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MINIREVIEW

Microbiology of the Phyllosphere

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The above-ground parts of plants are normally colonized by a variety of bacteria, yeasts, and fungi. While a few microbial species can be isolated from within plant tissues, many more are recovered from the surfaces of healthy plants. The aerial habitat colonized by these microbes is termed the phyllosphere, and the inhabitants are called epiphytes. While there has been some investigation of the colonists of buds and flowers (1, 48), most work on phyllosphere microbiology has focused on leaves, a more dominant aerial plant structure. Bacteria are by far the most numerous colonists of leaves, often being found in numbers averaging 10^6 to 10^7 cells/cm^2 (up to 10^8 cells/g) of leaf (1, 7, 41). Because of their numerical dominance on leaves, and because more information is available on the process of bacterial colonization of leaves, we focus on this group of microbes in this review.

Compared to most other bacterial habitats, there has been relatively little examination of phyllosphere microbiology. This is somewhat surprising given the abundance of plants in the world and the roles of various phyllosphere bacteria in the important processes discussed below. Leaves constitute a very large microbial habitat. It is estimated that the terrestrial leaf surface area that might be colonized by microbes is about 6.4×10^8 km^2 (83). Given the large number of bacteria on leaves in temperate regions of the world and that populations in tropical regions are probably even larger, the planetary phyllosphere bacterial population may be as large as 10^{26} cells (83). Clearly, in aggregate, these bacteria are sufficiently numerous to contribute in many processes of importance to global processes, as well as to the behavior of the individual plants on which they live.

In the following sections we review available information on the identities and properties of the bacterial colonists of plant surfaces as well as describe the nature of the leaf surface habitat that these bacteria colonize. We emphasize the results of studies using new molecular and microscopic tools that have provided new insights into both the identity and the behavior of epiphytes, as well as into the nature of the plant surfaces that they inhabit. Also, we review recent studies of the interactions of various epiphytic bacteria with plants, which suggest that there is much more interaction between them than in a strict commensalistic relationship, in which they have been traditionally considered to coexist. The interactions that occur between bacteria on leaves have also received considerable attention. We attempt to illustrate how new approaches to defining the nature of leaf surface habitats have helped us to understand the behavior and interactions of epiphytic bacteria as well as why leaves are a particularly suitable habitat for exploring processes in microbial ecology. More comprehensive reviews of phyllosphere microbiology also address other important features of this interesting association (1, 7, 8, 41, 47, 55, 73).

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MICROBIAL COMMUNITIES ON LEAVES

The microbial communities of leaves are diverse and include many different genera of bacteria, filamentous fungi, yeasts, algae, and, less frequently, protozoa and nematodes. Filamentous fungi are considered transient inhabitants of leaf surfaces, being present predominantly as spores, whereas rapidly sporulating species and yeasts colonize this habitat more actively (1). Bacteria are by far the most abundant inhabitants of the phyllosphere. Epiphytic bacterial populations differ sharply in size among and within plants of the same species, as well as in close proximity, and over short time scales (40) as well as over the growing season (26, 114). These considerable variations in population sizes are caused in great part by the large fluctuations in the physical and nutritional conditions characteristic of the phyllosphere. Additionally, plant species appear to influence the microbial carrying capacity of the leaf, since the total number of culturable bacteria recovered from broad-leaf plants such as cucumber and beans was significantly greater than that recovered from grasses or waxy broad-leaf plants (58, 89). Reflective of marked differences in the physicochemical environments of above-ground versus subterranean plant surfaces, the leaf bacterial flora differs substantially from that of roots. For example, pigmented bacteria, which are rarely found in the rhizosphere, dominate leaf surfaces (28, 103, 104), presumably because solar radiation influences the ecology of the phyllosphere (45, 112). The differential composition of leaf and root bacterial communities is further evidenced by the failure of common root colonizers such as *Rhizobium* (89) and *Azospirillum* (51) to become established on leaves.

Studies of the composition of bacterial communities on leaves have been numerous but rather limited in scope. It is generally believed that populations of culturable aerobic bacteria on leaves are dominated by a few genera. A few exhaustive studies of the variations in the microbial community of leaves over multiple time and space scales have provided important detailed knowledge about the identity and the ecology

of bacterial leaf inhabitants (22, 25, 26, 46, 114). Ercolani (26) made an extensive inventory of culturable aerobic bacteria isolated from the surface of olive leaves over six growing seasons and reported distinct bacterial community structures on leaves of the same age at a given time of the growing season. Thompson et al. (114) analyzed 1,236 bacterial strains from immature, mature, and senescent leaves of field-grown sugar beets over a complete growing season. They identified 78 species and 37 named and 12 unnamed genera of bacteria. Most importantly, like Ercolani (26), they found distinct patterns of microbial colonization at different times of the year, with bacterial community diversity being lowest during the warmest and driest months of the season and highest during the cooler and rainy months. Coincidentally, in both of the above-described studies, communities on young leaves were composed of a greater number of taxa than those of old leaves. Thus, specific natural environments of the phyllosphere apparently select for the presence of specific genotypes within the leaf bacterial community. This is further supported by the finding that the acquisition by *Pseudomonas fluorescens* of plasmids that are indigenous to the leaf microflora coincided with a specific maturation stage of the plant over two consecutive years (64). This indicated that traits carried on these plasmids conferred variable selective fitness to specific plasmid-bacterial host combinations during the growing season, possibly in response to changing conditions in the phyllosphere habitat (2).

The study of bacterial colonizers of leaves has been restricted mostly to aerobic culturable bacteria and also driven by the importance of investigating the ecology of plant-pathogenic bacteria because of their deleterious effect on plant productivity. Thus, the microbial ecology of the phyllosphere has been viewed mainly through the biology of gram-negative bacteria such as *Pseudomonas syringae* and *Erwinia* (*Pantoea*) spp., two of the most ubiquitous bacterial participants of phyllosphere communities. There is reason to believe, however, that the extreme fluctuations in the physicochemical environment of the phyllosphere over short time scales may select for bacterial species that have unusual and versatile traits that make them fit to colonize plant surfaces but have remained unculturable. Indeed, similar to other investigations of natural microbial consortia, a pioneering study by Yang et al. (123) has demonstrated that culture-independent methods reveal higher community complexity in the phyllosphere than conventional culture-based methods. Their study revealed that the majority of 16S rRNA sequences recovered from the leaf washings of various plant species were from bacteria not previously described as being found in the phyllosphere, with some sequences representing undescribed species (123).

In recent years, the association of multiple outbreaks of food-borne illness with fresh fruits and vegetables (17, 20, 36, 37, 82) has raised concern about the possible preharvest contamination of plants with human pathogens. Surveys of the occurrence of enteric pathogens on produce showed that *Salmonella* spp. and *Shigella* spp. were detected in up to 4% of the samples (http://www.cfsan.fda.gov). Two independent studies have shown that *Salmonella enterica* and *Escherichia coli* have the ability to colonize corn, bean, and cilantro plants under humid conditions, albeit to lower population levels than those of common bacterial epiphytes (13, 89). Unlike *P. syringae*, they failed to grow on leaves under dry conditions (13, 89). However, *S. enterica* survived dry conditions on cilantro leaves and recovered to achieve significant population sizes under subsequent wet conditions (13). The relative fitness of some human enteric pathogens in the phyllosphere, as well as the wide distribution on plants of *Enterococcus* spp. (90) and of common opportunistic pathogens such as *Pseudomonas aeruginosa* (19) and *Burkholderia cepacia* (3, 34), should prompt us to broaden our paradigms on plant microbial communities and their significance to the field of microbiology.

THE LEAF SURFACE AS A MICROBIAL HABITAT

The leaf surface has long been considered a hostile environment for bacterial colonists. The leaf surface is exposed to rapidly fluctuating temperature and relative humidity, as well as repeated alternation between presence and absence of free moisture due to rain and dew. The leaf also provides limited nutrient resources to bacterial colonists. While other habitats probably offer more extreme conditions of desiccation or temperature, etc., they may not be subject to such rapid and extreme fluctuations in these several physical conditions. Several factors may influence the microhabitat experienced by bacteria on leaves. First, the leaf itself is surrounded by a very thin laminar layer in which moisture emitted through stomata may be sequestered, thereby alleviating the water stress to which epiphytes are exposed. Second, some cells in a leaf bacterial population, particularly in plant-pathogenic populations, may not reside in exposed sites on the leaf surface but instead may at least locally invade the interior of the leaf, avoiding the stresses on the exterior of the leaf by residing in substomatal chambers or other interior locations (78, 118). Thus, while some phytopathogens may have the option of avoiding stresses, most other epiphytes apparently must tolerate them in some way (7, 8). This issue raises the question of what we mean when we refer to epiphytic bacteria, a term which usually conveys the image of surface colonists of leaves. While a large majority of all bacterial colonists of plants are easily washed from leaves or killed by nonpenetrating agents such as peroxide or UV light (118), it is perhaps more fitting that epiphytic colonization be perceived as one which is somewhat more three dimensional than the planar process sometimes imagined. Therefore, the actual conditions to which epiphytes are exposed on leaves are probably quite different from those estimated from large-scale measurements of irradiation, humidity, etc. We are only beginning to be able to assess what these conditions actually are, since the scale of the microhabitats that leaves present to bacteria is much smaller than that of even the smallest physical probes. Recent studies using a green fluorescence protein (GFP) reporter gene (*gfp*) linked to a promoter responsive to water availability revealed that the majority of *Pantoea agglomerans* cells on leaves did not experience the extreme water stress on dry leaves that might be expected, suggesting that the surface of the leaf is indeed buffered somewhat from the atmosphere away from the leaf (6).

The surfaces of most plants are very tortuous at the small scales at which interactions with bacteria will occur. Epidermal cells produce bulges and troughs that will determine the shape and size of low areas on the surface, which in turn will influence the shape and spread of water droplets on the plant (77). The first contact between immigrating bacteria and a leaf nor-

mally occurs at the plant cuticle. This waxy layer, which has different three-dimensional crystalline structures on different plant species and can change as leaves age, presumably in part due to microbial modifications (60, 61, 77), limits passive diffusion of nutrients and water vapor from the plant interior onto the surface and defines the hydrophobicity of the leaf. Thick waxy cuticles have thus been thought to interfere with bacterial colonization of plants by limiting diffusion of nutrients and inhibiting the wetting of the leaf surface. Using maize mutants having epicuticular waxes altered in density or composition as well as in crystal structure (9), Beattie (5) found that while attachment of bacterial cells to the cuticle was not substantially affected, bacterial establishment and maintenance on leaves was influenced in a complex manner; those glossy mutants with the fewest crystalline waxes were not the best hosts for epiphytic bacteria. Such results hint at small-scale interactions between the plant and bacterium that are not yet understood.

The availability of carbon-containing nutrients on leaves is a major determinant of epiphytic colonization. Bacterial communities on well-fertilized plants are limited by carbon availability and only secondarily by nitrogen availability (120, 121). Several studies have revealed that small amounts of nutrients can be washed from leaves (115). Simple sugars such as glucose, fructose, and sucrose are the dominant carbon sources on the plants that have been examined and are thought to simply leach from the interior of the plant (78, 115). Several lines of recent evidence suggest that nutrient availability on leaves is highly spatially heterogeneous. For example, chemical analysis revealed that about 0.2 to10 μg of sugar (enough to support the growth of 10^7 to 10^8 cells/leaf) could be washed from uncolonized bean leaves. Substantial residual sugars could also be washed from leaves heavily colonized by bacteria, suggesting that nutrient resources may be spatially sequestered from epiphytes (78). Molecular biosensors for different sugars have revealed a great deal about the chemical environment of the phyllosphere at the small spatial scales of relevance to microbes. A whole-cell bacterial biosensor, consisting of *Erwinia herbicola* cells harboring a sucrose- and fructose-responsive *scrY* promoter fused to a *gfp* or *inaZ* reporter gene, exhibits GFP fluorescence and ice nucleation in a sugar-dependent manner (80). Studies performed with this bioreporter in situ on plants revealed a high-level heterogeneity of apparent sucrose availability but an average sucrose availability of only about 20 μM on uncolonized moist bean leaves. The use of short-half-life variants of the *gfp* reporter gene with a fructose-responsive *fruB* promoter in another study provided unparalleled information on the process of consumption of nutrients by bacteria on plants (63). While nearly all fructose-utilizing bioreporter cells were engaged in consumption of fructose (as evidenced by GFP fluorescence) within 1 h after inoculation onto plants, this fraction dropped to less than 1% within 24 h, suggesting a highly heterogeneous availability of nutrients to individual cells (63). Furthermore, direct in planta examination of the bioreporter cells on leaves revealed that those cells that continued to consume sugars were generally in localized sites on the plant and not randomly dispersed across the leaf (63). Together, such observations suggest that most areas of a leaf harbor only small amounts of nutrients, and nutrients may be relatively abundant in only a few locations. Thus, most immigrants to a leaf may find themselves in an oligotrophic environment, with consequently limited growth and metabolic activity, whereas some cells encounter "oases" of relatively abundant nutrients. In contrast, and although in overall low abundance on leaves, iron is apparently not limiting to the growth of bacteria on leaves (75). However, a high variability in available Fe^{+3} on leaves was observed when a *gfp*-based iron biosensor strain of *P. syringae* was used, with many cells experiencing no iron limitation (50). Such heterogeneity in the phyllosphere environment as that described above places constraints on the patterns of competition and other interactions that can occur among phyllosphere bacteria. As discussed below, those few sites on a leaf where abundant nutrients are available due to localized leakage, such as from glandular trichomes or sites of injury, are where large bacterial aggregates also form (Fig. 1).

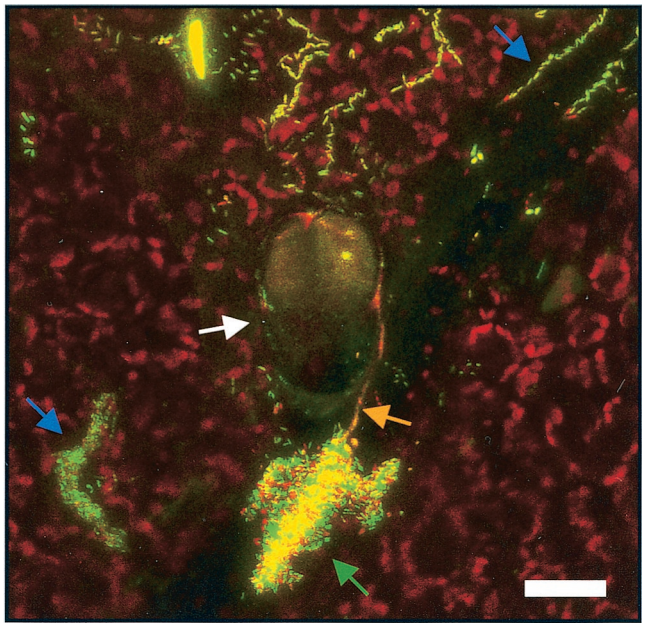

FIG. 1. Fluorescence micrograph of the natural microbial flora colonizing the bean phyllosphere. A large mixed bacterial aggregate (green arrow), which also includes a fungal hypha (orange arrow), has formed at the base of a glandular trichome (white arrow). Bacteria are present also at plant cell junctions and on veins (blue arrows). The red background originates from the autofluorescent chloroplasts within the leaf epidermal cells. The natural microbial flora was visualized by confocal laser scanning microscope-projected *z* series of the surface of a leaf from a bean plant grown in the field and subsequently incubated under humid conditions at 24°C. The microbial flora was stained with Live *Bac*Light (Molecular Probes, Inc., Eugene, Oreg.), which imparts green and red fluorescence to gram-negative and gram-positive bacteria, respectively. Bar = 20 μm.

Large fluxes of UV radiation are one of the most prominent features of the leaf surface environment to which epiphytes presumably have had to adapt. Indeed, a striking feature of epiphytic bacteria is the large proportion that are pigmented (28, 103); such pigmentation has been presumed to confer protection against UV radiation. A recent detailed examination of the epiphytic bacteria present on peanut plants exposed to high fluxes of UV revealed that most tolerated relatively large UV fluctuations (112). Interestingly, the relative proportion of UV-tolerant strains in the bacterial community in-

creased during those parts of the day when leaves were exposed to UV, and the most tolerant strains were those that produced pink or orange pigments (112). In *P. syringae*, a particularly fit epiphytic bacterial species, UV tolerance has been associated with UV-inducible plasmid-borne *rulAB* genes, which confer mutagenic DNA repair (53, 54, 107–109). Nearly all naturally occurring strains of this species have a functional *rulAB* locus, and those without this locus are much less tolerant of UV radiation on leaves. The presence of such adaptive traits on plasmids may be one means by which epiphytes maintain such elements and other conditionally beneficial genes in the population. The mutagenic DNA repair exhibited by epiphytes such as *P. syringae* may endow them with significant adaptive potential for genome evolution by transiently increasing the mutation rate in cells upon periodic exposure to UV (107). Further examination of traits harbored on indigenous plasmids in plant-associated bacteria should shed much light on other phenotypes that are important for an epiphytic lifestyle.

MICROBIAL MODIFICATION OF THE LEAF HABITAT

Alteration of plant surface properties. Multiple chemical and physical factors limit bacterial growth and survival in the phyllosphere. Thus, it might be expected that there should be selection for any bacterial phenotype that allows epiphytic bacteria to circumvent this limitation. Such phenotypes may include traits that confer on bacteria the ability to modify their microhabitat in order to increase nutrient availability on the phylloplane. For example, bacteria can increase the wettability of leaves by producing compounds with surfactant properties (16, 43, 86). Bunster et al. (16) found that this ability occurred in 50% of the *Pseudomonas* strains tested. Because of the hydrophobic nature of the cuticle, it is likely that increased wettability of these habitats allows solubilization and diffusion of substrates, making them more readily available to epiphytic bacteria. Alternatively, biosurfactants may facilitate the movement of bacteria on the phylloplane, as was suggested for tolaasin, a toxin produced by *Pseudomonas tolaasi* (43). The water film created by the surfactant could spread the bacteria across the leaf surface to areas where nutrients are more abundant. Thus, the production of biosurfactants may be one trait by which bacteria can alter their habitat to exploit it more efficiently.

Genetic determinants for the biosynthesis and secretion of the toxin syringomycin were found to be present in most strains of *P. syringae* pv. syringae, including many nonpathogenic strains of this species (96), which commonly can be recovered as epiphytes from a wide variety of plants. Syringomycin production is an important virulence factor in *P. syringae* pv. syringae (81). This toxin affects ion transport across the plant cell plasma membrane by inducing the formation of ion channels; this ion flux leads to the release of metabolites from the plant cells and ultimately to cell lysis (44). However, significant levels of pore-forming activity in plant cells were detected at concentrations of syringomycin much lower than that required for measurable cell lysis (44). Nonpathogenic strains of *P. syringae* may synthesize syringomycin in amounts that are insufficient to cause cell necrosis and disease but are high enough to trigger a low-level release of plant metabolites; in addition to its effect on plant cells, syringomycin acts as a potent biosurfactant (44) (Fig. 2A). These properties may explain why syringomycin production, a complex biosynthetic and regulatory process (10), was selected for in both pathogenic and nonpathogenic strains of *P. syringae*.

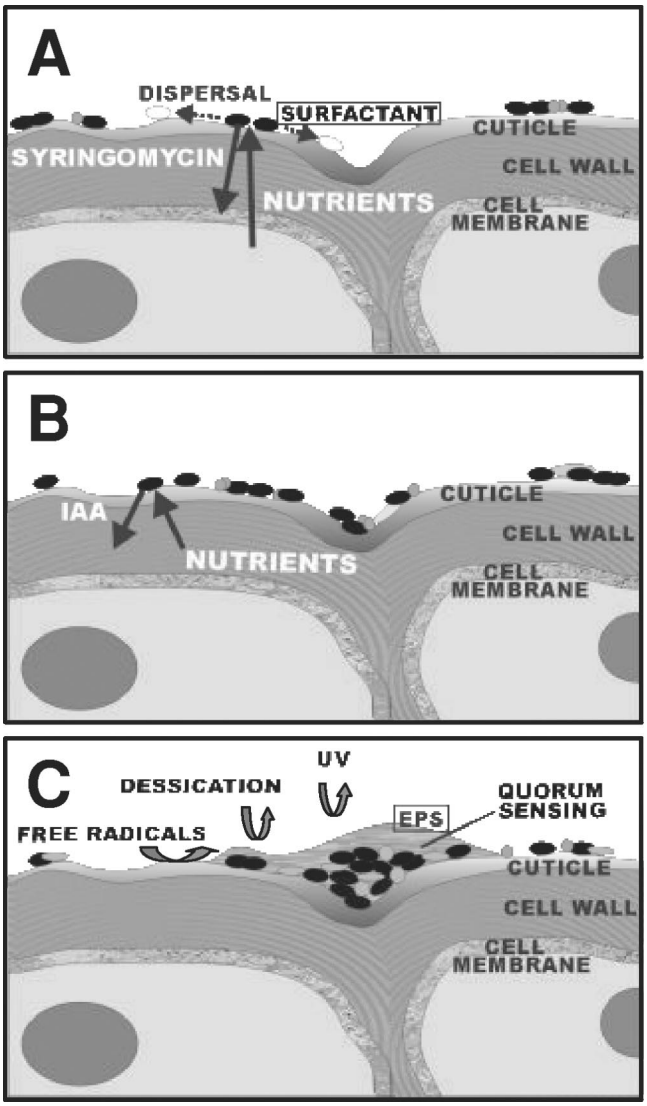

FIG. 2. Schematic diagram representing various hypothetical bacterial-habitat modifications in the phyllosphere, such as the release of nutrients from plant cells and bacterial cell dispersal effected by the production of syringomycin, which may act both as a phytotoxin and as a surfactant (A); the release of saccharides from the plant cell wall, caused by bacterial secretion of auxin (B); and protection from environmental stresses via production of EPS in bacterial aggregates (C).

In contrast to the production of biosurfactants and syringomycin, which appears to be restricted to *Pseudomonas* species, the biosynthesis of the plant growth regulator indole-3-acetic acid (IAA) is widespread among bacterial colonizers of the phyllosphere (14, 27, 32, 71). Although bacterial production of auxin is a major pathogenicity determinant in many bacteria inducing hyperplasia on plants (122), its role in other pathogenic and nonpathogenic plant-associated bacteria has not been determined. Because IAA is involved in many aspects of

plant development, it is of great interest that microbes which colonize plant surfaces have the ability to synthesize an auxin identical to that found in plants. In competition experiments, an IAA-producing strain of *Pantoea agglomerans* reached twice the population size of an isogenic IAA-deficient mutant on pear flowers in the field and on bean plants in the greenhouse (12). This increase in the ratio of the population size of the parental strain over that of the IAA-deficient mutant occurred only during periods of active colonization of the plants (12). IAA production in *Pantoea agglomerans* was also associated with increased fitness during periods of drought stress on plants (76). IAA promotes cell wall loosening at very low concentrations (116), and exogenously applied auxin stimulates the release of saccharides from the plant cell wall (31, 33). Because bacteria on plants are frequently nutrient limited, it was hypothesized that the greater epiphytic fitness of IAA-producing strains resulted from enhanced nutrient availability caused by increased leakage of saccharides from plant cells in their vicinity (Fig. 2B). There is also circumstantial evidence that the synthesis of cytokinin by *Methylobacterium* spp., a successful plant colonizer, has a role in its interaction with its plant host (42). Foliar applications of *Methylobacterium* spp. increased soybean yields; in return, the secretion of cytokinin, which is involved in plant cell division and expansion, may trigger the release of methanol from the plant cell wall and provide nutrients to *Methylobacterium* (42). Future studies with cytokinin-deficient mutants of *Methylobacterium* will help to elucidate the benefits of cytokinin production in plant colonization by this common epiphyte.

Cell density-dependent modifications. There exists considerable evidence that bacteria form large and heterogeneous aggregates on plant surfaces. Microscopic examinations of colonized leaves have revealed that on plant surfaces, many epiphytes occur in large mixed-bacterial-species aggregates that also harbor fungi (72, 84, 85) (Fig. 1). While large numbers of solitary bacterial cells occur on plants, a few large masses of apparently mixed bacterial species can be found. Initial results suggest that while uncommon, such aggregates can constitute between 30 and 80% of the total bacterial population on certain plant species (72; J.-M. Monier and S. E. Lindow, unpublished data). Such assemblages, with an extent and structure similar to those of biofilms that develop in aquatic habitats, are probably found only on long-lived leaves in moist climates such as the tropics or wet temperate regions such as the Pacific Northwest (1). The assemblages on most other plants, while of considerable size, might best be called aggregates (1). The formation of aggregates by bacteria on plants has major implications for the ability of these microbes to colonize and survive the harsh environment of the phyllosphere, as it may provide them with a means to modify their immediate environment in this habitat (Fig. 2C). The production of extracellular polysaccharide (EPS), which is considered to form a major part of the bacterial aggregate matrix (21), may benefit epiphytes in the phyllosphere. Given that water availability is likely one of the most highly fluctuating factors on leaf surfaces, the heavy EPS slime within aggregates may shield the bacteria from desiccation stress by buffering the matric and osmotic potentials of their surroundings. Additionally, EPS has a role in protecting plant-associated bacteria from reactive oxygen species (59), which are often encountered on plants. It has been demonstrated that aggregated bacteria resist oxidative stress better than planktonic bacteria (101). The synthesis of alginate by *P. syringae* is stimulated by desiccation stress (52, 98) and H_2O_2 (52) and contributes to the epiphytic fitness of this organism on dry leaves (124). The dense matrix surrounding cells within aggregates may also increase nutrient concentrations (21), a great benefit to epiphytic bacteria since the phyllosphere is overall a nutrient-poor environment (63). There is strong evidence that high cell density induces the expression of particular phenotypes (4, 93) and that cross talk via quorum-sensing signals occurs between different bacterial species (92). Many epiphytic bacterial species can produce *n*-acyl homoserine lactones that act to control cell density-dependent gene expression (18, 23). Given that recent results indicate that cells in aggregates are much more tolerant of desiccation stresses on leaves than are more solitary cells (72; Monier and Lindow, unpublished), cell density-dependent signaling may play a major role in the behavior of bacteria epiphytes. Thus, it is tempting to speculate that bacterial cells within aggregates may have the ability to modify their microenvironment on the plant surface by triggering the expression of traits by neighboring microbes to benefit their own fitness on plants. If cell-cell signaling via small molecules proves to be an important factor in regulating genes involved in epiphytic fitness, as it is in other habitats, then many new avenues for managing bacterial colonization of plants might be developed.

Plant-microbe interactions. Bacterial alteration of the host plant environment has been traditionally associated with plant-pathogenic bacteria. The ability of plant-pathogenic bacteria such as *P. syringae* to cause disease is strongly correlated with their epiphytic population sizes on leaves (66, 97, 105) and probably also with that subset of the population that is within symptomless leaves and, thus, in more intimate contact with plant cells (8, 118). Considerable attention has been given to the characterization of the molecular determinants that are involved in the interaction of *P. syringae* with its host, particularly the hypersensitive response and pathogenicity (*hrp*) genes. The *hrp* gene cluster encodes a type III protein secretion pathway and related proteins (35). Field studies by Hirano et al. (38, 39) of the role of *hrp* genes in the fitness of *P. syringae* on beans revealed that an intact type III secretion apparatus is required for the growth, and possibly the survival, of *P. syringae* in the phyllosphere. These results suggest that the secretion of particular molecules enables *P. syringae* to interact with and modulate the metabolism of plants to its advantage. The presence of a functional type III secretion pathway in *P. fluorescens* and *Pseudomonas putida* (94) prompts us to hypothesize that habitat modification by type III secretion may be one of the ecological strategies employed also by nonpathogenic bacterial colonizers of plant surfaces.

INTERACTIONS BETWEEN BACTERIA ON PLANTS

Much of what has been learned of the interactions of bacteria with each other on leaves has come from studies with the goal of exploiting such interactions to control plant diseases or frost injury caused by epiphytic bacteria. The composition of epiphytic bacterial communities can be strongly influenced by establishment of certain antagonistic bacteria at appropriate times during plant development. Perhaps the best-studied sys-

tem is biological control of frost injury of sensitive plants, such as flowers of deciduous fruit trees, leaves of sensitive herbaceous plants, and tropical plant species. Epiphytic bacterial species with ice nucleation activity (Ice^+ bacteria), such as *P. syringae*, contribute to frost injury to these plants by reducing their ability to supercool and avoid damaging ice formation (67, 68, 70). Since the ice nucleation temperature of these plants increases with increasing population sizes of Ice^+ bacteria, a strategy of frost control is to reduce the numbers of Ice^+ bacteria on plants at the onset of cold temperatures. Fortunately, the population size of Ice^+ bacteria on young tissues such as flowers or young leaves of most plants is very small and increases with time due to the immigration of bacterial cells followed by their growth on plants (68, 70). Preemptive competitive exclusion of Ice^+ bacteria with naturally occurring non-ice nucleation-active bacteria has proven to be an effective and practical means of frost control (70, 74). If applied to plants before they become colonized by other bacteria, such antagonists rapidly multiply and compete for limiting resources, presumably carbon compounds (119, 120); antagonism via the production of antimicrobial compounds appears to be an uncommon mechanism of interaction of bacteria on leaf surfaces (69). Recombinant Ice^- bacteria, the first recombinant microorganisms released into the open environment in field experiments, were used to test the specificity with which competitive exclusion of Ice^+ bacteria occurred (70, 99). These studies of the competition between isogenic and heterologous Ice^+ bacteria under field conditions revealed that a similarity in resource requirements maximized the competitive exclusion of immigrant bacteria (70) and verified greenhouse study data suggesting that bacteria differed in their ability to exclude others from a leaf (56, 67). Management of frost injury by reducing Ice^+ bacterial population size has become an important new method of frost control; lyophilized preparations of *P. fluorescens* strain A506 are now commercially available (Blightban A506; Nufarm Americas, Inc., Sugar Land, Tex.) for spray application to a variety of crop plants for frost control.

Nonchemical management of fire blight disease is perhaps the most advanced example of biological control of disease, and it draws directly from detailed studies of the ecology of both the pathogen and antagonists on plants (48). This potentially devastating disease of the pear and apple, caused by the bacterium *Erwinia amylovora*, is typical of most bacterial diseases in that the pathogen establishes itself on susceptible plant tissues (flowers in the case of fire blight) before infection occurs. Detailed studies of the ecology of the pathogen as well as of potential antagonists have led to nonchemical means of disease control, thus reducing the need for the frequent applications of antibiotics, such as streptomycin and oxytetracycline, normally used for disease control. Prior colonization of the stigmatic surface of flowers with nonpathogenic bacteria such as *P. fluorescens* strain A506 and *Pantoea agglomerans* strains can greatly inhibit colonization by *E. amylovora*, leading to a substantial reduction in disease (48, 74, 95, 102, 119). These antagonists were also shown to move readily from inoculated to noninoculated flowers, thereby facilitating biocontrol in flowers that otherwise would support relatively few other indigenous bacteria (49, 88). Several other recent studies have revealed the in vitro antagonism and/or competitive interactions of potential antagonistic bacteria with bacterial and fungal plant pathogens on plants (15, 57, 79, 106, 117, 125, 126). They illustrate the considerable potential for further development of useful biological control organisms for diverse diseases.

The aggregated nature of bacterial epiphytes on leaves, presumably at sites of relative nutrient abundance, may explain the remarkably high rates of plasmid transfer observed among phyllosphere bacteria. The transfer of plasmid RP1 from donor to recipient *P. syringae* cells on leaves occurred at frequencies as high as 40% after inoculation of these bacteria onto bean leaves (11). Surprisingly, the rate of transfer on plants exposed to high relative humidities was equal to the plasmid transfer rate on plants in low-relative-humidity environments, whereas the metabolic activity of the cells was lower at low relative humidities (11). In an ingenious experiment in which plasmid transfer from a *P. putida* strain on leaves could be visualized by green fluorescence of recipient cells due to the derepression of a *gfp* reporter gene, as much as 33% of the recipient population had acquired a derivative of a TOL plasmid (87). The 30-fold-higher rate of plasmid transfer on leaves compared to membrane surfaces was ascribed to the aggregation of bacterial cells that occurred at the junction of plant epidermal cells, thus facilitating exchange (87). Such laboratory studies help explain the very high rates of acquisition of indigenous mercury resistance plasmids by a genetically marked strain of *P. fluorescens* after it was introduced onto plant surfaces (65). Given that the communities of bacteria on plants undergo substantial compositional changes during a growing season (24, 62, 65, 113) and that epiphytic bacterial species harbor a diversity of plasmids (63, 64, 110, 111), the potential for extensive mixing of genes in these communities seems large. Together, these observations indicate that compared to other habitats, such as the soil, rates of plasmid transfer on leaves are very high and may make the genetic and phenotypic stability of inocula introduced onto plants unpredictable with time. It is tempting to speculate that the nutrient-rich but localized leaf sites that support cell aggregates and at which bacteria at least transiently retain high levels of metabolic activity are also the sites at which gene transfer occurs. If so, this would suggest that leaves are at least transiently less oligotrophic than other habitats, such as soil. It also suggests that leaf surfaces are hot spots for horizontal dissemination of genetic information and, therefore, are important breeding grounds for microbial diversity (73).

CONCLUSIONS

The phyllosphere is both scientifically and economically an important habitat in which to study microbial ecology. Epiphytes are involved in processes as large in scale as the carbon cycle (intercepting carbon compounds released directly from plants or removed by sucking arthropods [100]) and the nitrogen cycles (nitrification of ammonium pollutants intercepted by plants [91]; nitrogen fixation [29, 30]) to processes affecting the health of individual plants. Because of the importance of many phyllosphere microbial inhabitants to plant health, there will likely be many practical applications that result from a better understanding of the interactions of microbes with plants and among themselves. This enhanced knowledge may

contribute also to our understanding of the ecology of human-pathogenic bacteria on plant surfaces and provide new insights for the development of prevention or control strategies to manage preharvest contamination of crops with enteric pathogens.

The phyllosphere has many features that make it an excellent habitat in which to study microbial ecology. Leaves are clean, and microbes can be observed directly on leaves, enabling the use of powerful new microscopic techniques to measure microbial identity, activity, and gene expression. To test models of microbial behavior, we can easily change the nature of the habitat conditions on the plants on which bacteria live by genetically altering plants. Plants can be readily grown without epiphytic microbial communities, allowing us to readily manipulate their inhabitants, while communities can be made as simple or complex as needed by simple inoculation. In addition, important microbial processes, such as immigration, and ecological models, such as island biogeography, can be readily explored in epiphytic bacterial systems. Thus, phyllosphere microbiology has much to offer to the field of microbial ecology and promises to contribute to more effective and less environmentally damaging means of plant protection.

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