticulitermes speratus* and *Macrotermes gilvus*. This diversity can vary from one termite type to another (Hongoh et al. 2006; Fall et al. 2007). *Drosophila*, the fruit fly very familiar to geneticists, hosts Proteobacteria (*E. coli*, *Pseudomonas*, *Sphingomonas*), *Bacteroidetes*, and *Mycoplasma* (Mateos et al. 2006). Besides the parasites (*Plasmodium*) or viruses (dengue or Chikungunya) they transmit, mosquitoes belonging to genera *Anopheles* and *Aedes* harbor many acetic acid bacteria including *Asaia*, *Acetobacter*, *Gluconobacter*, and *Sphingomonas* (Favia et al. 2007) or even genera *Aeromonas*, *Acidovorax*, *Bacillus*, *Paenibacillus*, *Pseudomonas*, and *Stenotrophomonas* (Lindh et al. 2005).

Although little studied, worms also host microbes, especially bacteria. For example, filarial nematodes such as *Onchocerca volvulus* (etiologic agent of onchocerciasis or river blindness) and *Brugia malayi* (etiologic agent of lymphatic filariasis such as elephantiasis) shelter in ovarian tissues the bacteria of genus *Wolbachia* that synthesize polysaccharides in infected animals, which constitutes an inflammatory factor aggravating disease (Saint André et al. 2002; Taylor 2003). As it will be described later on, the entomophagous nematodes (feeding on arthropods) of the genera *Steinernema* and *Heterorhabditis* host enterobacteria of the genera *Xenorhabdus* and *Photorhabdus,* respectively, with which they establish symbiotic associations (Forst et al. 1997).

11.4.2.2 Abundance and Location of Microbes

Microbial populations vary in size according to their intracellular or extracellular localization, the surface or inner organ colonized, and depending on the host considered. These numbers can reach relatively high values in certain organs of human and animals. In healthy adult men, densities are $10^{6–7}$ bacteria per cm^2 of skin (Roth and James 1988), $10^{11–12}$ bacteria per ml in the colon (Whitman et al. 1998), and 10^{14} bacteria in the entire intestinal tract. The microbes of the human body account for 10 times the total number of somatic and germ cells. The bacterial densities can greatly increase in cases of bacteremia or sepsis.

Cytological observations showed that in some insects, bacteria can also be localized in specialized cells called bacteriocytes (or bacteriomes) and in oocytes, which promotes vertical transmission from parents to offspring (Baumann et al. 2000). This is the case of bacteria *Buchnera* and *Rickettsia* in aphids or *Wolbachia* and *Spiroplasma* in many Diptera and Hymenoptera (Fig. 11.13). The whitefly *Bemisia tabaci*, the vector of viral pathogens of many plants, can host in a single cell many different bacterial genera, including *Arsenophonus*, *Cardinium*, *Hamiltonella*, *Portiera*, *Rickettsia*, and *Wolbachia* (Gottlieb et al. 2006). The bacteriocytes of the beetle *Sitophilus oryzae* (Fig. 11.13), a pest of stored grain, contain an average of 10^3 bacteria per cell or 10^6 bacteria per insect (Heddi et al. 1999). Apart from these few fragmentary data on insect models, investigations on the associated microbiota remain very limited despite their important role in the adaptation and development of invertebrates. A large effort is under way to study the microbes of insects since many of the latter are devastating pests of crops or vectors of pathogens that cause serious diseases in plants, animals, and humans.

11.4.3 Types of Interactions Between Microorganisms and Animals/Humans

Microbes that establish interactions with animals mostly have environmental origins (air, water, soil, food), but some of them move from one animal to another. An exemplary case of inter-animal microbial traffic is the transmission of the microbiota from mother to newborn. The vast majority of microorganisms are harmless and some can even be used as nutrients for animals. Fungivorous nematodes eat fungi, while bacterivorous ones feed on bacteria, up to 10^6 bacterial cells per day (Blanc et al. 2006). In soils, this predation may significantly alter the composition and structure of microbial communities and therefore the biological functioning of soil, as microorganisms play an important role in various processes such as decomposition of organic matter, solubilization of phosphates, nitrogen-fixing symbiosis, or denitrification. Other microorganisms have spectacular effects on their host, such as light organ differentiation in the squid *Euprymna scolopes* or manipulation of invertebrate reproduction by the bacterium *Wolbachia*.

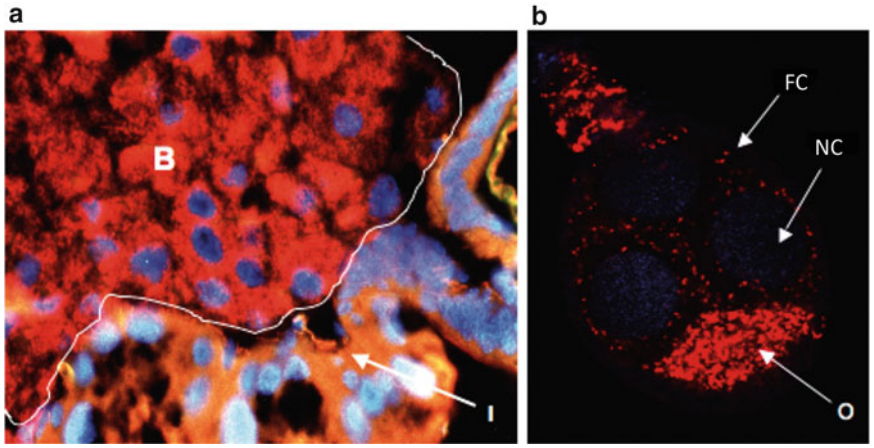


Fig. 11.13 Location of symbiotic bacteria in the tissues of insects by fluorescence in situ hybridization. (**a**) Bacteriome delimited by the white line containing (B) bacteria-filled bacteriocytes SOPE (*red*) *Sitophilus*. The intestine (I) is located in the lower level (Photo A. Heddi, Insects and Interactions Functional Biology, INSA, Villeurbanne, France). (**b**) Egg chamber of the mosquito *Aedes albopictus* with *Wolbachia* (*red*) in the follicular cells (FC), nurse cells (NC), and the oocyte (O) (Photo P. Mavingui and D. Voronin, UMR CNRS 5557 Microbial Ecology, Villeurbanne, France). Bacteria are detected using specific oligonucleotidic probes coupled to the rhodamine. The cell nuclei are stained (*blue*) with DAPI

The type and evolution of relationships between microorganisms and animals, especially along the parasitism-mutualism continuum, depend on the mode of transmission of the microbe (Edwald 1987). Horizontal transmission, by contact between infected and uninfected individuals, tends to favor the evolution to parasitism with intermediate levels of virulence resulting from a trade-off between maximum exploitation of the host and the probability of transmission of the microbe. In contrast, vertical transmission from parents to offspring promotes a reduction in virulence and evolution toward mutualism, fitness depending then on host reproduction. Examples of interactions sustainable or not between microbes and animals are presented hereafter, as well as the mechanisms involved and consequences on the evolution and adaptation of interacting entities.

11.4.3.1 Parasitic Interactions

Microorganisms are the cause of most diseases of man and animals. From the 1,500 microbial pathogens identified in man, over half would be zoonotic (i.e., of animal origin), and many of them are transmitted by hematophagous arthropods (Woolhouse et al. 2005). Nosological entities (microorganisms responsible for a disease) are involved in zoonoses or anthropozoonoses (animal diseases transmissible to humans), in epizooties (animal epidemics), and in human pathologies. These diseases include prion disease (bovine spongiform encephalopathy or BSE), bacterioses (tuberculosis, leprosy, pneumonia), fungal infections (ringworm, candida), parasitoses (malaria, filariasis, sleeping sickness), and various virus diseases (influenza, acquired immunodeficiency syndrome or AIDS, severe acute respiratory syndrome, or SARS, Ebola). Apart from the viruses, in terms of prevalence and incidence, bacteria represent the largest group involved in human and animal diseases; fungal diseases are a minority (*cf.* Chap. 15).

Microbes and Infectious Diseases

Since their appearance on earth about 3 billion years ago (*cf.* Chap. 4), microbes have caused major epidemics and pandemics in humans and animals. Examples of infectious diseases include cholera since antiquity, the plague in the fourteenth century, or tuberculosis that decimated human populations in the nineteenth century and that is unfortunately reemerging in recent years. The Spanish flu of the early twentieth century caused many damages estimated at more than 40 million people. Since its discovery in 1981, AIDS has become pandemic and continues to cause victims. Animals are not spared with outbreaks of diseases such as BSE (or mad cow disease), which is a form that causes the Creutzfeldt–Jakob disease to humans. Since the 1960s, the foot and mouth disease and swine fever have killed and led to the slaughter of millions of animals. Recently, the outbreak of avian influenza due to the highly pathogenic avian influenza virus H5N1 has killed or led to the culling of millions of poultry.

Many parasitoses are among the most devastating infectious diseases in humans and animals, of which vector-borne diseases occupy an important part. One of them is the Chagas disease caused by *Trypanosoma cruzi* transmitted by blood-sucking bugs. Trypanosomiasis is endemic in tropical South and Central America, infecting 300,000 people each year and causing 13,000 deaths. Schistosomiasis is itself considered the second most important parasitic

infection after malaria. This disease is caused by flatworms mainly *Schistosoma haematobium*, *S. japonicum*, and *S. mansoni* and is contracted in water infested with larvae that develop in freshwater snails. Schistosomiasis is endemic in 76 countries mainly located in Africa, South America, Caribbean islands, eastern Mediterranean, and Southeast Asia. More than 600 million people are at risk of infection and 200 million are infected with schistosomiasis, with 20 million cases of severe illness.

Malaria also deserves to be cited as an example since it is the first global pandemic parasitosis. In fact, 107 countries are at risk of malaria, representing three billion people (46 % of the estimated world population of 6.5 billion people). An estimated 350–500 million clinical malaria episodes annually cause the death of 1.5–2.5 million people, including many children (Murray et al. 2012). *Plasmodium*, the causative agent of malaria, is transmitted by mosquitoes of the genus *Anopheles* that suffer only marginally from the parasitic infection. A negative effect of the parasite on fertility of the host mosquito has been identified (Ahmed and Hurd 2006). In humans, *Plasmodium* infects red blood and liver cells during its development cycle, which is one of the most complex cycles in the parasitic world (Box 11.4, Fig. 11.14).

Emergence and Reemergence of Diseases

In the context of epidemics, many new diseases are termed emerging diseases. An emerging disease means a disease whose incidence increases significantly in a given population in a given region, compared to the usual situation of this disease. An exemplary emerging disease is the toxic shock syndrome caused by diffusion into the body of toxins produced by the bacterium *Staphylococcus aureus*, more commonly known as golden staph. Toxic shock syndrome is increasing especially among women as a result of using tampons (during the menstrual cycle), which increases the risk of infection. Avian influenza, as indicated above with highly pathogenic H5N1 virus, decimates sensitive wild birds and poultry and is emerging in different regions of the world. Under certain conditions, the H5N1 virus can cross the species barrier and cause fatal infections in humans, especially in Southeast Asia where the proximity between human population and livestock is the major risk of contamination.

Reemerging diseases are those that reappear after a silent period, short or long, and often in a different form, sometimes more severe. The AIDS pandemic causes multiple microbial coinfections involved in the reemergence of diseases such as tuberculosis. Epidemics of chikungunya or dengue hemorrhagic fevers, human diseases due to viral agents transmitted by blood-sucking mosquitoes, reemerge after long silent periods (7–30 years) in the regions of Africa and Southeast Asia. In 2005, a new strain of highly pathogenic Chikungunya virus carrying a mutation at position 221 (alanine substitution by valine) of the envelope protein E1 was involved in severe and debilitating symptoms as well as in human deaths in the islands of the Indian Ocean (Schuffenecker et al. 2006).

Factors involved in disease emergence and reemergence can vary considerably and are generally poorly known, but there are many risk factors worsening epidemiological changes. These factors include general environmental perturbations (global warming) that will interfere with the circulation pattern of infectious agents and their vectors, urbanization and increased human populations that lead to human impact on the environment, aging of human populations that become weakly immunocompetent, and new land uses and practices. Manufacture of animal meal from the carcass of dead animals, potentially contaminated with prion, and its use in animal feed are one of the causes of the emergence and reemergence of mad cow disease. As well as viruses, prion is an entity that is not independent of the host cell for its multiplication. It should be noted that the prion protein (PRoteinaceous Infectious particle ONly) is present naturally in a nonpathogenic form in mammals (including humans), where it is involved in the development of the nervous system of the embryo. It would also play a protective role against oxidative stress and programmed cell death. It is the mutated form of the prion protein that becomes pathogenic. With the ability to multiply exponentially and self aggregate, the mutated prion destroys neuronal cells and causes deposits in the brain.

International trade can lead to the spread of pathogens, as suggested for H5N1 avian influenza outbreaks which reflect the poultry trade on the Trans-Siberian route (Gautier-Clerc et al. 2007). Other causes are related to the intrinsic adaptive capacity of infectious agents and their ability to acquire genetic information by horizontal transfer (*cf.* Chap. 12). These genetic events may lead to the selection of resistance to biocides (antibiotics and other antimicrobials), which poses serious problems in human and veterinary medicine (Box 11.5).

The Conflictual Nature of Animal–Microbe Interactions

Any pathology arises from uncontrolled conflictual interactions between potentially infectious agents and their hosts. The example of commensal bacteria in animals including humans is striking. These bacteria are called normal flora, confined to specific areas and live in balance with the immune system of healthy individuals. Upon environmental (rain, cold, heat) or immune (injury, poisoning) disturbance, the balance of the interaction can be broken, and the resulting imbalance can lead to the expression of a more or less severe disease in the healthy individual carrier. The infectious agent escapes the control of the host, colonizes unusual tissues and reaches excessive numbers, produces toxins, and can eventually kill its host.

Box 11.4: Cycle of *Plasmodium*, the Etiologic Agent of Malaria

Patrick Mavingui

Injection of *Plasmodium* sporozoites infectious forms is performed by infected females *Anopheles* mosquito as they bite. The sporozoites migrate to the liver via the blood or lymphatic circulation, invade hepatocytes, and differentiate into schizonts that release merozoites into the blood (Fig. 11.14a). In some species, *Plasmodium ovale* and *P. vivax*, a stage known as cryptozoic remains hidden in the liver before waking up several months or years later to restart the cycle. Merozoites infect red blood cells where they differentiate as characteristic rings called amoeboid or trophozoites. The active parasites (merozoites) and erythrocytes pass from the blood to invade new erythrocytes. The sexual stages are then produced and can again be drawn by a female *Anopheles* mosquito following a bite of the infected individual.

In mosquitoes (Fig. 11.14b), the replication cycle takes place in the gut. Gametocytes differentiate into gametes, fuse to generate entire zygote ookinetes which differentiate by passing the barrier peritoneal epithelial cells, change in oocyst which releases a large number of sporozoites. Infective sporozoites migrate through the hemolymph of the mosquito where they are ready to be injected into the blood of the next host and the cycle restart.

Malaria fevers are due to activities of parasites in the blood, which lyse erythroid cells and release toxic substances.

Corresponding to the complexity of the infectious cycle of *Plasmodium*, the triggered immune response is equally complex: activation of macrophages and NK cells (natural killer) that recruit and activate other immune cells such as neutrophils (Baratin et al. 2005). The complexity of the life cycle of these parasites and the high variability of associated antigens makes it difficult to develop a vaccine.

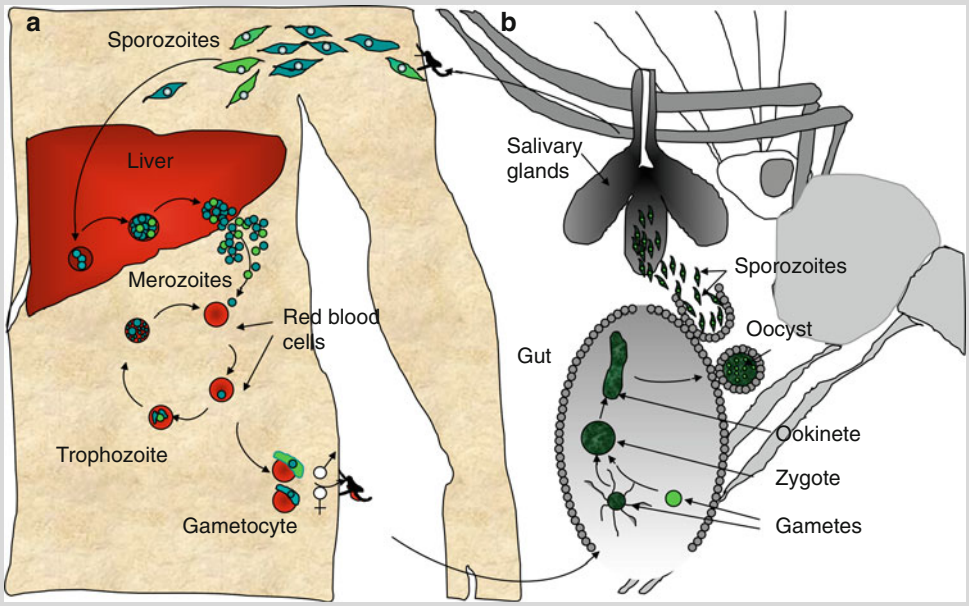


Fig. 11.14 Cycle of *Plasmodium*. (**a**) In human; (**b**) in the mosquito. The female mosquito bites humans and injects *Plasmodium* under the form of sporozoites. They move to the liver, where they develop through several stages into merozoites that invade and multiply in red blood cells, via the trophozoite form. Up to 10 % of red blood cells can thus be infected. The clinical symptoms of malaria, including fever and chills, anemia, and cerebral malaria are associated with infected red blood cells, and most current drugs target this stage of the life cycle. The merozoites of a subpopulation of infected red blood cells develop into gametocytes. Following a sting on an infected individual, the mosquito sucks gametocytes (gametocytes) contained in the blood, which differentiate into male and female gametes. In the gut of the mosquito, the gametes fuse to form a zygote. The zygote develops into ookinetes that pass through the intestinal wall and forms an oocyst filled with sporozoites. When the oocyst bursts, it releases sporozoites that will migrate to the salivary glands (Adapted from Wirth 2002)

Box 11.5: Antibiotic Resistance and Infectious Diseases

Patrick Mavingui

An antibiotic is a chemical substance, natural or synthetic, capable of compromising the growth of bacteria (antibacterial antibiotic) or fungi (antifungal antibiotic). Antibiotics act on specific targets by inhibiting or disrupting biosynthetic pathways essential for the development of microbes. Different microbial taxa such as Actinobacteria and some eukaryotic microorganisms are capable of producing antibiotics (*cf.* Chap. 9). Following major use in human and veterinary therapeutics to fight against microbial infections, the emergence of antibiotic resistance of pathogenic microorganisms, usually sensitive, has become a major public health problem.

Several factors are at the origin of the current resistance. First, they include genetic factors intrinsic to the microbes. Random mutations at the DNA level that alter the targets of antibiotics (mutational resistance) are infrequent and represent only 10–20 % of the resistance encountered in hospitals. The nonspecific mechanisms such as efflux pumps or the production of matrix polysaccharides are also involved in tolerance to antibiotics. Second, there is resistance acquired by horizontal transfer of plasmids carrying the genes for antibiotic resistance (hydrolytic or inactivating enzymes). They are the most numerous and correspond to 80–90 % of resistant isolates in the clinic. This is the case of *Staphylococcus aureus* MRSA (methicillin resistant) and XDR *Mycobacterium tuberculosis* (ultra-resistant TB). Extrinsic predisposing factors are those that facilitate the passage and movement of resistant microbes found in the environment (air, water, soil), plants, and animals. The massive use of antibiotics in different sectors of human activity (human and veterinary medicine, feed, food, etc.) is to be related to the proliferation of resistance, as evidenced by the high frequency of resistant strains in hospitals where antibiotics are commonly used.

The resurgence of multiple resistances in microbes requires the constant development of new antimicrobial molecules but also the application of more appropriate preventive strategies. The use of antibiotics should be carefully considered and rigorously applied.

Box 11.6: AIDS and Its Opportunistic Infectious Process

Patrick Mavingui

The Syndrome of Acquired Immune Deficiency or AIDS is caused by infection by the HIV (Human Immunodeficiency Virus) retrovirus identified by the team of Luc Montagnier (Barré-Sinoussi et al. 1983), which earned him and Françoise Barré-Sinoussi the Nobel Prize for Medicine in 2008. HIV is transmitted through body fluids (blood, breast milk, vaginal secretions, semen). Since its discovery in 1981, AIDS has reached pandemic levels because it has killed more than 25 million people, and 40 million people live with HIV in almost all regions of the world (UNAIDS, January 2006 http://www.unaids.org). In the absence of a vaccine, the disease progression can be delayed for several years by the administration of an antiretroviral triple therapy and improved hygiene of people with HIV. Uncontrolled, the disease leads to a weakening of the immune system and opens the door to opportunistic infections responsible for diseases such as pneumonia and Kaposi's sarcoma that kill infected patients. Many fungi are among the opportunistic pathogens and include the genera *Candida* which causes candidiasis; *Cryptococcus* responsible for cryptococcosis; *Histoplasma*, the causative agent of histoplasmosis; or *Aspergillus* causing aspergillosis. New fungal pathogens are increasingly identified. For some species, the pathogenesis mechanisms are still unknown and cause problems. There are also a large number of bacteria involved in these opportunistic infections (*cf.* Chap. 15).

A dramatic example is that of immunocompromised patients (AIDS (Box 11.6), cystic fibrosis, transplantation, etc.) who are victims of nosocomial infections (from the Greek "nosokomeone", which means hospital and describes what is contracted in hospital) by opportunistic microbial taxa (bacteria, fungi, parasites). In these individuals whose immune system is affected, opportunistic enterobacteria, usually nonpathogenic, such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, or the *Burkholderia cepacia* complex (Vandamme et al. 2007) colonize organs such as the lungs, where they multiply to very high densities, produce biofilms

that clog arteries, and cause tissue necrosis. Biofilms consist of microbial communities of cells attached to a surface (cell matrix) and generally embedded in a viscous substance rich in polysaccharides. Biofilms protect bacteria against phagocytosis by immune cells and prevent the penetration of biocides used to treat patients, at the same time promoting systemic infections and chronic diseases. Some microbes, such as atypical mycobacteria involved in opportunistic lung infections, spread and survive in macrophages, which are immune cells of the host paradoxically specialized in the fight against infectious agents (Sundaramurthy and Pieters 2007).

Another amazing case of weakening of the innate immunity system with a disease outcome in insects lies in the criminal alliance between the entomophagous nematodes *Steinernema* and *Heterorhabditis* and the entomopathogenic bacteria *Xenorhabdus* and *Photorhabdus*, respectively (Goodrich-Blair and Clarke 2007). *Xenorhabdus* and *Photorhabdus* bacteria live symbiotically in intestinal vesicles of the nematodes. When the larvae of nematodes, living freely in the soil, encounter and infect an insect host, they metamorphose into the adult stage, which weakens the immune system of the insect. Bacteria are then released into the blood of the immunocompromised insect and act as opportunistic agents. They multiply and release through their secretion systems toxins that cause septicemia and toxemia, causing the death of the insect. Necrotic tissues in turn are eaten by the entomophagous nematode. Inoculation of the asymbiotic nematode or the bacterium alone does not lead to death of the insect whose immune system can then effectively eliminate the intruders.

In contrary to opportunistic pathogens that exploit immune vulnerability of hosts to cause damage, certain groups of bacteria such as enteropathogens (*Campylobacter, E. coli, Legionella, Listeria, Salmonella, Yersinia*) responsible for diarrheal crises that affect hundreds of millions of people around the world (of which millions will die) are natural pathogens with invasive capacity and production of toxins, which give them a virulent character (Cossart and Sansonetti 2004).

11.4.3.2 Virulence Factors of Pathogens

One of the first virulence factors shared by pathogens of animals such as enteropathogenic bacteria is the ability to overcome the physical barrier (skin and mucosa) through an intimate attachment via structures such as adhesins (Ofek et al. 2003).

Adhesins are the first virulence factors of pathogenic bacteria involved in the infection process (Mainil 2013). They interact with the components, called receptors, present on the surface of eukaryotic cells or on the surface of the extracellular matrix leading to the attachment of interacting cells. Dozens of bacterial adhesins are known and three types of adhesin–receptor interactions with a certain degree of specificity have been described. Bacterial lectins are fimbriae-like structures or have fibrillar proteic nature, which are classified according to their hemagglutination properties, and carbohydrate receptors are components of glycoproteins or glycolipids of cell membranes of the host animal. The second type includes proteins of the cell wall or membrane associated or not to bacterial lipopolysaccharides and peptidoglycans and protein receptors of cytoplasmic membranes of the host cells. Finally, hydrophobins, present on the surface of both bacteria (adhesins) and eukaryotic cells (receptors), are mostly composed of lipid or hydrophobic domains of proteins.

The adhesion of bacteria to the surface of host cells is followed by injection of invasive effector molecules via secretion systems. These secretion systems constitute the second group of virulence factors, which play a role as important as adhesins in the establishment of interactions between microorganisms and hosts. Seven secretion systems, designated type I to type VII (Fig. 11.15) (Abdallah et al. 2007; Filloux et al. 2008), have been described and characterized mainly in pathogenic bacteria and to a lesser extent in the mutualists (Thanassi and Hultgren 2000). The type I secretion system (or ABC transporters) is involved in the transport of a wide variety of substrates (proteins, sugars, lipids) and many processes of bacterial life *per se*. This is by far the most represented secretion system found in many bacteria. Type II (or typical sec-dependent secretion) and type V (or autotransporters) systems are mainly used for the secretion of enzymes and/or other proteins (Sandkvist 2001). The type III (T3SS) and type IV (T4SS) secretion systems have evolved for interaction with other organisms (Cascales and Christie 2003). The T3SS develops a path allowing the bacterium to inject cytotoxic or cyto-destabilizing factors directly into the cytoplasm of the target cell. The T4SS, meanwhile, allows the translocation of nucleoprotein complexes to hosts, either directly inside the target cell or via the extracellular medium (Llosa and O'Callaghan 2004). Mammal cells such as human cells but also plant cells are potential targets, providing an agronomic and medical interest to the study of these systems. Pili formed by the T3SS and T4SS are also involved in the attachment process of bacteria to target cells. The relationships between bacteria and host cells (animal or plant) via the T3SS and T4SS often have a pathogenic outcome and sometimes a mutualistic character (Dale et al. 2002).

Like the bacterial symbiotic islands, genetic determinants of virulence factors of pathogenic bacteria can be located in a region of the genome designated pathogenicity island (or PAI) (Hacker and Kaper 2000). Discovered for the first time in pathogenic *E. coli,* genome analysis showed the presence of PAIs in many other bacterial pathogens of animals. With a

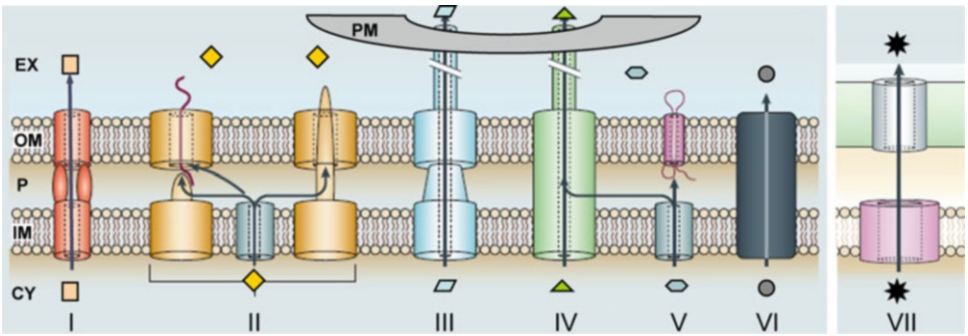


Fig. 11.15 The bacterial secretion systems. In Gram-negative bacteria, the six secretion systems described (named types I to VI) are anchored into internal (IM) and outer (OM) membranes and periplasm (P). Usually, types I, III, IV, and VI carry effectors from the cytoplasm (CY) to the cell surface or to the external environment (EX) in a single step. Types III and IV produce a syringe which will cross the plasma membrane (PM) of the host cell, thereby discharging directly effectors into the cytosol. Types II and V carry the effector molecules in two steps, passing through the periplasm (P) via Sec or Tat. The newly described type VII in Gram-positive bacteria would carry effectors in a single step. *Arrows* indicate the route followed by transported effectors (Adapted from Abdallah et al. 2007 and Filloux et al. 2008)

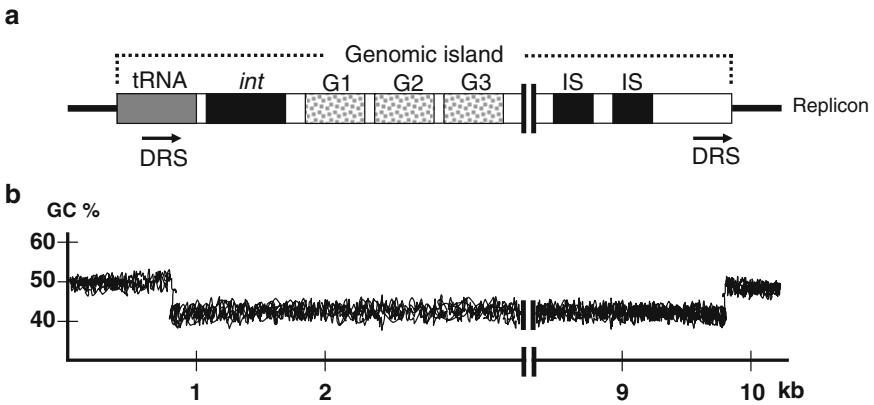


Fig. 11.16 Schematic representation of a genomic island carried by a bacterial replicon. (**a**) The genetic map of the DNA fragment transferred contains a tRNA and two direct repeat sequences (DRS) at the ends. (**b**) The GC content of the island is generally different from that of the entire replicon. Int, integrase; G1 to G3, genes encoding specific functions; IS, insertion sequence; kb, kilobase (Adapted from Hacker and Carniel 2001)

size varying from 10 to 200 kilobases (Fig. 11.16), the PAIs carry genes that encode virulence proteins (adhesins, protein secretion machinery, toxins, invasins, etc.). There is typically a transfer RNA (tRNA) that serves as the site of insertion of PAIs in a genome, sequence repeats generated during the events of horizontal transfer, or different genetic elements (integrases, transposases, insertion sequences) known to provide mobility features of gene modules (Hacker and Carniel 2001). PAIs can move from one region of the bacterial genome to another or be transferred from one strain to another by horizontal transfer. Some PAIs and other virulence factors are in fact carried by mobile elements such as plasmids of bacteria *Shigella* and *Yersinia* (Parsot and Sansonetti 1999) or bacteriophages of enteric bacteria and *Vibrio cholerae* (Karolis et al. 1999). Environmental factors (temperature, pH, osmolarity, oxygen partial pressure, etc.) affect positively (activation) or negatively (inhibition) the expression of virulence genes and thus the pathogenicity of invasive bacteria (Altier 2005).

The case of enteropathogenic bacteria is exemplary; its toxins are injected into target cells through T3SS systems and/or T4SS inducing perturbations of cellular processes and suppression of their defenses. For example, the IpaB shiga toxin of *Shigella* and its *Salmonella* counterpart SipB induce apoptosis (a form of programmed cell death; *cf.* Sect. 10.4.2) in macrophages involved in immune defense (Hersh et al. 1999). Through bypassing the host defenses, enteric bacteria cause septicemia and toxemia resulting in inflammation and diarrhea that allow microbial growth and ecological success.

In addition to toxins excreted by classical secretion systems, metabolic enzymes are increasingly found on the surface of the cell walls of pathogenic microorganisms, without the mode of secretion clearly established. Recent studies indicate the involvement of some excreted glycolytic

enzymes in microbial virulence. These enzymes include glyceraldehyde-3-phosphate dehydrogenase (GAPGH), enolase, aldolase, and pyruvate kinase in staphylococci, mycobacteria or the fungus *Candida albicans* (Pancholi and Chhatwal 2003).

11.4.3.3 Mutualistic Interactions

Relationships between animals and their "normal" microbial communities may evolve toward mutualism, with sometimes spectacular effects on the host biology. These mutualistic associations are maintained stably and sustainably by fitness gains of partners because of their interdependence, as already observed and noted by Charles Darwin (1859). Genetic studies suggest population coevolution cases for mutualistic associations which are often highly specific (Futayama 1986). The phenomenon of coevolution refers to transformations that occur during the evolution of species and the resulting reciprocal influences of interacting partners. Mainly studied in bipartite relations, coevolution may involve mutualistic associations as well as multi-parasitic ones. They may include, among others, transfer mechanisms and interspecies genomic coadaptations, but also qualitative and quantitative controls of microbial communities by the host. Mutualistic interactions between microorganisms and invertebrates are the most studied at the molecular and evolutionary levels, especially insect–bacteria endosymbioses.

Insect Nutritional Symbioses

The example of aphids (Homoptera, Insecta) and their symbiotic bacteria is one of the best described. These insect pests attack almost all plant species feeding off their phloem rich in carbohydrates but low in amino acids. This imbalance is compensated by a mutualistic association with symbiotic bacteria *Buchnera*, which are housed in specific organs of the host, bacteriomes, or bacteriocytes where they play nutritional functions (Douglas 1998). *Buchnera* genomes contain multiple copies of genes that encode the biosynthesis of essential amino acids such as tryptophan and appropriate gene regulators (Shigenobu et al. 2000). The intracellular localization of the bacteria facilitates the transfer of microbial compounds produced to the host. In return, the host provides the bacteria with energy for growth in an environment free of competitors.

Like most obligate intracellular symbionts, unable to grow outside the host cell, the genome of *Buchnera* has undergone a significant size reduction by eliminating a large number of genes (for membranes, metabolism, etc.), whose functions are performed by the aphid. It is possible to identify genes lost in *Buchnera* by comparative analysis with the genome of its closest relative, the bacterial saprophyte *E. coli* (Moran and Mira 2001). From an evolutionary point of view, the mutual dependence between aphids and *Buchnera* dates back nearly 200 million years and the congruence of phylogenies of the partners reflects a coevolutionary process supported by vertical transmission of *Buchnera* across generations of aphids (van Ham et al. 2003). In addition to *Buchnera*, aphids are hosts of many bacteria termed secondary symbionts. The role of these clandestine passengers is now better understood. Some of these secondary symbionts are involved in tolerance to heat stress, and resistance to parasitoids, which are insects that develop as aliens at the expense of other insects they parasitize, which content they consume and kill (Oliver et al. 2003; Dunbar et al. 2007; Moran et al. 2008).

Fig. 11.17 Low- and high-magnification views of the giant worm *Riftia pachyptila* (Copyright: Ifremer-Nautile/Campagne Mescal 2010)

A spectacular multipartner symbiotic interaction in the world of insects is that between social ants of the genus *Atta* and some fungi (Bacci et al. 1995). Unlike humans and herbivorous animals that depend on endosymbiotic microbial partners to digest cellulolysic compounds of the plants they eat, ants have adopted an ectosymbiotic strategy that is surprising but effective. They grow in their nest cellulolytic fungi of the genera *Leucoagaricus* and *Lepiota* that they feed with plant material brought from the outside, often also causing considerable damage in the tropics. Fungi decompose the plant material, use it as nutrient, and develop numerous hyphae that can grow to represent large biomass. In return, ants, exclusively mycophagous, feed the fungal hyphae. Even more remarkable, these ants establish a symbiotic association with bacteria of the genus *Burkholderia* that produce an antibiotic involved in the fight against other fungi that contaminate nests (Santos et al. 2004).

Symbiosis with Marine Animals

The discovery of the giant worm, *Riftia pachyptila* (Fig. 11.17), present in hydrothermal vents has permitted to study an equally singular nutritional symbiosis (Jones 1981; Cavanaugh et al. 1981). This annelid devoid of a digestive tract has a specialized organ, the trophosome, which hosts symbiotic sulfur-oxidizing bacteria belonging to the Proteobacteria (Distel et al. 1988). To feed,

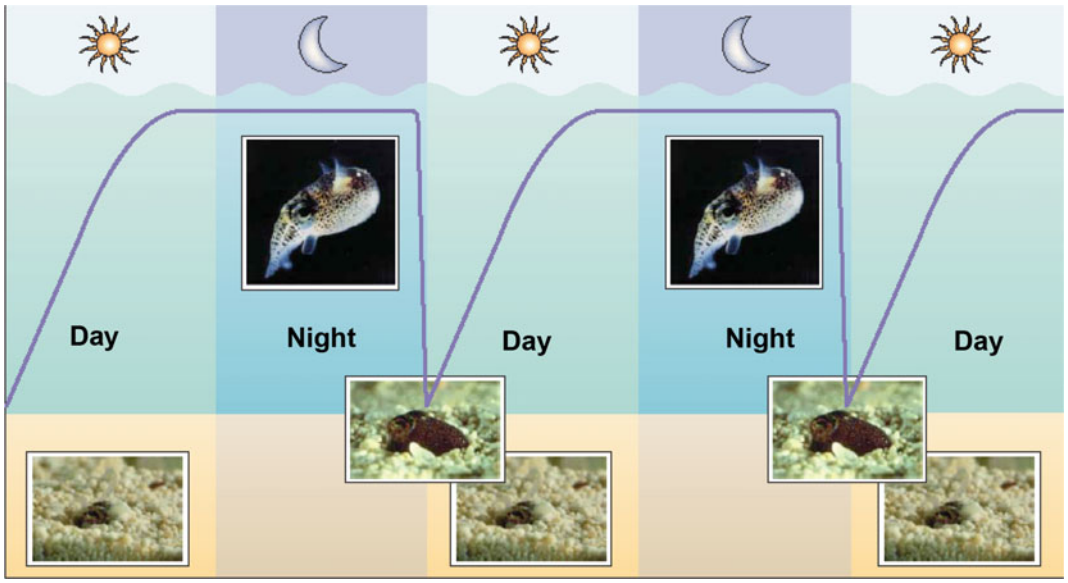


Fig. 11.18 Symbiosis between the squid *Euprymna scolopes* and *Vibrio fischeri* and the phenomenon of chemiluminescence. The *E. scolopes* squid hosts in its light organ the domesticated bacterium *V. fischeri* whose density increases over the day to reach a maximum after dark (blue curve), thereby producing a strong luminescence. This behavior allows the squid to hunt at night while escaping predators by camouflage. At daybreak, almost all (~90 %) bacteria are expelled into the environment; *E. scolopes* buries itself in the sand. During the day, the remaining bacterial population (~10 %) in the light organ is multiplied in order to reach again the maximum density required to produce luminescence at night (Adapted from Nyholm and McFall-Ngai 2004)

R. pachyptila absorbs through its gill filaments oxygen and hydrogen sulfide that are transported via the blood to the trophosome, where bacteria perform chemosynthesis, generating organic matter necessary for the growth of the host (*cf.* Chap. 10).

Another exemplary mutual interaction of the marine world is observed in the association between the squid *Euprymna scolopes* and the alphaproteobacterium *Vibrio fischeri* (Nyholm and McFall-Ngai 2004). This squid that lives mainly on the seabed is exposed to predation when swimming back to the surface at night. To escape predators, *E. scolopes* emits a ventral light that serves as camouflage concealing its silhouette against the light of the stars. The emission of light by the squid results from the phenomenon of bioluminescence produced by *V. fischeri* (formerly *Photobacterium fischeri*) located in a specialized light organ (photophore) of the host (Fig. 11.18). Although the initiation of morphogenesis of the photophore occurs without the intervention of the bacterium, the latter is involved in postembryonic maturation among others acting on actin filaments of the cytoskeleton (Montgomery and McFall-Ngai 1994; Kimbell and McFall-Ngai 2004). In the photophore, *V. fischeri* can reach high densities in the order of 10^{10} cells per ml, and the light is emitted at a certain bacterial density (or quorum perceived by homoserine lactone-like molecules; *cf.* Chap. 9). At dusk, *E. scolopes* expels almost all bacteria and quorum (optimal number) required for light emission is reached again at night.

Many other bacteria are found associated with marine animals, particularly in oceanic ridge ecosystems, whose functions are gradually beginning to be elucidated. This is the case of sulfur-oxidizing and methanotrophic gammaproteobacteria in clams of Mytilidae group *Bathymodiolus*. These symbiotic bacteria can reach 10^{10} to 10^{11} cells per g of gill tissue (Yamamoto et al. 2002) where they use sulfide, thiosulfate, and methane as energy sources (Nelson and Fisher 1995). These symbiotic chemosynthetic systems are a significant component of the carbon cycle in the ocean, and organic matter resulting from autotrophy, whether thiotrophy and/or methanotrophy (*cf.* Chap. 14), is a major source of carbon for animals that populate these particular environments.

Microbe-Vertebrate Mutualism

In vertebrates, the mutualistic associations between the indigenous microbiota of the intestine and the animals are clearly established in the carbon metabolism of ruminants and monogastric coprophages. The microbiota of the intestinal tract is structured in trophic network that plays an essential role in the degradation of polysaccharides such as cellulose and lignin of plant material ingested by the hosts (Zhanq et al. 2007). From this mutualistic association, ruminants and coprophages draw energy as carbohydrates, while microbes are protected in a germ-free biotope and are supplied with nutrients.

The mutualistic nature of the human intestinal microbiota has often been deduced from the data obtained in animals.

Indeed, mammalian cells are also unable to digest most polysaccharides of plant origin except starch, which therefore depends on the human microbiota. Active anaerobic intestinal bacteria in the gut, anoxic, are similar between humans and animals. Recent work confirms the existence of mutualistic bacteria in the human gut. Indeed, the genomes of the obligate anaerobic bacteria *Bacteroides*, the dominant human gut bacterial genus, confirm the presence of genetic determinants and proteins involved in the degradation of polysaccharide compounds (Xu et al. 2003). In addition, inoculation to laboratory mice devoid of microbiota and maintained under axenic conditions, of strain *Bacteroides thetaiotaomicron* VPI-5482 isolated from the human intestine, leads to a pleiotropic effect: activation of carbon metabolism and storage of lipid compounds (Bäckhed et al. 2004), stimulation of cells producing antimicrobial compounds, and microvilli of the intestinal wall (Stappenbeck et al. 2002; Hooper et al. 2003). Another effect called **probiotic*** was supported by some data. The concept of probiotics has been proposed by the Russian Nobel Prize winner Elie Metchnikoff. It is defined as any living microorganism which, when ingested at a certain amount, exerts beneficial effects beyond basic nutritional functions. The probiotic microorganisms most frequently cited are lactobacilli and bifidobacteria, and the reported effects range from changing the intestinal ecology to the stimulation of the immune system or the decrease of the risk of cancer (Ouwehand et al. 2002). The probiotic issue has generated many controversies.

The prevalence and persistence across generations of microbes that play an important role in nutrition, in immunity, and in development are clear proof of the mutualistic nature of animal-microbe associations.

11.4.3.4 Commensalism, Parasitism, and Other Interactions with Intermediate Phenotypes

Overview

A large number of microorganisms that live on the surface and in the internal organs of animals benefit from this habitat without producing observable effects in their hosts. These are microorganisms known as commensals, among which are mainly bacteria such as *Pseudomonas, Acinetobacter*, and *Bacillus* in insects or *E. coli, Staphylococcus, Corynebacterium,* and *Lactobacilli* in mammals. Some of these bacterial taxa are also, as noted above, the pathogenic or mutualistic flora. Commensal microbiota that colonizes the surface of a given organ can prevent through a physical barrier effect the colonization of other bacteria, including pathogens. In contrast, the commensal microflora may represent a hazard to animal health as some groups carry genes for antibiotic resistance that may be acquired by pathogens by transformation, conjugation, or transduction. Transfer of antibiotic resistance genes between bacteria of animal and human origin has been identified in the gastrointestinal tract of animals (Moubareck et al. 2003). It has been seen that many commensal microbes colonize the skin or integument of animals. Some of them are involved in the production of volatile and odorous molecules that may have a direct or indirect impact on health. This is particularly the case of compounds of microbial origin that act as attractants toward mosquitoes (Brady et al. 1997) whose bites cause viral or parasitic infections.

Genomics can allow comparative research on the differences and similarities at the genetic and functional relationship between commensal microbes, parasites, and mutualistic animals. The distinction between these interactions is sometimes difficult to establish. Whether pathogenic or mutualistic, commensal or parasitic, microorganisms share common genetic determinants and adaptations that allow them to colonize many environments (Hentschel et al. 2000; Goebel and Gross 2001). The similarity of the processes involved in the expression of a bipartite or multipartite symbiotic interaction can lead to intermediary phenotypes that are not always obvious to position in traditional categories (pathogenesis, parasitism, cooperation, mutualism, etc.).

Wolbachia and Phenotypic Pleiotropy

The case of bacteria of genus *Wolbachia* is exemplary because they establish associations with their hosts yielding multiple phenotypes (**pleiotropy***) which lie in the parasitism–mutualism continuum. These alphaproteobacteria of the order *Rickettsiales* infect filarial nematodes of the family *Onchocercidae* and many arthropods such as mites, crustaceans, and insects (Werren et al. 1995; Bandi et al. 2001). *Wolbachia* is mutualistic of filarial nematodes that include human pathogens such as *Onchocerca volvulus* involved in human onchocerciais or river blindness or *Wuchereria bancrofti* or *Brugia malayi* both responsible for elephantiasis. Bacteria are located in the hypodermal cells of the lateral chords of larvae but also in the ovaries in adult females and are thus transmitted transovarially to offsprings (Taylor et al. 1999; Fisher et al. 2011). Polysaccharides of *Wolbachia* represent an aggravating factor in inflammatory processes associated with filariasis (Saint André et al. 2002). The elimination of *Wolbachia* by antibiotics (rifampin, tetracycline) from infected nematodes leads to inhibition of embryogenesis and larval development (Bandi et al. 2001). Therapeutic trials combining antifilarial drugs (albendazole and ivermectin) and antibiotics (tetracyclines) have been conducted with some success against infection with *Wuchereria bancrofti* (Turner et al. 2006; Johnston and Taylor 2007).

In arthropods, *Wolbachia* induces multiple effects to the hosts. A pathogenic effect has been shown in drosophila; *Wolbachia* strain *w*MelPop is able to grow at high densities in nervous tissue and causes fatal bacteremia (McGraw et al. 2002). However, in most cases, *Wolbachia* manipulates the reproduction of their arthropod hosts. Four effects of

reproductive manipulation are known: feminization of genetic males, male-killing by larval development arrest, parthenogenesis in haplodiploid Hymenoptera, and cytoplasmic incompatibility, which is a non-fertile cross between infected males with *Wolbachia* and uninfected female or carrying a different strain of *Wolbachia* (Werren et al. 1999). *Wolbachia* is thus qualified as a parasite of the reproduction of its host. These reproductive manipulations have resulted in biased sex ratios disadvantaging males, thus generating more females that transmit *Wolbachia* transovarially to their descendants. This explains the high prevalence of *Wolbachia* in natural populations of arthropods, estimated at up to 76 % in insects (Jeyaprakash and Hoy 2000; Hilgenboecker et al. 2008). A phenotype of mutual dependence was found in some arthropods where *Wolbachia* is required for normal reproduction or development. In the hymenopteran *Asobara tabida*, *Wolbachia* has become necessary for oogenesis in females (Dedeine et al. 2001). The elimination of *Wolbachia* by antibiotics generates asymbiotic individuals completely devoid of oocytes, and ovarian tissues showed early manifestations of apoptosis (Pannebakker et al. 2007). The bedbug, *Cimex lectularius*, hosts *Wolbachia* in a bacteriome and the two partners seem to establish a nutritional mutualistic symbiosis through the provisioning of vitamins (Hosokawa et al. 2010). Remarkably, *Wolbachia* is also able to protect drosophila against viral pathogens (Hedges et al. 2008), and in some cases this bacterium inhibits the transmission of pathogens by the mosquitoes (Moreira et al. 2009; Mousson et al. 2012).

There is a unique situation where the same bacterium induces various effects on the hosts, differing in nature and intensity, either on their physiology or reproduction, and is ranged in a continuum from parasitism to mutualism. These associations thus raise many questions in evolutionary biology (evolution of mutualism, coevolution, role in the speciation of hosts), epidemiology (diffusion process), cell biology (cellular targets, signaling), and molecular biology (structure, functioning, and evolution of the genome). The mechanisms underlying these interactions are unknown. Effectors secreted via the *Wolbachia* T4SS (Rancès et al. 2008) have been proposed. Many genes encoding proteins with ankyrin domains known to be involved in protein–protein interactions are potential candidates present in complete genomes of *Wolbachia* (Wu et al. 2004; Foster et al. 2005; Klasson et al. 2008). Transfers of *Wolbachia* genes to insects and from the insect to the bacteria have been reported following the sequencing of genomes (Hotopp et al. 2007; Klasson et al. 2009).

11.4.4 Defense and Counteroffensive in Microbe–Animal Interactions

Animals interacting with microbes have developed a set of mechanisms and weapons to contain or eliminate potentially infectious agents. These mechanisms include phagocytosis by immune cells such as macrophages, secretion of toxic compounds, or inhibitors such as cytokines and reactive oxygen species. Discovered in drosophila and in many other arthropods and vertebrates, the production of antimicrobial peptides is part of the humoral response against microbial infection. The synthesis of antimicrobial peptides is regulated by molecules of the nuclear factors kappa B (NF-kappaB) family, such as DIF and Relish in *Drosophila melanogaster*. Nuclear factor DIF is mainly activated in response to fungal infection or by Gram-positive bacteria, whereas Relish plays a role during infection by Gram-negative bacteria (Ferrandon et al. 2007).

Programmed cell death (PCD) is a manifestation of defense commonly observed in response to infection by microbes, either pathogenic or mutualistic (Williams 1994; Vavre et al. 2008). In contrast, many microorganisms have developed mechanisms to circumvent the host defenses by detoxification, immunosuppression, or molecular mimicry for their survival and proliferation (Silver et al. 2007).

11.4.4.1 PCD as a Means of Defense of Animals

PCD is an integral part of the development program of higher organisms. **Apoptosis*** is the best known and described PCD in insects, nematodes, and mammals (Jacobson et al. 1997). It takes place during development and is involved in the elimination of damaged cells and in maintaining the homeostasis of immune cells (Vaux et al. 1994). Apoptosis involves the caspase enzymes that are activated by many stimuli. Two apoptotic pathways are known: the extrinsic pathway is triggered by TNFR family receptors (Tumor Necrosis Factor Receptor) and the intrinsic pathway that is activated by intracellular signals such as oxidative stress and involves the disruption of mitochondria (Fig. 11.19). From a phenotypic point of view, apoptotic cells have their nuclear DNA fragmented.

In addition to its role in development, apoptosis is involved in the defense against microbes and participates in the elimination of infected cells. Its induction is initiated after contact and perception by host cell receptors of microbial components or structures designated MAMPs (microbe-associated molecular patterns) or PAMPs (pathogen-associated molecular patterns). These components that are specific to microorganisms are composed of lipopolysaccharide (LPS), peptidoglycan (PGN), and lipoteichoic acids of the cell walls. Host cell receptors that are on the front line for detecting MAMPs and PAMPS are designated PRRs (pattern recognition receptors), the most reknown being the Toll-like receptors or TLRs. Discovered first in the innate antibacterial response in drosophila (Hoffmann 2003), TLR homologues were subsequently identified in other animals including mammals (Medzhitov et al. 1997). TLRs are also involved in the induction of apoptosis in

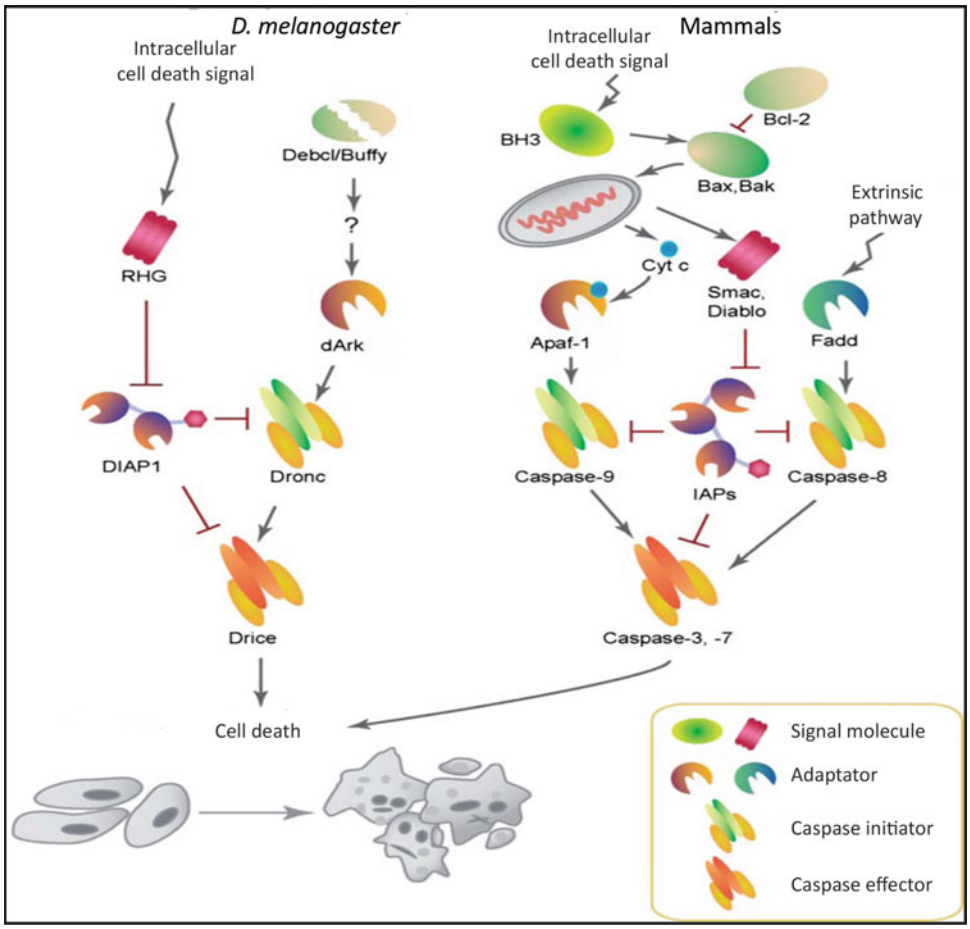


Fig. 11.19 Comparison of apoptotic pathways between drosophila and mammals. In *D. melanogaster,* dArk adapter (protein homologous to Apaf-1 in mammals) activates the initiator caspase Dronc. Proteins having pro- and antiapoptotic domains Debcl and Buffy, belonging to the Bcl-2 family, may regulate the activation through dArk, but this step has not been demonstrated. The apoptosis inhibitor protein, DIAP-1, negatively regulates activity of Dronc and of the caspase effector Drice. The RGH family proteins (such as Rpr, Hid and Grim) activate apoptosis by inhibiting the action of DIAP-1 on caspases. In mammals, the intrinsic pathway is characterized by the activation of caspase 9 (functional homologue of Dronc) by Apaf-1. But the activity of Apaf-1 depends on the Bcl-2 family proteins: Bax and Bak have a proapoptotic activity, while Bcl-2 inhibits this activity to prevent apoptosis. Intracellular death signals activate BH3 domain proteins, thereby promoting activity of Bax and Bak. The latter form pores in the mitochondrial membrane resulting in the release of proteins that either promote the activation of the Smac/Diablo complex, preventing the action of the inhibitor of apoptosis proteins (IAPs), or activate Apaf-1 (role of cytochrome C). In the extrinsic pathway, the cell death receptor is activated by its specific ligand which causes the recruitment of the FADD adapter. FADD can then activate caspase 8, an initiator caspase. Finally, the two routes meet in the activation of effector caspases (such as caspases 3 and 7, Drice homologues) that lead to cell death (Adapted from Hay and Guo 2006)

response to microbial infection (Salaun et al. 2007). For example, the perception of bacterial LPS by TLR4 on endothelial cells leads to the induction of apoptosis (Bannerman and Goldblum 1997; Aliprantis et al. 1999). In mammalian cells such as mice, apoptosis mediated by TLR4 confers resistance to infection due to the perception of the pneumococcal virulence factor, pneumolysin (Srivastava et al. 2005). To eliminate or stop the progression of the infection with *Shigella* or *Mycobacterium*, infected macrophages can undergo apoptosis resulting in concomitant death of these bacterial pathogens (Zychlinsky and Sansonetti 1997).

In insects, the involvement of apoptosis in the defense mechanism was shown for the first time in response to infection of the butterfly *Spodoptera frugiperda* by baculovirus (Clarke and Clem 2003). Phenomena associated with apoptotic caspase activity were observed in mosquitoes infected with *Plasmodium* (Hurd et al. 2006).

11.4.4.2 Modulation of Apoptosis by Microorganisms

Microbial pathogens have developed various mechanisms to inhibit apoptosis in host cells to facilitate their replication and persistence. Microbes can also induce apoptosis in immune cells to destroy these cells thereby facilitating their escape. The ability to circumvent host defenses by modulating apoptosis is found in various microbial taxa,

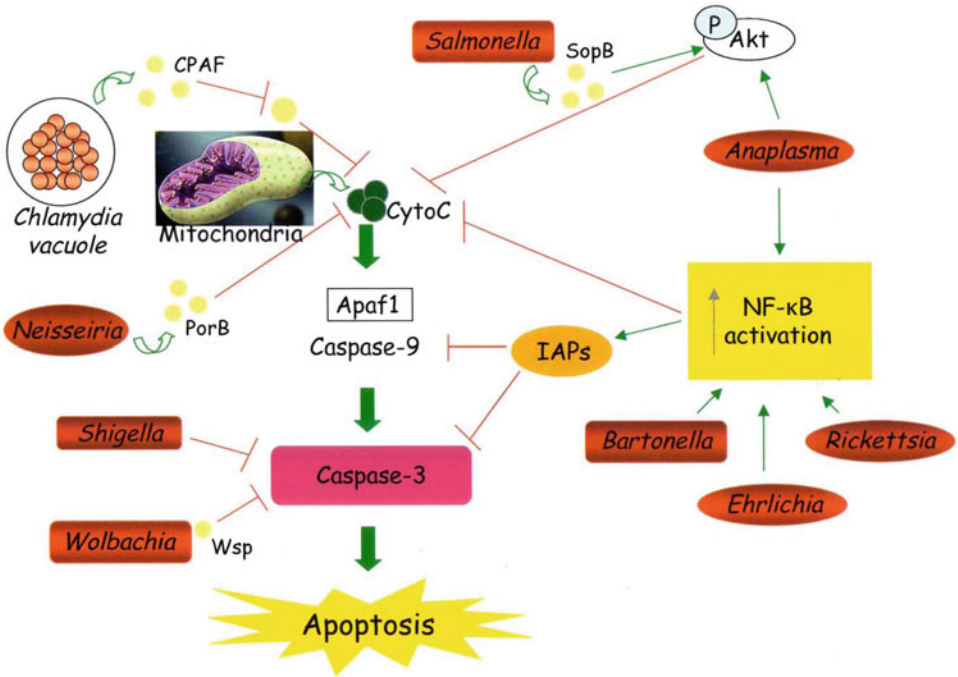


Fig. 11.20 Different bacterial mechanisms involved in the inhibition of apoptosis. *Chlamydia* sp. secretes the CPAF (Chlamydial Protease/proteasome-like Activity Factor) that has proteasomal activity and degrades a proapoptotic protein, thereby preventing the output of cytochrome C in mitochondria. *Neisseria* sp. secretes the PorB protein which inhibits the release of cytochrome C. *Salmonella enterica* SopB secretes the protein that activates the phosphatidylinositol 3-kinase pathway-Akt (PI3K/Akt) inhibiting the release of cytochrome C. *Anaplasma phagocytophilum* activates the PI3K-Akt and NF-kappaB activation allows inhibitor of apoptosis proteins (IAPs). *Bartonella* sp., *Ehrlichia chaffeensis*, and *Rickettsia rickettsii* activate NF-kappaB. *Shigella flexneri* inhibits caspase activation. *Wolbachia* may inhibit the activation of caspase-3 using proteins such as WSP or certain proteins with ankyrin domains. *Red lines*: inhibition of apoptosis. *Green arrows*: activation of apoptosis inhibitory pathways (Adapted from Faherty and Maurelli 2008)

especially in intracellular microbes (Roulston et al. 1999; Gao and Abu Kwaid 2000).

Inhibition of Apoptosis by Microorganisms

Commensal and pathogenic bacteria can inactivate apoptosis by modulating the extrinsic and intrinsic pathways (Fig. 11.20). Facultative intracellular bacterium *Mycobacterium tuberculosis*, the causative agent of chronic tuberculosis, can prevent apoptosis in macrophages where it multiplies and persists. To this end, *Mycobacterium* increases the production of TNF receptors which in turn activate the antiapoptotic pathway NF-kappaB (Balcewicz-Sablinska et al. 1998). Another facultative intracellular bacterium, *Bartonella henselae*, involved in bacillary peliosis, prevents endothelial cell apoptosis by inhibiting caspase activation and DNA fragmentation (Kirby and Nekorchuk 2002). Obligate intracellular bacteria of the genus *Chlamydia*, involved in various human infectious diseases, can protect infected cells against apoptosis during the early phase of infection by blocking the proapoptotic caspases or by preventing the release of cytochrome c from mitochondria (Fan et al. 1998). Similarly, strict intracellular pathogens belonging to the order *Rickettsiales* are able to inhibit apoptosis. This is the case of *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever, which inhibits apoptosis of infected cells by activation of NF-kappaB (Clifton et al. 1998), while *Anaplasma phagocytophilum*, responsible of human anaplasmosis, prevents apoptosis of infected neutrophils through the transcriptional control of the antiapoptotic BFL1 and inhibition of the activation of apoptosis mediated by mitochondrial caspase 3 (Ge et al. 2005). Recalling that, as indicated above, the invasive success of the symbiotic bacteria *Wolbachia* would involve inhibition of apoptosis among other traits.

Finally, protozoan parasites such as *Leishmania*, *Plasmodium*, *Toxoplasma*, and *Trypanosoma* have antiapoptotic capabilities that allow them to invade mammalian cells by modulating different known control points of apoptosis, including Bcl-2 pathways and NF-kappaB (Heussler et al. 2001).

Activation of Apoptosis by Microbes

Activation of apoptosis in host cells often allows microbes to destroy immune cells or escape from infected target cells (Weinrauch and Zychlinsky 1999). Again caspase-mitochondria-dependent pathways are concerned. Upon infection of macrophages by mycobacteria, apoptosis is induced after attachment to TLR2 and activation of

proapoptotic pathways and caspase-1 TNF-alpha (Rojas et al. 1999). Exemplary cases of apoptosis induced by microbes involved pathogenic *Shigella* and intracellular *Salmonella*. To escape from macrophages, these bacteria excrete effectors via a secretion system directly into the cytoplasm of the host cell (Mills et al. 1997). Injected toxins activate caspase-1 and induce apoptosis, thus facilitating systemic infection as demonstrated in mice (Monack et al. 1998).

Functional Duality: Inhibition and Activation of Apoptosis

Many microbial pathogens can use pro- and antiapoptotic activities to promote their replication and diffusion in the host cells. This seemingly paradoxical behavior is found in many viruses and some bacteria such as *Chlamydia* and mycobacteria (Miyairi and Byrne 2006). It is suggested that the antiapoptotic activity allows replication and generation of enough infectious entities in primo-infected host cells. Then, apoptosis is activated in the latter stages of infection to facilitate the spread of microbes to other surrounding host cells. Cell tropism has been observed in *Chlamydia* that has a functional duality: inhibition of apoptosis occurs primarily in phagocytic cells in which infectious agents multiply, while apoptosis is activated in immune response cells such as T cells (Miyairi and Byrne 2006). The complex interactions that occur during the modulation of apoptosis often involve the expression of many genes. However, in some cases the product of a single gene of microbial origin may be at the origin of apoptosis modulation. First shown in baculovirus (Clarke and Clem 2003), the involvement of a single gene in the control of apoptosis has been found in many other pathogens. For example, in *Photorhabdus*, the *mcf* gene alone is sufficient to induce apoptosis in insect cells (Daborn et al. 2002). In a similar way, the *nuoG* virulence gene in *Mycobacterium tuberculosis* is sufficient to inhibit apoptosis of host cells (Velmurugan et al. 2007).

11.4.5 Applications in the Interactions Between Microorganisms and Animals

11.4.5.1 Microbes and Nutrition

Microbes play an important role in human and animal nutrition. Following the ingestion of food by animals, the natural process of digestion through the action of enzymes leads to the conversion of macromolecules into simple compounds (sugars, amino acids) that can be assimilated and that are involved in the production of the energy needed for growth and development of organisms. However, we have seen that through digestive enzymes eukaryotic cells are unable to degrade certain macromolecules of plant origin, such as cellulose or chitin. This function is then performed by microbial communities (bacteria, fungi, protozoa) of the digestive tract.

The products of metabolism of the microbiota also contribute to animal nutrition, such as amino acids and various vitamins (B, C), whose deficiencies are the cause of many diseases such as anemia or beriberi. Food industries use microorganisms to produce these essential compounds that can be incorporated as dietary supplements.

Finally, microbial fermentation has been used since antiquity in the manufacture of dairy products (milk, yogurt, cheese), but also in the production of alcoholic beverages such as beer and wine, which excessive consumption may in turn adversely affect human health.

11.4.5.2 Microbes and Health

In the field of human and animal health, antibiotics of bacterial origin are the major molecules used in treatments against nosocomial and community-acquired infections. The group of Actinobacteria, in particular, the genus *Streptomyces*, is the major producer of antibiotics (glycosides, penicillins, tetracyclines, *cf.* Chap. 9), antifungals (nystatin, amphotericin B), and immunosuppressants such as cyclosporine used during organ transplantations. Some anticancer compounds are also produced by microbes. Other microbial molecules used for public health purposes include dextrans, a substitute for blood plasma, numerous steroids for hormone treatments. Microbes themselves, once inactivated by heat or ionizing radiation, can be used in immunization.

Many infectious diseases are transmitted by blood-sucking arthropods such as mosquitoes, ticks, and lice. The absence of a vaccine against many of these diseases renders indispensable the control of arthropod vectors. Spores of the bacterium *Bacillus thuringiensis* have been used since the 1950s to fight against a large number of crop pests and vectors of animal and human pathogens. However, vectors increasingly develop resistance toward the products used, and, on the one hand, the production of new chemical compounds is hindered by the financial costs for research and development and, on the other hand, by the increasing risk linked to the toxicity of these products, both for human and animal populations but also for the environment. In this context, the exploitation of microbial communities as biopesticides for vector control, in addition to genetic and chemical agents, is a strategy globally promoted worldwide. Knowledge of interaction mechanisms and effector molecules of pathogens can lead to the development of antagonistic molecules that block infection or spreading of infectious agents. It should be recalled that the commensal microflora that colonizes the surface of a given organ such as the skin can prevent by simple physical barrier the colonization by pathogens.

Finally, it should be noted, in addition to many cancer-causing chemicals, that microorganisms are also responsible, at least for the initial mechanisms, of cancer diseases. For example, the bacterium *Helicobacter pylori*, which infects about two-thirds of the world population, plays a major role in the development of gastric cancer. Human papilloma virus (HPV) is itself responsible for almost all cervical cancers, while Kaposi's sarcoma is linked to infection with the virus of the herpes family (HHV8, Human Herpes Virus 8). Progress in the identification and characterization of these microbial pathogens as well as knowledge of infectious processes and physiopathology can exploit the microbes own weapons to develop effective therapies against these diseases that too often prove fatal.

11.4.6 Epilogue

Interactions between microorganisms and animals are part of a continuum from parasitism to mutualism. They generate associated entities whose properties often exceed the sum of preexisting structures in the interacting partners. The complexity of associations is a major driver of the evolution and adaptation of species. A better understanding of the interactions between microorganisms and animals in the environment where they operate provides knowledge with multiple outcomes in all areas. We can mention basic biological functions of genes and their products, as well as regulatory networks at the molecular and cellular levels. We can also quote medicine in the development of diagnostics and the development of new therapies against infectious diseases including recurrent reemerging ones or biotechnology on the production of antimicrobial molecules or interest in food and livestock production. The advent of DNA sequencing of the complete genomes of microorganisms and their animal hosts, including the human genome, provides new insights for understanding the interactions between microbes and animals, which should be optimized to the benefit of humans and its environment.

11.5 Conclusion

Biotic interactions, whether transient or sustainable and intraspecific or interspecific, have played and continue to play a major role in the development of microorganisms and macroorganisms with which they are associated, substantially altering the structure and the level of organization. They were decisive in the evolution of organisms, as exemplified by the endosymbiosis as the origin of the eukaryotic cell structures, and have contributed to the adaptation and biodiversity of interacting organisms. Microorganisms interact with each other but also with mobile genetic elements and macroorganisms occupying the same ecosystems. In addition, biotic interactions involve a large variety of entities, unicellular and multicellular, and concern different levels of complexity, starting from the molecule and the gene through the cell, tissues, and organs to the shape of organisms.

Microorganisms stand prominently in the associations between living entities as they are involved in all types of known biotic interactions. The complexity of these interactions and the importance of multiple interactions make them difficult to understand, and many of them are difficult to describe with the scientific terminology currently available. Moreover, the meaning of concepts has changed significantly over time, including improved knowledge, and the meaning of terms describing biotic interactions can vary significantly from one discipline to another.

The importance of biotic interactions implies that microorganisms must first be defined by the number of interactions in which they participate (and thus their social life) and microbial communities (and possibly associated with eukaryotic hosts) in terms of networks of interactions. In food webs, microorganisms provide essential metabolic functions, from primary production to decomposition, and they are essential to the mobilization of flow of matter and energy in the environment. Interactions between microorganisms and multicellular eukaryotes also have a major impact on the health of plants, animals, and human in ecosystems and on ecosystem health itself.

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