9 Symbiosis of Plants, Animals, and Microbes

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INTRODUCTION

A diversity of plants, animals, and microbes on Earth abounds due to evolution, climate, competition, and symbiosis. Single-cell species such as microorganisms are assumed to have evolved initially. Over time, plants and animals established and flourished. As each new kingdom of life came about, the ecosystem on Earth became more complex and the bionic components became more interactive. Symbiosis, in a broad definition, is "the living together in an intimate association of two or more dissimilar organisms." Symbiosis can result in a relationship in which both organisms benefit. Nitrogen fixation by legumes is a consequence of microbes that fix nitrogen and plants that supply simple carbons. Plants and fungi have established a cooperation in which the plant provides nutrients and the fungi provide alkaloids to deter predation and allow for greater drought tolerance. More generally, plants and herbivores have essentially co-evolved such that the action of herbivores on plants can lead to greater diversity and dispersion of seed. Complex cellulose degradation of plant material

by herbivores is accomplished by specialized bacteria in gastrointestinal compartments that are optimally maintained by each host animal for bacterial growth. Within the mammalian digestive tract, commensal microorganisms can provide energy, amino acids, and vitamins for the host, and provide protection against parasitic microorganisms. This chapter focuses on environmental sustainability of the many symbiotic relationships among plants, animals, and microbes that enhance our global food production.

LIFE ON EARTH

Life on Earth is complex and interactive, with organisms forming populations, which in turn form communities, or ecosystems, both locally and globally. The ecology is defined by the interactions between species and their composition within that system that drives natural selection, evolution, and genetic composition. The fitness for survival of an organism in any ecosystem is not dependent solely upon the species, but includes the interactions of other organisms with that species. Interactions between and among species within an ecosystem can be simple or complex, competitive or beneficial, predatory or symbiotic. Within a similar order, such as plants or animals, competition for resources can select for the better-fit species under one set of conditions, whereas predation results in one species consuming another.

Humans, through the development of agriculture, have identified and exploited different species for food production. Consequently, our desire for better production has often required control of the ecosystem. More importantly, the usefulness of a particular plant or animal species is often dependent on interactions with other species, including plant, animal, or microbial organisms. In agriculture, humans control competitive and predatory interactions to minimize the impact of competitive or predatory species on the agricultural species of interest. In contrast, symbiotic relationships are often encouraged and many production traits of interest are the result of symbiotic interactions.

Symbiosis is defined as two different species "living together." These close interactions between two species are often long-term and, for the most part, beneficial to one or more of the symbionts. There are numerous examples of symbiosis in agriculture. Agriculture in a broad sense involves a symbiotic relationship between humans and plants or animals. Humans plant, fertilize, control weeds and pests, and protect crops. Humans also nurture, feed, and protect livestock. The crops and livestock benefit from human interaction by being more productive and, in turn, they are utilized for food, clothing, shelter, and other human needs. Of more importance are symbioses, particularly interactions of lower order organisms, for example, microorganisms, which can impart health or disease in higher organisms.

PARASITISM AND PATHOGENICITY

Symbiotic relationships can be further defined or characterized by the type and level of interaction (see Figure 9.1). *Parasitism* describes a system in which one species benefits at the expense of another over time. *Pathogenic* relationships are often acute interactions in which one species specifically infects and benefits at the expense, and even death, of another. *Commensalism* describes a system in which one species benefits, but not at the expense of the other. *Mutualism* describes a system in which both species benefit. Within these types of symbiotic interactions, the level of interaction can be close contact between the symbionts (ectosymbiosis or exosymbiosis) or it can include one symbiont living inside the other (endosymbiosis).

Exploitation of a host can result in symbiosis that is parasitic in nature, and in production agriculture, these relationships can be costly. In *endosymbiotic* interactions, immature insects and parasitic microbes, such as protozoa or bacteria, can reside in a host for periods of time and compete for nutrients. In *exosymbiotic* instances, parasites, such as pests or insects, can persistently remove nutrients from the host. Regardless of the level of interaction, the loss of nutrients often results in lower yields of crops or reduced performance by the animal. When the parasitic relationship results
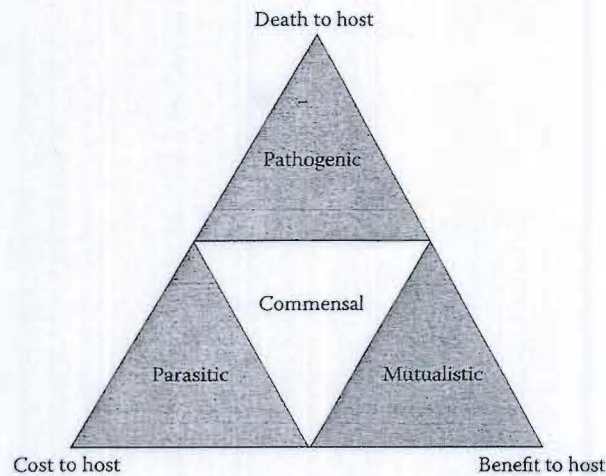
FIGURE 9.1   Trophic relationship between host and symbiont. Each corner of the triangle represents the key interactions, with the peak denoting a necrotrophic relationship that results in host death and the base denoting biotrophic relationships that require survival of the host.

in death of the host, the interaction is *necrotrophic*, whereas in a relationship that requires survival of the host, the interaction is *biotrophic*. The parasitic and pathogenic relationships are costly to agriculture and future efforts will be needed to control these relationships while not affecting the environment negatively.

INSECTS AND PARASITISM WITH PLANTS AND ANIMALS

Plants account for most of the living biomass on Earth, and parasites, insects in particular, have evolved closely with plant hosts (Rosomer and Stoffolano, 1997). *Phytophagous* ("plant eating" or herbivores) insects (anthropods) are the most abundant insects and include locusts, which are not selective and will consume most green plants (*polyphagous*), whereas the western corn rootworm is selective in consuming one species or genus (*monophagous*). Most plant parasites target a variety of plants (*oligotrophic*). Phytophagous insects may be specific in the anatomy of plant tissue that is targeted, and every plant tissue or anatomical part is susceptible to one or more phytophagous insects. These insect herbivores play a key role in the evolution of plant structure and response to herbivory, or being eaten (Stout, Thaler, and Thomma, 2006). Plants signal injury via two main pathways. One pathway is a systemically acquired response utilizing salicylic acid in responses to acute structural damage to elicit antimicrobial release and control microbial entry, and to deter insect attack. The second pathway is an induced resistance response utilizing jasmonic acid in response to chronic herbivory to deter the insect or hinder its development or reproduction. In contrast to the phytophagous insects, predatory insects such as spiders prey on other insects, particularly the phytophagous insects, and they can be beneficial to agriculture. Even the lowly household pest, the Asian cockroach, can be beneficial because it preys on bollworm and armyworm pests of agricultural crops.

Zoophagous insects extract nutrients from a living animal host and represent a broad group of parasitic insects, including the predatory insects that feed on other insects (Rosomer and Stoffolano, 1997). Most zoophagous insects that feed on vertebrate animals are biotrophic and live on the external surface (*exoparasitic*). Among animals common to production agriculture, suckling lice (Anoplura), chewing lice (Mallophaga), flies and mosquitoes (Diptera), ticks (Ixodidea), and fleas (Siphonaptera) are common parasitic insects and most are blood feeders *(hematophagous)*. Zoophagous insects have adapted a variety of host interactions. Lice can spend their entire lifecycle on a single host and are typically species specific. Host-specific zoophagous insects have often

co-evolved with the host species and in some cases have evolved special structures for feeding on the host. Other zoophagous insects, such as fleas, may only live on the host for a short period of their lifecycle. Free living zoophagous insects, such as mosquitoes and flies, may only utilize the mammalian host for meals and not live on the host per se. Individually, the zoophagous insect may be insignificant relative to the much larger mass of the host, but collectively, these parasites can carry disease and in large numbers over extended periods can be a nutrient drain to the host. Identifying and exploiting predatory insects that target blood-feeding zoophagous insects would reduce the therapeutic use of pesticides for pest control in animal agriculture, but no significant predatory insect has been identified.

MICROBES AND PARASITISM/PATHOGENICITY WITH PLANTS AND ANIMALS

Parasitism, in its strictest definition, describes an interaction of one organism surviving at the expense of the other. However, when microbes are involved, the level of interaction is less clear and the definition less strict. To some extent, the definition of a parasite may depend on the point of view and the depth of understanding of the symbiosis. In *biotrophic* parasitism, what one may perceive as one organism surviving at the expense of the other may, in reality, be an interaction in which both organisms benefit, but the full symbiosis is not known. In addition, some interactions may be mutualistic (both organisms benefit) at certain times, but parasitic at other times. In contrast, pathogenic organisms represent examples of necrotrophic parasitism, but not all pathogenic microbes cause death of the host. Regardless, pathogen interaction represents greater loss to the host and needs to be considered differently than a parasitic interaction. Among plants and animals, particularly those of agricultural importance, a number of parasitic and pathogenic microbes are important relative to disease and host health, but a thorough discussion is beyond the scope of this chapter. A few interactions of economic importance will be discussed. A further note regarding animals: A number of pathogens may survive and multiply in one host as a commensal and not cause disease, but can cause disease or death in another host. Understanding these zoonotic pathogens is important not only to animal health, but also to providing a safe food and water supply to humans.

Plant Microbial Parasites and Pathogens

Plants are the most abundant form of terrestrial life and are plagued by numerous opportunistic organisms colonizing the leaves, stems, and roots. Fungi are associated with spotting, rusting, wilting, and rotting of plants. Fungi in the phylum of Ascomycota (commonly called Ascomycetes) are a diverse group known for a sac structure and include important decomposers in nature and sources for important medicinal uses. Species associated with plant disease include *Aspergillus*, *Fusarium*, *Thielaviopsis*, *Uncinula*, and *Verticillium*. Fungi in the phylum of Basidiomycota (commonly called Basidiomycetes) are a diverse group known for a "club" or "fruiting" structure, and include plant disease-causing species of *Rhizoctonia*, *Phakospora*, and *Puccinia*. Oomycetes are small eukaryotic organisms, or protists, that are fungal-like, include species of *Pythium* and *Phytophthora*, and are associated with rusts, rots, and blights. Numerous bacteria cause diseases in plants, but species belonging to *Agrobacterium*, *Burkholderia*, *Clavibacter*, *Erwinia*, *Phytoplasma*, *Pseudomonas*, *Spiroplasma*, and *Xanthomonas* can cause significant damage or death to plants. Disruption of plant colonization is important for control of many diseases caused by microbes.

Animal Microbial Parasites and Pathogens

Animals are susceptible to a number of microbes that colonize and alter the health of the host. Animals represent nutrient-dense targets for opportunistic organisms, and bacteria, protozoa, fungi, protists (protozoa), and helminths (parasitic worms) have adapted opportunistic lifestyles that target animals. Primary targets include the pulmonary and digestive systems, but invasive microbes can penetrate the skin where lesions or abrasions have occurred. Among animals of agricultural importance, vaccinations and antibiotics have reduced the incidence of parasites and pathogens. However,

concern regarding the use of antibiotics in animals and the potential consequences of antibiotic resistance reducing antibiotic effectiveness in humans has led to mandated reductions in antibiotic use in animal agriculture. Alternatives to antibiotics include prebiotics (dietary component that alters microbial composition) and probiotics (microbial additive to alter microbial composition) in animal diets. Microbial interactions, specifically in the intestinal tract, that may reduce parasites and pathogens are discussed next.

Animals and Microsporidia

Microsporidia are unicellular organisms and intracellular parasites found in all major animal groups (Williams, 2009). Microsporidia are a common parasite in insects and fish, and a particular problem for farm-raised fish. Infection is associated with chronic, persistent illness and the parasite, although not directly lethal, has been shown to result in 30% mortality in farmed salmon. In most cases, the host exhibits reduced weight, vigor, and fertility. In addition to fertility issues, transmission can be vertical to the offspring, particularly in insect and crustacean hosts, and this parasite can change the sex of hosts via suppression of androgenic gland development.

These organisms represent a large group of microbes that are related to fungi phylogenetically, but they are atypical fungi in cell structure. Formerly thought of as protists and called microspora, these eukaryotic organisms lack mitochondria (they have mitosomes), are non-motile, and form spores with thick cell walls that can survive outside the hosts for years. Shifts in pH can prime the spores to germinate and inject the microsporidia into the host cell, which is typically a mucosal epithelial cell. Once colonized in the host, the parasite can exploit the host cell for nutrients and energy. Exploitation unique to microsporidia is the gathering of host cell mitochondria and accumulating the mitochondrial ATP, which may have allowed this eukaryotic organism to shed its endogenous mitochondria long ago.

Animals and Zoonotic Bacterial Pathogens

Zoonotic pathogens are transmissible between animals and humans (Wells and Varel, 2005). Plants may harbor pathogens harmful to humans, but most originate from animal sources. In animals, a variety of zoonotic pathogens has been observed, and in many cases, the pathogen may not be harmful to the animal, but may cause much harm to infected humans. Species of *Salmonella*, *Campylobacter*, *Enterococcus*, *Escherichia*, and *Yersinia* are excreted by animals and are potential pathogens to humans.

Many of these human-pathogenic bacteria can reside in production animals with little or no obvious signs of disease. In particular, *Escherichia coli* O157:H7 can reside in the bovine gastrointestinal tract of some animals for weeks at numbers greater than 100,000 organisms per gram feces, but less than 10 organisms can cause severe gastroenteritis, and even death, in humans. In the case of *E. coli* O157:H7, the zoonotic pathogen does not cause disease and provides little benefit to the animal carrier, but does compete for nutrients. In contrast, *Salmonella* are disease-causing organisms in humans and production animals, and some *Salmonella* strains have evolved adaptations for different hosts. In recent years, *Salmonella* serotypes Typhimurium, Enteritidis, Newport, and Heidelberg account for nearly 50% of the serotypes found in humans, and most likely originate from animal sources. *Salmonella* serotype Typhimurium is most often found in cattle and swine; Heidelberg and Enteritidis are found in chickens; and Newport is most often found in cattle. Controlling pathogen incidence and load in animal reservoirs is important for the safety of the environment, water, and human food, and understanding how the host diet or its gastrointestinal ecology may deter colonization of these pathogens is important for sustainable agriculture.

COMMENSALISM AND MUTUALISM

Typically, symbiosis is thought of as interactions that impart no negative effect to either symbiont, and these interactions can be described as commensal or mutual. These interactions are beneficial

to one (commensalism) or both (mutualism) species. In agriculture, we promote or select for these types of interactions that benefit the crop or animal. Although strictly defined as commensal or mutual, these symbiotic interactions are not clearly distinct, and the symbiotic relationship can have shades of both types of symbiotic interactions. In some interactions, the benefit to one species is obvious whereas the benefit to both species may not be as clear. The sustainability of agriculture for future generations is highly dependent on identifying and maximizing commensal and mutual relationships that improve agricultural production while minimizing the environmental footprint.

COMMENSALISM AND MUTUALISM AMONG PLANTS AND ANIMALS

Plants represent a significant amount of biomass on Earth, and are subject to a variety of interactions. As mentioned previously, phytophagous insects target plants and represent a parasitic type of interaction. However, plants attract and utilize insects, such as bees and butterflies, for pollination and these interactions distribute plant genetic material in the form of pollen. Ants also play an important mutualistic role with dispersion of plant seeds (*myrmecochory*) in the terrestrial ecosystem, particularly for flowering plants.

Animals have evolved to exploit plants as well, and in a balanced ecosystem, this interaction can be viewed as commensal to animals, if not possibly mutual to both plant and animal. Animals differ in their dietary adaptations. Carnivores are predominantly meat eaters and have little capacity to utilize plant material, but omnivores, which also cannot digest plant material, may feed on fruiting bodies of plants and, consequently, distribute seeds in the stool. Herbivores in contrast, and ruminants in particular, have developed the capacity to digest and utilize plant biomass for their own nutrient needs. These latter adaptations involve microbial symbioses, to be discussed later, but in relation to the plant, they can be commensal in nature.

Mammalian herbivores differ in their grazing strategies, and thus, can differ in how they affect or benefit the plant or forage consumed (Asner et al., 2009; Augustine and McNaughton, 2004; Bailey, 2005; Kant and Baldwin, 2007; Parker, Burkepile, and Hay, 2006; Rinella and Hileman, 2009). In feeding studies, herbivores tend to selectively consume exotic (non-native) plants over the native plants from their environment. In a larger ecosystem, native herbivores can suppress invasive plants by selectively consuming more of the exotic plants. This action will result in retardation of invasive exotic plants. In contrast, exotic (non-native) herbivores are more likely to selectively graze the native (but exotic to them) plants. This selection can result in greater abundance and overpopulation of exotic plants. In ecosystems that are more complex, such as the African savannas, the combination of native browsers and bulk-feeders retards shrub and woody plant encroachment onto rangelands used for agricultural grazing. On rangelands in the western United States, goats and sheep can improve grasslands-production by selectively consuming pines, junipers, and forbs (herbaceous flowering plants) that invade these grazing areas.

The negative implications of animals on plants are widely recognized. Animal movements can trample plants and grazing by herbivores can result in plant injury and loss of the plants' reproductive organs. However, omnivores and mammalian herbivores can benefit plants by dispersing seeds (Pakeman, Digneffe, and Small, 2002). Some plants have evolved structures such as hooks to facilitate attachment to animals for dispersion by *exozoochory*, or transport outside the animal, whereas other plants have evolved to utilize *endozoochory*, or transport by animal ingestion. Fruit-bearing plants often have fleshy fruit to attract a variety of birds and mammals that consume (*frugivory*), transport, and defecate the seeds, a process known as *direct endozoochory*. Likewise, mammalian herbivores graze and consume plants (*herbivory*) including the seeds of those plants, and transport those seeds until defecated, a process known as *indirect endozoochory*. The movement of the seed through the digestive tract may damage the seed and prevent germination, but the nutrient-rich environment is likely more conducive to germination and growth for those seeds that survive. Grasses and plants, particularly annuals, may be widely distributed in dung from herbivores, but plant species with smaller seeds typical of many pasture weeds are better adapted to surviving the

gastrointestinal tract. Whether or not seeds are dispersed by an animal, hoof action by animals disrupts the soil surface and can serve to bury seeds for later germination. In agriculture, properly managing forage lands and foraging animals will minimize the environmental impact of animal agriculture and sustain a productive system.

MICROBES AND MUTUALISM/COMMENSALISM WITH PLANTS

In horticulture, combating plant diseases that arise from microbial infections has been the plant/microbial interaction of greatest interest. In recent years, the diversity and economic importance of mutualistic interactions between plants and microbes have been recognized, but with the exception of nitrogen fixation, these interactions have not been as well studied. In plants, the *rhizosphere* describes the soil around the plant roots and this ecosystem represents a community of bacteria, fungi, protozoa, and nematodes that interact with each other and the plant roots (Barea et al., 2005; Berg and Smalla, 2009; Mocali and Benedetti, 2010). The biotic factors that affect the community structure include plant species and cultivar, stage of plant development and health, and animal activity. Abiotic factors include climate, geography, soil type, and amendments made because of human activities (e.g., pesticides, fungicides, or herbicides). The plant may control the predominant interactions via exudates from the roots, which can serve as signals for beneficial bacteria. Exudates include ions, oxygen, water, mucilage, and carbon compounds. The carbon excreted by roots can be variable, but can account for more than 25% of the carbon fixed by the plant. Plants can benefit from microbial interactions due to the release of phytohormones, availability of nutrients, micronutrients, and minerals, increased tolerance to stress, and biocontrol of pathogens. Soil-specific inhibition of the plant pathogens *Fusarium*, *Gaeumannomyces*, *Rhizoctonia*, *Pythium*, and *Phytophthora* is in part due to indigenous rhizosphere microbes.

Nitrogen Assimilation

Carbon and nitrogen are the building blocks of life on Earth. Plants use photosynthesis to transform light energy and carbon dioxide into carbon building blocks. However, plants, like all other eukaryotes, cannot directly assimilate nitrogen, and require nitrogen in the form of nitrates or ammonia for nitrogen assimilation. The biological ability to fix nitrogen to ammonia is limited to prokaryotes that express nitrogenase enzymes (Barea et al., 2005; Hurek and Reinhold-Hurek, 2003; Lindström et al., 2010). Numerous free-living bacteria in the soil (e.g., *Azobacter*, *Clostridium*, *Klebsiella*, and *Rhodospirillum*) have developed abilities to fix nitrogen, which can diffuse to surrounding plants. A variety of plants has evolved extracellular symbiotic (*epiphytic*) relationships with nitrogen-fixing cyanobacteria, most of which involve heterocysts, or cavities formed in the leaf, to house the bacteria (e.g., *Anabeana azollae* and the waterfern *Azolla*). A few flowering woody shrubs and trees have adapted intracellular symbiotic (*endophytic*) strategies with the filamentous antinomycete *Frankia* in large root nodules, but this interaction is limited to some species of angiosperms in the plant kingdom. More agriculturally important plants in the legume family (*Fabaceae*) have widely evolved symbiotic relationships to exploit microorganisms. In general, these plants have specialized nodules in their roots where atmospheric nitrogen is fixed and assimilated by a variety of rhizobia bacteria. Rhizobia are Gram-negative rod-shaped bacteria and the nitrogen-fixing species are distributed among *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium* groups.

The symbiosis with legumes and rhizobia occurs in specialized root tissue called *nodules*. The development of this symbiosis begins with plants secreting an exudate from the root hairs that chemotactically attracts the rhizobia. The rhizobia colonize and multiply on the root hair. Flavonoids produced by the plant induce the nodulation (*nod*) genes in the rhizobia to produce Nod factors, which result in a sequential series of plant host reactions that result in internalization of the bacteria and nodule development. Specificity between legume species and bacterial species is determined by modifications to the Nod factors that are encoded by host-specific nodulation genes. Nitrogen fixation genes (*nif* and *fix*) encoded by the bacteria are triggered and the highly conserved nitrogenase

and accessory proteins are produced. The reduction of nitrogen to ammonia is energy intensive (requiring 16 mole ATP per 1 mole NH_3 produced). The rhizobia require readily available oxygen for catabolism, but the nodules have low oxygen content. To compensate, the host plant produces leghemoglobin to deliver oxygen to the rhizobia. This symbiosis can account for more than 50% of all biologically fixed nitrogen in agriculture, and modern cropping systems implementing a legume in rotation can derive significant savings in nitrogen fertilizer applications.

The ability to fix nitrogen in agricultural crops may not be limited solely to legumes and their mutualistic bacteria (Bhattacharjee, Singh, and Mukhopadhyay, 2008; Hurek and Reinhold-Hurek, 2003; Steenhoudt and Vanderleyden, 2000). The most important agricultural crops are grasses (family Poaceae) and recent evidence suggests that bacterial species belonging to *Azospirillum*, *Acetobacter*, *Herbaspirillum*, and *Azoarcus* may form mutualistic relationships with some of these plants. Mutualistic relationships between nitrogen-fixing bacteria and grasses may be concentrated in the tropic regions, and rice is one agricultural crop that may benefit from endophytic bacteria that can fix nitrogen. Some sugar cane varieties in Brazil and Kaller grass common to saline soils in south-central Asia appear to assimilate most of their nitrogen from nitrogen-fixing endophytes, such as *Azoarcus* spp. Nitrogen-fixing bacteria in grasses may be epiphytic or endophytic. Unlike legumes, the bacterial endophytes in the grasses are not housed in specialized structures, but are free-living in the plants' extracellular spaces. In the case of *Azospirillum*, the bacteria first swarm and attach to the root surface and secrete polysaccharides, essentially anchoring the bacteria to the root in a biofilm. Not all *Azospirillum* can internalize, but as noted with *Azoarcus* spp., cellulolytic enzymes appear to aid in their internalization into the plant root.

Arbuscular Mycorrhiza

Fungal-plant mutualistic interactions in the rhizosphere represent a diversity of interactions by a group of fungal taxa and over 90% of plant species (Bonfante and Genre, 2010). Interactions with trees account for the bulk of the variety of interactions with fungi, utilizing ectomycorrhizal mechanisms in the root hair in which the fungal mycelium are extracellular. Most vascular plants have evolved endosymbiotic interactions with arbuscular mycorrhiza, a common fungus in soil. This relationship appears to have occurred early in the development of land plants and represents the most widespread type of symbiosis in nature. Members of the fungi phylum Glomeromycota are part of the soil matrix, and their hyphae can infect the root hair and form arbuscule structures (endomycorrhizobial) in the plant root cells to exchange nutrients. Without the roots of plants, these microorganisms would be unable to complete their lifecycle and they would die. These fungi benefit the host plant by providing additional phosphorus, but can also provide additional micronutrients and water due to the increased surface area of the filamentous mycorrhiza hyphae widely distributed through the soil, whereas the plant can provide the fungi with carbon, often the sole source of carbon, for the arbuscular mycorrhiza. Disruption of the rhizosphere, or the soil surrounding the plant root system, can disrupt the mycorrhiza hyphal network and impede the symbiosis-based development. In particular, tillage, fungicides, and application of phosphorus fertilizers are modern practices that negate the potential benefits of arbuscular mycorrhiza by disrupting fungal growth and minimizing infection of the host plant. In contrast, this symbiosis could be managed and exploited by farming systems where inputs are minimal, such as organic farms, to improve plant growth and crop yields.

Additional Plant/Microbe Interactions

Understanding of the plant/microbial interactions in the rhizosphere is slowly increasing as research in sustainable agricultural systems matures and newer technologies come online (Newton et al., 2010). Varieties of wheat may selectively support the growth of beneficial bacteria, such as *Pseudomonas* species, which in turn may make minerals and nutrients more available to the plant roots and suppress plant pathogens. Consequently, subsequent wheat varieties of different genotypes may not perform as well in the same field if different rhizosphere ecology

is needed. In particular, it has been noted that older wheat cultivars appear to be colonized by a variety of rhizobacteria and more recently developed cultivars are associated with members of Proteobacteria, such as *Pseudomonas*. Mutualistic interactions for production crops such as maize, grasses, barley, and oat cultivars may include microbes *Agrobacterium* sp., *Bacillus* sp., *Burkholderia* sp., *Pseudomonas* sp., *Paenibacillus* sp., and *Streptomyces* sp., but additional enrichments for members of the rhizosphere community are likely to exist. Plant root exudates could play a determining role in selecting mutualistic microorganisms, but microbes have to signal back to the plant to initiate colonization. In general, motile soil bacteria, such as *Pseudomonas* strains, appear to be predominant because motility offers a competitive advantage in colonizing the plant and establishing the symbiosis.

Pathogen Suppression

The identification and potential for mutualism between plants and microbes to be exploited in production agriculture has yet to be fully determined. Research to identify beneficial bacteria and fungi will be difficult, but the rewards could be invaluable (Newton et al., 2010). In particular, suppression of plant pathogens by commensal or mutualistic microbes in the rhizosphere is a viable opportunity. Biocontrol by bacteria, such as *Pseudomonas*, *Agrobacterium*, *Bacillus*, *Streptomyces*, and *Burkholderia* strains, or by non-pathogenic fungi, such as *Trichoderma*, *Pythium*, and *Fusarium*, against plant pathogens may be a useful preventative system to control plant pathogens or reduce the damage inflicted by the pathogen. Numerous mechanisms may explain the antagonisms, and in nature, more than one may be involved. Putative mechanisms may involve competitive exclusion for colonization sites, stimulation of plant defense systems, niche nutrient competition and depletion (in particular, iron sequestering), inhibition via antimicrobials, degradation of virulence factors, and parasitism.

Fungal Endophytes and Plants

Most of the mutalistic microbes described in the previous sections are endophytes (intracellular in the plant) found in the rhizosphere where they may provide nutrients or prevent microbial pathogens. In the stem and leaf, which are primordial (aerial or *phyllosphere*) portions of the grasses (family Poaceae), symbiotic relationships with fungi (family *Clavicipitaceae*) have evolved (Schardl, Leuchtmann, and Spiering, 2004). In numerous examples with endophyte fungi, the specific fungal strain is transmitted with the seed from the host plant. Many of these relationships are commensal or mutualistic, and in the latter case, the fungal endophyte enhances root development, drought tolerance, and resistance to herbivory by insects and animals. Alkaloids, including lolines, peramine, indolediterpenes, and ergotamines, produced by the endophyte fungi can possess antimicrobial activities that reduce pathogen colonization and can be toxic to insect pests that may forage the host plant. The indolediterpenes and ergotamines can also be a problem for foraging livestock and have been implicated in toxicities observed with ryegrass and tall fescue. In these cases, the mutualistic relationship that benefits plant growth and fitness has become a costly problem to grazing livestock.

Modern Agriculture and Symbiosis with Plants

Historically, consideration of mutualistic microbes has played little role in crop production. However, as the understanding of the mutualism grows, the selection of mutualistic relationships with microbes will likely expand. Understanding plant physiology and the function and role of *R*-genes (which encode proteins for disease-resistance), immune peptides and proteins, flavonoids, and other microbial effectors against parasites, pathogens, and mutualistic microbes, as well as the mechanisms by which the symbiotic microbes control plant defenses, will provide the foundation for proper selection of appropriate mutualistic benefactors. However, as noted with endophyte fungi, the implications for foraging animals where the plant host may be used for grazing livestock must also be considered when selecting mutualistic partners.

MICROBES AND MUTUALISM/COMMENSALISM WITH ANIMALS

Microbes are ubiquitous in nature, but the animal has provided the microbe with the most opportunity. Pathogenic or parasitic microbes, as discussed previously, have evolved to take advantage of an animal host, but a greater level of interaction has evolved between microbes and animals in which both organisms benefit to some degree. In mammals, it is widely recognized that microbes play a role in digestion, but mutualistic and commensal relationships are known even in insects. Termites harbor symbiotic protozoa and other cellulolytic microbes to digest wood cellulose; leafcutter ants nurture a fungus to digest freshly cut leaves; and even the plant parasitic aphid has co-evolved with a bacterium (*Buchnera aphidicola*) that provides the sap-sucking aphid host a source of amino acids (Degnan et al., 2010). Of greater interest with the aphid is the propensity to utilize other bacterial strains to manipulate sex ratios and protect against natural enemies, such as wasps and fungal parasites. In these examples, mutualism with microbes has provided a competitive advantage to parasitic organisms that can be damaging to agriculture.

Gastrointestinal Tracts of Production Animals

The gastrointestinal tract (GIT) begins at the mouth and ends at the anus. Animals ingest and digest food for energy and growth, and the gastrointestinal tract provides a system for the consumption, mastication, digestion, and absorption of nutrients to fuel these needs. The gastrointestinal system can vary from species to species of animals, particularly in the upper GIT (oral cavity to stomach). Compartmentalizations of the esophagus and stomach have allowed dietary specialization in some animals (pregastric digestion and fermentation, see Table 9.1). The lower GIT can also vary in size, but common to most animals are the small intestine, large intestine, and colon. These regions of

TABLE 9.1
Relationships between Dietary Strategy for Mammalian Host and Microbiota Types

Herbivores		Omnivore	Carnivore	Microbiota Type
Foregut Fermenters	**Hindgut Fermenters**			
Cow, sheep, and giraffe				Type 1: Foregut fermenters, such as ruminants, that consume forage materials
	Horse and rhinoceros			Type 2: Hindgut fermenters that consume forage materials
Columbine monkey	Gorilla and orangutan			Type 3: Pseudo-ruminant and hindgut fermenters, includes foliovores and omnivores
		Chimpanzee, human, baboon, spider monkey, and lemur		Type 4: Simple stomached mammals, includes omnivores and frugivores
	Giant panda and red panda	Brown bear	Polar bear, dog, hyena, and lion	Type 5: Simple stomached mammals, mostly carnivores but includes mammals with extensive dietary range

Source: Adapted from Ley, R.E., C.A. Lozupone, M. Hamady et al. 2008. Worlds within worlds: Evolution of the vertebrate gut microbiota. *Nat. Rev. Microbiol.* 6(10):776–788.

Note: Microbiota type is based on the cumulative microbial composition of feces sampled from a variety of mammalian hosts.

the GIT are active in the digestion and absorption processes. Of particular interest for some animal species is the developed cecum, which allows for dietary specialization (postgastric digestion and fermentation).

At birth, the GIT in mammals is sterile, but that quickly changes unless the newborn is delivered by Cesarean section and maintained germ-free. The lumens of the lower gastrointestinal tissues are nutrient-rich and packed with not only digesta, but also bacteria that are degrading and utilizing ingested nutrients. The GIT is an open system and susceptible to microorganisms from outside the host; however, the predominant microflora in the GIT are often permanent residents and, in some cases, unique to certain animal species. The bacterial population in the GIT can outnumber the host cells by as much as 10 to 1, and the populated tract is now commonly recognized as an organ. The microflora can be a source of energy, amino acids, and vitamins; and these bacteria can function to modulate the immune system, regulate the function of the intestinal tissues, and prohibit pathogen colonization.

Pathogens and other opportunistic bacteria can affect animal performance, and prohibition of pathogen colonization by commensal or probiotic strains provides an important benefit to the host animal. The beneficial bacteria can operate by several mechanisms, including competitive exclusion, antimicrobial production (e.g., bacteriocins), and occupation of colonization sites.

Competitive exclusion, or Gause's Law, describes a principle of ecology in which competing species cannot co-exist with the same resources if all other factors are constant, and one organism will out-compete the other for nutrients to the point that the other becomes extinct or evolves. Antimicrobial compounds can be produced by bacteria to inhibit another species, and the most common compounds are proteinaceous bacteriocins. Bacteriocins have been classified as Class I, IIa, IIb, IIc, and III based on synthesis, biochemistry, and mechanism of action. However, categorization of bacteriocins can depend on a number of factors, including mechanism of action and producing species. For example, colicins and microcins are typically produced by *Escherichia coli*; lantibiotics are produced by lactic acid bacteria; and subtilin is produced by *Bacillus subtilis*. Colonization involves attaching or invading the epithelial tissue. Bacteria as a whole express a variety of extracellular proteins for binding different glycoconjugates and epithelial cell components.

Abundant nutrients feed a diverse microflora and recent technologies should allow researchers to understand better the strong relationship between host and gastrointestinal microflora. Phylogenetic analyses utilize sequence information from cell DNA or proteins to study relatedness or classification of different strains, species, genus, or higher orders. In microbiology, the 16s RNA gene is commonly used for classification of related bacteria. The 16s RNA gene sequences are interwoven with conserved and variable regions, and sequencing a specific region allows the study of the diversity in a sample of microflora. In the mammalian lower GIT, the predominant microflora is bacteria, and of the approximately 24 phyla of bacteria, the lower intestine is predominated by the phyla Bacteroidetes and Firmicutes. Overall, most Bacteroidetes in the distal intestine belong to the genera *Bacteroides*, whereas most Firmicutes belong to genera *Clostridium*, *Enterococcus*, *Lactobacillus*, *Peptostreptococcus*, and *Ruminococcus*. Minor phyla of abundance in the intestine, such as Actinobacteria and Fusobacteria, are represented by genera *Bifidobacterium* and *Fusobacterium*, respectively. The abundance of bacteria can vary by animal species and by location from the small intestine to the colon, with species of *Lactobacillus* predominant in the jejunum region of the small intestine and species of *Bacteroides* and *Clostridium* being predominant in other regions.

Historically, studies of microflora have involved isolation and culturing of bacteria. However, these studies are time consuming and not all bacteria are easily cultured. Modern molecular methods for DNA amplification and sequencing have provided a different view of bacterial niches and recent studies of the human intestinal microflora have provided a better understanding of the symbiosis in the intestine (Eckburg et al., 2005; Ley et al., 2008). Obesity in mice has been associated with higher levels of the phyla Firmicutes and lower Bacteroidetes, and when germ-free mice were inoculated with microbes from obese mice, the animals exhibited weight gain and lower food intake. In humans, obese subjects exhibit similar patterns compared to lean subjects, and

imparting a dietary regime to obese subjects altered the microflora to higher Bacteroidetes and lower Firmicutes. Changes in microflora composition are believed to be associated with changes in the energy balance in the intestinal tract. Specific changes in bacterial genera or species have not been reported, but based on results of these recent studies, modulation of gastrointestinal microflora may affect weight gain, adipogenesis, and lean accretion.

Establishment of the gastrointestinal microflora is important to the host (March, 1979; Ratcliffe, 1991). Initial inhabitants in mammals are those ingested during passage at birth and from the mother's skin when suckling. Additional bacteria are ingested from the environment, and over time, the gastrointestinal microflora stabilizes. Milk from the mother provides antimicrobial factors to reduce pathogen risk in the neonate, and the newborn is specialized in digesting and absorbing the nutrient-rich milk. Initial colonizers include coliforms (including *E. coli*), clostridia, and streptococci, and are found in stomach and small intestinal contents. Species of *Lactobacillus* and other lactic acid bacteria soon predominate in these tissues and colonize significant portions of the small intestinal mucosa. The small intestine is a major colonization site for pathogenic *E. coli* associated with diarrhea in young mammals (enteropathogenic or enterotoxigenic *E. coli*; EPEC or ETEC, respectively), and the bacteriocins and exclusion by colonized indigenous flora, in particular the *Lactobacillus* spp., are major factors in reducing bacterial disease.

The stomach has several distinct tissue regions, and the acids produced by the secretory regions are lethal to many bacteria. In the monogastric stomach, the bacterial populations are highest after meals (1000 to 1,000,000 colony forming units per gram of luminal contents) when stomach acid is diluted (Katouli and Wallgren, 2005). Bacterial populations are lowest after digestion is complete, with bacteria often undetectable in luminal contents of the stomach. Many of the observed luminal bacteria may originate with the food or feed, or are dislodged from the upper GIT when food is chewed and swallowed. Regardless, bacteria observed in the stomach contents of the piglet are sparse relative to other regions of the GIT. The non-secreting regions harbor a number of bacteria, and the bacterial flora present are mostly attached to the stomach epithelial surface or embedded in these tissue linings. *Lactobacillus* spp. is most often isolated, although *E. coli* and species of *Streptococcus, Eubacterium, Bifidobacterium, Staphylococcus, Clostridium,* and *Bacteroides* have been isolated. Although their numbers may be small, these commensal colonizers such as *Lactobacillus* spp. may reduce ulcerations by excluding or preventing colonization by *Helicobacter pyloris* (humans), *H. suis* (swine), and *H. bovis* (ruminants), and similar mucosal irritants. Numerous bacteria have been tested *in vitro*, including *L. johnsonii, L. acidophilus, L. reuteri, L. gasseria, Weisella confusa,* and *Bacillus subtilus*. Effective beneficial commensal and probiotic bacteria in the stomach would have to tolerate low pH and rapid luminal turnover, and need to colonize epithelial surface glycolipids targeted by bacterial irritants in stomachs such as *H. pylori*.

Colonization of the stomach is not limited to the monogastric stomach. Numerous animals have evolved specialized stomachs. In particular, compartmentalization of the stomach regions has led to diversity and food specializations in mammals to exploit microbial interactions (Russell and Rychlik, 2001). In particular, mammals that derive some nutrients from pregastric fermentations have evolved to exploit utilization of plant forages and fiber in their diets. The rumen is one such compartmentalization that will be discussed in detail later. Regardless of the animal species, a compartment equivalent to the gastric stomach, or abomasum, serves as a barrier to transient and pathogenic bacteria that would otherwise invade the lower nutrient-rich GIT.

The small intestine is common to most animals and has three physiological regions—the duodenum, jejunum, and ileum—each with distinct roles in digestion and absorption. The duodenum is a primary site for secretions of bile and enzymes that aid digestion. In contrast, the jejunum and ileum are important for absorption of nutrients. Overall, the small intestine has a fast passage rate for digesta, compared to the regions of the lower intestine, and the lumenal contents have fewer bacteria. The commensal bacteria in the small intestine, such as *Lactobacillus* spp., are most often attached to the intestinal epithelial lining and sloughed into the lumen, with the jejunum and ileum being primary sites for bacterial colonization.

The piglet has one of the most frequently studied small intestine systems due to similarities with that of humans (Katouli and Wallgren, 2005). Commensal *Lactobacillus* spp. in the small intestine most often cultured from the piglet include *L. fermentum, L. acidophilus,* and *L. delbrueckii.* In comparison, molecular fingerprinting has more recently identified *L. mucosae, L. delbrueckii, L. salivarius,* and *L. johnsonii* as being most abundant in weaned piglets. Phylogenetically similar species have been observed with young cattle and poultry. These lactobacilli are typically resistant to bile and other intestinal secretions, and bind to the mucosa via mucin and epithelial binding proteins. Many lactobacilli produce antimicrobial compounds, commonly referred to as bacteriocins, and specifically known as lantobiotics for these bacteria. In addition, colonization by *Lactobacillus* spp. may alter host defensive responses, cytokine release, and immune activity. *Bifidobacterium* spp. can also generate similar responses in humans, cattle, and poultry, but these bacteria are rarely abundant in swine.

The large intestine is common to most animals and has three separate regions—the cecum, the colon, and the rectum—each of which aid in absorption of nutrients and water. The cecum is a region of divergent evolution that has allowed for specialization by the host animal. Amphibians lack any cecal structure, and fish have "pyloric ceca," or out-pockets, along the intestine but not a defined cecum. In most animals, with the exception of amphibians and fish, the cecum is a pouch of the large intestine located at the connection between the small intestine and the large intestine. Birds have two ceca, whereas most mammals have only one cecum. The primary function of the cecum is to provide space for post-gastric fermentation and for absorption of volatile fatty acids. Therefore, the cecum varies in size, with specialized herbivores having a large voluminous cecum and carnivores having a small cecum, or in these latter animals, essentially a blind pouch at the proximal end of the colon with a small appendix tube in some cases.

Bacteria in the lower GIT are predominantly strict anaerobes belonging to the Firmicutes and Bacteroidetes phyla at concentrations of 10^{10} to 10^{11} per gram of lumenal content, but can vary between host animal species, with host diet, and from one host GIT region to another (Allison et al., 1979; Katouli and Wallgren, 2005; Robinson, Allison, and Bucklin, 1981). The bulk of microbial diversity is found in the lower GIT, with estimates of 400+ autochthonous, or indigenous, strains in the ecosystem. Colonization of the cecum after birth appears to assist with the development of the immune system, even in carnivores and humans that lack a developed cecum. In the developed cecum, the microfloras for the young pig and the laying hen have been characterized by a number of studies. In classical anaerobic studies with isolated strains, the swine cecal bacterial strains were characterized as predominantly *Prevotella* sp. and *Selenomonas ruminantium,* whereas culture-independent techniques detected not only an abundance of *Prevotella* sp. but also higher levels of low G+C microorganism related to the diverse group of Gram-positive bacteria including *Clostridium* (Leser et al., 2002). In the hen, recent culture-independent techniques recognized *Prevotella/ Bacteroides* members as the predominant genera in the fed hen, and *Bacteroides* as the predominant genera in hens during molting induced by withholding feed (Callaway et al., 2009). The cecum may also harbor certain pathogens, with *Salmonella* sp. and *Clostridium difficile* detectable at high levels in swine and molting hens. In the GIT of swine, *E. coli* and related coliforms (Proteobacteria) tend to be at their highest concentrations in the cecum and decrease in concentration with passage through the colon. Cultured lactobacilli are found at their highest level in the small intestine, and appear to decrease in amount through the cecum and colon. The microflora in the colon, like the cecum, includes variable levels of *Prevotella, Bacteroides, Clostridium,* and *Lactobacilli* sp., but also includes *Eubacterium* and *Enterococci* sp. not always observed in the cecum.

The microflora in the lower GIT is beneficial to the host in several ways (Wells and Varel, 2005). Autochthonous bacterial strains colonize the mucosal layer and serve as a primary deterrent to pathogen colonization and entry. The volatile fatty acids generated by microflora fermenting fiber in the cecum and colon can contribute 20 to 30% of the total caloric requirement of omnivores and herbivores (Bergman, 1990). In particular, butyrate is a primary energy source for enterocytes, and butyrate-producing bacteria represent an important functional group of diverse genera (*Eubacterium, Roseburia,* and *Faecelibacterium* sp.) that promote intestinal growth, development,

and health (Louis and Flint, 2009). Microbial activity also leads to vitamin synthesis; however, the impact is limited for some vitamins due to poor absorption from the lower GIT. Animals reared germ-free require vitamin K supplementation, but normally raised animals do not, and germ-free animals require more B vitamins in their diet. The lower intestinal tract has limited ability-to absorb amino acids and, in swine, lysine from microbial activity may contribute 10% of a young pig's requirements and most of a grown pig's needs. Coprophagia (consumption of feces) has been observed in a variety of animals; however, the rabbit, like many hares and picas, has adapted a unique version in which cecal contents are passed directly thorough the colon and the "soft feces," or cecotropes, are re-ingested to extract additional protein and vitamins arising from the initial microbial activity in the cecum.

Pregastric Fermentation and the Ruminant

Animals differ in their abilities to digest foods, and some animals have developed specialized regions of the GIT to exploit microorganisms for digestion, fermentation, or production of nutrients. Pregastric compartmentalization allows for microbial activity prior to the digestion and absorption of nutrients by the host animal. Mammals lack enzymes to break down fiber and digest forages, but microorganisms have these enzymes and can perform these activities. In addition, the host animal can digest the microorganisms as they pass into the lower GIT, and these microorganisms are rich in proteins that have amino acid profiles to meet the host animal's requirements.

Ruminants, in particular, have evolved strong symbiotic relationships with microbes for these purposes, and cattle, sheep, goats, and deer are species that provide most of the meat (>50%) and milk (>90%) consumed by humans. Typically described as having four stomachs, the ruminant animal actually has four specialized compartments of the stomach (Hungate, 1966; Russell and Rychlik, 2001). The rumen is the largest compartment, accounting for 15% of the total empty weight of the GIT. This large voluminous compartment is the primary site for microbial activity. In ruminant animals predominantly consuming forage, the products of microbial fermentations are volatile fatty acids (acetate, propionate, and butyrate are the most abundant) and gases (carbon dioxide and methane). The volatile fatty acids from the rumen can account for up to 70% of the host energy requirements (Bergman, 1990), and the host has adopted metabolic pathways to utilize the volatile fatty acids produced. Most of the glucose used by tissues of ruminant animals originates from propionate conversion by the liver. Microbial proteins produced in the rumen can account for 40 to 90% of the animal's protein requirements, and the animal has a protein requirement similar to the amino acid composition of microbial proteins in rumen fluid (Bergen and Wu, 2009; Reynolds and Kristensen, 2008; Wells and Russell, 1996).

To accommodate the microbial activity and fermentations, ruminant animals continuously pass saliva rich in sodium carbonate into the rumen to buffer the acid product. Nitrogen, in the form of urea, continuously flows into the rumen through the saliva and from the blood in the epithelial tissue of the rumen. Urea is rapidly hydrolyzed in the rumen to ammonia and this free ammonia is important in the nitrogen cycle between the rumen microbes and host. The microbes in the rumen have adapted to using sodium gradients across their membranes to drive nutrient uptake systems and to using ammonia as a predominant source of nitrogen for microbial protein synthesis.

The microbial flora in the rumen is a complex milieu of bacteria, protozoa, and some fungi, many of which can be diet specific and unique in nature to the rumen ecosystem (Hungate, 1966; Russell and Rychlik, 2001). Bacteria constitute the bulk of the rumen microbial mass and functionality of the rumen is dependent on the bacterial composition. Forages are predominantly cellulose and hemicellulose in structure and are digested in the rumen by a combination of several bacteria, including *Fibrobacter succinogenes, Ruminococcus albus, R. flavefaciens, Eubacterium ruminantium, Prevotella rumincola, P. albensis, P. brevis, P. bryantii, Butyrivibrio fibrisolvens,* and *Selenomonas ruminantium.* In ruminant animals being fed concentrate diets, predominant bacteria may include *Ruminobacter amylophilus, Succinomonas amylolytica, Streptococcus bovis, Lactobacillus* sp., *Succinovibrio dextrinosolvens, Megasphaera elsdenii, Prevotella* sp., *Butyrivibrio fibrisolvens,* and *S. ruminantium.* Other

important ruminal bacteria include *Lachnospira multiparus* (pectinolytic), *Anaerovibrio lipolytica* (lipolytic), *Peptostreptococcus anaerobius* (aminophilic), *Clostridium aminophilum* (aminophilic), *C. sticklandii* (aminophilic), *Wolinella succinogenes* (organic acid utilizer), *Methanobrevibacter ruminantium* (methanogen), *Methanomicrobium* sp. (methanogen), *Methanobacterium* sp. (methanogen), and *Methanosarcina* sp. (methanogen). Propionate is important for glucose homeostasis in the host, and production in the rumen is directly, via propionate production, or indirectly, via succinate production, associated with the strains of *F. succinogenes*, *R. flavefaciens*, *Ruminobacter amylophilus*, *S. amylolytica*, the numerous *Prevotella* sp., and *S. ruminantium*, which can convert the ruminal succinate to propionate. Butyrate is important for milk fat synthesis in dairy ruminants, and is produced as a primary metabolite by *Butyrivibrio fibrosolvens* and numerous varieties of *Clostridium*.

While generally recognized as mutualistic, some of the ruminal bacteria can be detrimental to animal performance. *Streptococcus bovis* is associated with rapid lactic acid accumulation and rumen acidosis in grain-fed cattle, while lactate-utilizing bacteria such as *Megasphaera elsdenii* and *Selenomonas ruminatium* can reduce lactic acid accumulation. Opportunistic organisms like *Fusobacterium necrophorum* can also utilize lactate and infect rumen ulcers arising from even minor bouts of acidosis. Another group of ruminal bacteria, the methanogens, provides no net energy to the ruminant animal, and their production of methane represents both an energy loss to the animal and the generation of significant greenhouse gases associated with global warming.

Ruminal protozoa and fungi are less studied components of the rumen milieu, but still important in rumen ecology and animal production (Trinci et al., 1994; Veira, 1986). The protozoa observed in ruminal fluid are, with a few exceptions, unique to the rumen. Rumen protozoa can account for up to 40% of the microbial biomass, and defaunation, or the elimination of the protozoan population, can alter rumen fermentation. Protozoa are highly mobile and attach to feed particles and rumen wall surface, which reduces the washout rate and minimizes protozoan contribution to net rumen output. The rumen protozoa ingest and digest a number of ruminal bacteria for a source of protein and nutrients. Methanogens appear to colonize the body surface of protozoa (Figure 9.2), and appear to have established an intra-ruminal mutualistic relationship with protozoa that predominantly produce acetate and hydrogen gas. Defaunation often results in less energy losses to nitrogen recycling and methane production, but no significant reductions in rumen digestion are apparent because rumen bacteria fill the niche or void.

Rumen protozoa are mostly ciliated protozoa, belonging to either holotrichs or entodiniomorphs. Flagellated protozoa are present, but at low numbers in the rumen (Hungate, 1966; Veira, 1986).
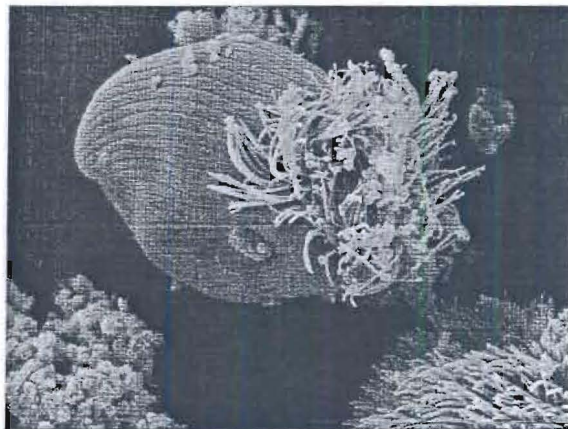

FIGURE 9.2   Microbes attached to the surface of protozoa isolated from the rumen of cow-fed forage. This is an example of a symbiosis within a symbiosis, denoting the complexity of research to determine cost-benefit to symbiotic interactions. (Micrograph by Sharon Franklin and Mark Rasmussen, National Animal Disease Center, ARS, USDA.)

The holotrichs are covered nearly entirely with cilia and comprised of *Isotricha* and *Dasytrichia* species, which are the predominant types observed in the rumen of grazing animals. The entodiniomorphs have cilia localized in specialized bands called syncilia to aid in food ingestion and locomotion. The entodiniomorphs are in greater variety and the abundance of specific genera is dependent on the host diet. The entodiniomorph groups consist of morphologically distinct species of *Entodinium*, *Epidinium*, *Ophryoscolex*, *Diplodium*, *Eudiplodium*, and *Polyplastron*, of which *Diplodium*, *Eudiplodium*, and *Polyplastron* have cellulolytic activities and may play a role in fiber digestion. Many of the holotrichs and entodiniopmorphs can ingest and accumulate starch granules. Strains of *Entodium* are more tolerant of rumen acidity and are most abundant in rumens of animals fed high grain diets. When protozoa accumulate starch, rapid digestion and production of lactic acid is reduced, thereby alleviating clinical and subclinical rumen acidosis.

Anaerobic fungi have been isolated from pregastric and postgastric herbivorous animals, but are most often observed in ruminants consuming high-fiber diets (Trinci et al., 1994). Vegetative fungi, or the thallus-forming bodies associated with colonization and degradation, are present in rumen at levels lower than protozoa. However, these unique microorganisms have adapted to foraging animals and many types are adept at digesting fiber with the invasive filamentous rhizoids, particularly for the most recalcitrant types of cellulose that many bacteria have difficulty digesting. The Neocallimastigaceae family of fungi is the sole family of the phylum Neocallimastigomycota, which includes six genera, including *Anaeromyces*, *Caecomyces*, *Cyllamyces*, *Orpinomyces*, *Piromyces*, and *Neocallimastix*. The *Neocallimastix* are the best described and most often reported filamentous fungi in the rumen. Anaerobic rumen fungi lack mitochondria and, like the ciliated protozoa, use specialized hydrogenosomes that produce hydrogen gas, which, in turn, is converted to methane by rumen methanogens. The presence of fibrolytic species of ruminal bacteria and anaerobic fungi are often associated with increased fiber degradation and utilization.

Birds, some fish, and reptiles have developed compartmented stomachs. The two compartments include the proventriculus, or true stomach, which is secretory, and the ventriculus, or gizzard, which is a muscular stomach for grinding food. Many birds have a muscular pouch preceding the proventriculus called a crop for storing food, but this compartment is an adaptation of the esophagus and not a compartment of the stomach. Since food is stored in the crop, fermentation by microbes is likely to occur. Herbivorous birds like the hoatzin specialize in eating leaves of trees (foliovores) and the crop in these birds contains a diverse microbial ecosystem predominated by Firmicutes and Bacteroidetes that digest the leaves and provide the host with fermentation products for energy and microbial cells for protein (Godoy-Vitorino et al., 2010). In contrast, commercial agricultural birds, such as the chicken and turkey, have crops adapted to omnivorous diets and the crop of these birds is predominantly colonized by species of *Lactobacillus*, similar to the ileum and jejunum (Hilmi et al., 2007). The *Lactobacillus* strains appear to be influential in minimizing colonization by pathogenic *E. coli* and *Salmonella* strains.

SYMBIOSIS AND EVOLUTION IN ANIMALS

The GIT is one of the best-studied and most-described symbiotic ecosystems. Host adaptations, such as foregut and hindgut fermenters, are obvious for the host to exploit the power of microbial enzymes. However, the complexity of the system and diversity of the microbiota have precluded an understanding of the strength of the host-microbial interaction. Pyrosequencing of complex microbiota samples has allowed a quantifiable measure of diversity and abundance for different microbial members, and studies of feces from intercrossed mice lines identified 13 regions in the mouse genome associated with abundance of one or more of the bacterial groups analyzed (Benson et al., 2010). Mouse chromosomes 1, 7, and 10 contain a number of genomic regions with strong associations to genus groups and higher orders. Concerning individual species, no specific species relationship was observed. However, strong association between a *Lactobacillus johnsonii/gasseri* group and chromosomes 7 and 14 were found, but none with *L. reuteri* or *L. animalis/murinus* group, and

results suggest a microbiota associated with heritable genetic factors. Many of the genes within the identified genomic regions are associated with mucosal immunity.

In biology, the hologenome theory of evolution has been proposed and recognizes the close relationship between symbiotic partners, or *holobiont*, and that this affects the combined genome, or *hologenome*, of the partners (Zilber-Rosenberg and Rosenberg, 2008). The theory is based on generalizations that animals and plants establish symbiotic relationships with microbes, that symbiotic microbes are transmitted between generations, that the relationship affects the holobiont, and that variations in the hologenome can result from changes in the host or the genome of microbes. Thus, as proposed by this theory, evolutionary pressure on the host may be compensated not only by the host, but also by the symbionts that comprise the holobiont. In periods of rapid environmental change, quick adaptation by a versatile symbiont would be beneficial to the host and allow for survival, if not expansion, of the host into the new ecosystem.

Bacteroides thetaiotaomicron is a host-adapted microorganism that can predominate in the GIT of humans. This Gram-negative organism has a completed genome sequence and analyses have revealed a diverse arsenal of genes adept at digesting complex polysaccharides, acquiring nutrients, and producing surface adherence factors for colonization and complex regulatory mechanisms to control and modulate gene expression (Comstock and Coyne, 2003). In addition, this organism is adept at assimilating mobile DNA elements that transmit from cell-to-cell via transposons and plasmids, and the plasticity of this genome indicates the versatility that the microbe has evolved to remain a strong host-adapted symbiont. Similar relationships exist between *B. vulgatus* and *B. distasonis* and the human distal intestine, and it is likely that additional relationships between microbes and their host will be forthcoming as molecular tools and modern sciences tease apart the relationships and understand the genomic traits driving symbioses (Xu et al., 2007).

SUMMARY AND CONCLUSIONS

Modern agriculture has led to significant improvements in efficiency of food production. The use of fertilizers, herbicides, fungicides, pesticides, antibiotics, and growth promoters has been instrumental in feeding the world. However, natural selection has established interactions that are often overlooked or disregarded. These symbiotic relationships could potentially reduce our use of synthetic agents and promote a more sustainable productive agricultural system. In addition, dedicating research efforts to better describe and understand beneficial symbiosis would allow more opportunities to exploit mutualistic relationships when they arise. Planting crops in particular rotations and with minimal tillage may sustain rhizospere interactions that promote healthiness and growth of plants. Herbivores, such as the ruminant, have historically benefitted from a variety of symbioses
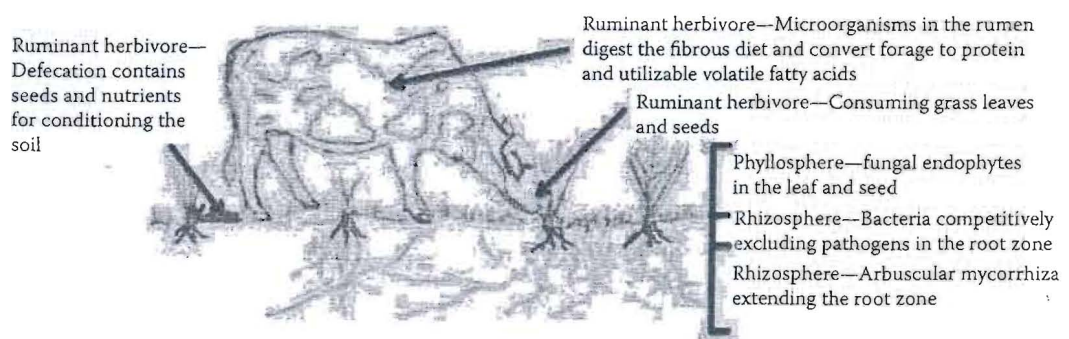

Ruminant herbivore—
Defecation contains
seeds and nutrients
for conditioning the
soil

Ruminant herbivore—Microorganisms in the rumen
digest the fibrous diet and convert forage to protein
and utilizable volatile fatty acids

Ruminant herbivore—Consuming grass leaves
and seeds

Phyllosphere—fungal endophytes
in the leaf and seed

Rhizosphere—Bacteria competitively
excluding pathogens in the root zone

Rhizosphere—Arbuscular mycorrhiza
extending the root zone

FIGURE 9.3   An ecosystem denoting symbiotic relationships of importance to animal agriculture.

(Figure 9.3), and there are many opportunities to enhance our understanding of these relationships to better utilize non-cultivatable land for animal production. Nature has provided humans with an arsenal of microbes and we need to understand better how to use them with modern practices.

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Animal Welfare in Animal Agriculture

Husbandry, Stewardship, and Sustainability in Animal Production

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