

1 The State of the Art

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As we enter the second century of research on associative and symbiotic microorganisms, it is heartening to see that attention is increasingly focused on the functions of these organisms in the natural and semi-natural systems in which it evolved. This volume, while encapsulating the spirit of the new adventure, also provides two further opportunities. It enables us to assess the strength of the platform from which we launch into this challenging area and to identify which experimental approaches might provide the most realistic evaluation of the roles played by surface microorganisms in natural communities. The long and difficult climb towards understanding the impacts of the microflora upon the species composition and dynamics, above and below ground, of plant communities is just beginning. This volume demonstrates both the strength and the weakness of the position from which we launch into the future. The strength may be that we have much precise information about microbial function under simplified conditions. The weakness, on the other hand, is that we have, as yet, little reliable information about the extent to which these functions are expressed under relevant, essentially multi-factorial circumstances of the kind that prevail in nature. The plant carries its major microbial community on its entire exposed surfaces, from apical tip to root cap. These plant surfaces represent an oozing, flaking layer of integument which discharges a wide range of substances that support a vast number of spatially discrete and specialized microbial communities, including parasites and symbionts, which can have a major impact on plant growth and development. In today's scenario the plant surface is considered as a dynamic adaptable envelope, flexible in both its own right and the first barrier between the moist, concentrated, balanced plant cell and a hostile ever-changing external environment. It is well known that the microbial diversity on the plant surface and in the soil habitats is much greater compared to the insight using cultivation techniques. Manipulation of the plant surface microflora to improve its health is a desirable and much needed goal in plant microbiology. However, efforts to exploit this type of biological control have frequently been impeded because of major

technical difficulties that must be overcome in order to fully understand the microbial ecology of this ecosystem, especially the lack of ability to extract in situ data that are both informative and quantifiable at spatial scales relevant to the ecological niches of the microorganisms involved. The entire volume is divided into five broad sections.

The combining aspect of the chapters in sections A and B are microbial communities in their interactions with higher plants. The communities are mainly dominated by a few species, however, a large number of other species may be equally important, although they are present only in the range of 1% of the total population or less. Experimental studies concentrate, of course, on the major components of the communities. These representatives are also used for biotechnology purposes such as seed inoculation by *Pseudomonas* and *Bacillus* control strains (Chap. 2). The interactions of methanogens and methanotrophs independent of the plant photosynthesis and the plant root ecology is a major contribution to the global CH₄ cycle. These communities are especially present in anoxic sites in wetlands such as flooded rice fields. The different carbon sources affect the CH₄ to CO₂ ratio, an important aspect for the impact of different root components on the microbial communities in the rhizosphere, as described in Chapter 3. Abiotic factors also influence the colonization of *Pseudomonas fluorescens* on seeds and include, besides growth substrates, also temperature, soil humidity and pH (Chap. 3). The dynamics of microorganism populations in the rhizosphere is a topic where a large number of research groups worldwide are involved. This is related to the huge amount of organic carbon exudated from plant roots into the rhizosphere, in the order of 10% or more of the total carbon assimilation by photosynthesis in higher plants. All major nutrient cycles such as the carbon cycle, the nitrogen cycle, the sulfur cycle, the phosphorus cycle and the cycle for micronutrients are much more active in this rhizosphere soil compared to the bulk soil. The enormous diversity in this microhabitat is increased by the fact that many different plant families and species exudate different sets of components into the soil. In addition, the composition of lignins and hemicellulose in the cell walls can be quite different, leading to a different composition of the rhizosphere communities (Chap. 4). More information on the major groups of microorganisms in soils in general are covered in Chapter 5, describing especially the impact of microorganisms on plant development by mycorrhiza species, actinorhiza species, plant growth-promoting rhizobacteria (PGPR), phosphate-solubilizing microorganisms and the important group of lignocellulolytic microorganisms.

Biotic signals from the microsymbionts inducing symbiosis and nodule development in legumes are even more specific in determining the interaction of the plants with their specific associated bacteria such as *Bradyrhizobium japonicum*, *Mesorhizobium loti*, *Sinorhizobium meliloti*, *Rhizobium tropici* or *Rhizobium etli*. Flavonoids and nod factors (lipochitoooligosaccharides) are the major components of the chemical language, in which the

microsymbionts and the host plants communicate to each other. The signalling concept studied in this type of symbiosis is equally complicated as the mammalian notch homologues and the integrin-adhesion-receptor signalling in other multicellular organisms (Chap. 6). A large stimulus for on-going and future research in the area of plant surface microbiology will be available from the use of already completed genome projects and on-going genome projects for prokaryotic and eukaryotic organisms. At present, about 145 genome projects are finished and more than 580 projects are on-going (<http://wit.integratedgenomics.com/GOLD/gold.html>). A list of completed genomes present in the public data bases, available in June 2003, is presented in Table 1. It is interesting to note that plant symbiotic and parasitic bacteria such as *Bradyrhizobium japonicum*, *Mesorhizobium loti*, *Sinorhizobium meliloti* and *Pseudomonas syringae* have the largest prokaryotic genomes. On the other side, there are some animal pathogenic organisms like *Rickettsia*

Table 1. Complete genomes present in the public DataBases, June 2003 (<http://wit.integratedgenomics.com/GOLD/gold.html>)

Organism	Size (kb)	ORF number
Archaeal		
<i>Methanosarcina mazei</i>	4,096	3,371 orfs MAP
<i>Methanobacterium thermoautotrophicum</i>	1,751	1,918 orfs MAP
Bacterial		
<i>Bradyrhizobium japonicum</i>	9,105	8,317 orfs MAP
<i>Mesorhizobium loti</i>	7,596	6,752 orfs MAP
<i>Sinorhizobium meliloti</i>	6,690	6,205 orfs MAP
<i>Nostoc</i> sp. PCC 7120	6,413	5,366 orfs MAP
<i>Pseudomonas syringae</i>	6,397	5,615 orfs MAP
<i>Pseudomonas aeruginosa</i>	6,264	5,570 orfs MAP
<i>Escherichia coli</i> 0157:H7, Sakai	5,594	5,448 orfs MAP
<i>Xanthomonas campestris</i> pv. <i>Campestris</i>	5,076	4,182 orfs MAP
<i>Agrobacterium tumefaciens</i>	4,915	5,402 orfs MAP
<i>Bacillus subtilis</i>	4,214	4,099 orfs MAP
<i>Escherichia coli</i> 0157:H7, EDL.933	4,100	5,283 orfs MAP
<i>Nitrosomonas europaeae</i>	2,812	2,573 orfs MAP
<i>Borrelia burgdorferi</i> B 31	1,230	1,256 orfs MAP
<i>Rickettsia prowazekii</i>	1,111	834 orfs MAP
<i>Chlamydia trachomatis</i>	1,042	896 orfs MAP
Eukaryal		
<i>Oryza sativa</i> L. ssp. <i>indica</i>	420,000	50,000 orfs
<i>Oryza sativa</i> ssp. <i>japonica</i>	420,000	50,000 orfs
<i>Arabidopsis thaliana</i>	115,428	25,498 orfs
<i>Neurospora crassa</i>	43,000	10,082 orfs
<i>Schizosaccharomyces pombe</i>	14,000	4,824 orfs
<i>Saccharomyces cerevisiae</i>	12,069	6,294 orfs

proWazekii with only 1.1 Mb, *Chlamydia trachomatis* with 1.04 Mb and *Borelia burgdorferi* with 1.23 Mb.

Bacillus thuringiensis and Bt transgenic plants are an example for biotechnology concentrated on a small number of well-studied soil microorganisms. The bio-insecticide protein is present only at a certain stage of sporulation in these organisms. Under natural conditions the spores have only a very limited survival time with less than 20 % presence after 24 h (Chap. 7). The toxin from *Bacillus thuringiensis* released from transgenic plants in the soil is much more stable with 25 % still present after 120 days. The toxin is protected from degradation by linkage and adsorption to clay minerals. Many other important signal molecules produced by plants and microorganisms in the soil may also have very different half-life times by specific adsorption to soil minerals. The impact of increasing concentrations of these toxins in soils due to this bio-control technique has not been sufficiently studied. Increases and decreases of specific subpopulations of soil microorganisms have been reported (Chap. 7). The other side of interactions, promotion instead of inhibition, is a topic of Chapter 8, which studies the mechanisms of plant growth-promoting rhizobacteria by phytohormones such as auxin and ethylene. An intermediate of ethylene synthesis is 1-aminocyclopropane-1-carboxylic acid (ACC). Microorganisms with an ACC deaminase gene increase stress tolerance of several plant species (Chap. 8). Compared to the rhizosphere, the communities in the phyllosphere have been studied less. The main reason is that the plant exudation from the rhizodermis is much larger than from the epidermis, due to the cuticles limiting carbon supply to the leaf surfaces. In contrast to bacteria, fungi have the ability to penetrate the cuticles and get access to carbon supplies (Chap. 9). Future work may concentrate especially on conditions where oligotrophic situations persist and genotypes adapted to these conditions may be present and not been recognized so far. The presence of animals in the interface of plants and microorganisms is another important aspect of communities, with the example of the Clavicipitaceae. It is very interesting to note that species of this family predominantly infect insects or the ancestors of grass-infecting species (Chap. 10). By sophisticated mechanisms, the fungi modify the plant tissues for nutrient acquisition. The shift from pathogenic interaction to mutualistic interaction in some species is a general aspect related to symbiosis and phytopathology. A completely new field of research has been developed, using the interaction of genetically modified plants (GMP) with microbial communities or specific microorganisms (Chap. 11). In the list of GMP species, important crop plants such as potatoes, maize, cotton, tobacco and alfalfa are used. The aspect of horizontal gene transfer (HGT) from GMP plants to associated bacterial species and fungal species is a topic for several biotechnology research projects.

Section C deals with interactions between plants, fungi, and bacteria. The plant root constitutes an environment which forms the basis for multiple relationships with microorganisms. Fine roots of most plants are associated with

symbiotic fungi, which facilitate uptake of nutrients and water. An example of such a symbiotic interaction (termed mycorrhiza), which occurs mainly with roots of trees in temperate and alpine regions is ectomycorrhiza. The formation of the resulting symbiotic structure is commonly associated with changes in root morphology. Properties of the root surface are obviously an important parameter which determines the establishment of the physical contact with soil fungi. Chapter 12 gives an overview about the current knowledge on this topic with regard to the interaction of soil bacteria and ectomycorrhiza-forming fungi. This includes recent data on the effects of a co-cultivation of a range of soil bacteria (Actinomycetes) with an important and widely distributed ectomycorrhiza-forming fungus, *Amanita muscaria*, as part of a model system. A specific topic is the interference of a bacterial strain, which highly promotes fungal growth with the protein complement of the latter. Chapter 13 deals with respective root properties such as type of root (long/short root) and surface chemistry. Here, hydrophobic cuticle layers obviously play an important role in hyphal attachment. In addition, compatible fungi are able to penetrate and digest this layer. How far this process is involved in altering the morphology of fungal hyphae when inside the root cortex (Hartig net formation) is discussed. As the data presented in this chapter originate mainly from ultrastructural investigations, possible pitfalls of such studies are also addressed.

An integral part of root–fungus associations are soil bacteria. These can support the development of the root/fungus interaction by improving fungal root colonization, the availability of nutrients, or by producing exudates (e.g., antibiotics) which can prevent attacks of pathogenic microorganisms. While ectomycorrhizas only constitute a small fraction of all root/fungus interactions known, another form of this symbiosis, namely endomycorrhiza, dominates by far, and facilitates nutrient uptake of many crop plants. Fungi forming this type of mycorrhiza can usually not be cultured in the absence of a plant root. Chapter 14 focuses on structural studies of the interaction of these fungi with their host plants. Electron microscopy reveals interaction-specific structures such as fungal deposits and interactive vesicles, which can be used for diagnostic purposes. *Piriformospora indica* is possibly an exception because this fungus can be cultivated separately and forms structures comparable to those of endomycorrhizas. Chapter 15 deals with the diverse interactions of this fungus with roots from a variety of plants (from bryophytes to a wide range of angiosperms) and various groups of soil microorganisms, including bacteria of the rhizosphere (compare also Chap. 12) and other soil fungi such as *Aspergillus* or *Gaeumannomyces* (root pathogen).

Interactions between smut fungi and their plant hosts are another topic of Section C. The term “smut fungus” characterizes fungi sharing similar organization and life strategies. As these fungi can considerably reduce crop yields, they are of economic importance. Most of them are members of the Ustilaginomycetes, which comprise a large number of species. Fungi can also