Phyllosphere microbiology

Steven E Lindow*† and Johan HJ Leveau*‡

Aerial plant surfaces harbor large numbers of microbes, some of which are deleterious to plants whereas others are benign or beneficial. Commercial formulations of bacteria antagonistic to plant pathogenic microbes and ice nucleation active bacteria have been utilized as an environmentally safe method to manage plant disease and to prevent frost damage. Molecular genetic tools, microscopic examination and whole-cell bacterial biosensors have provided extensive information on these microbes, their complex associations and their habitat. The aerial habitat influenced by plants, termed the phyllosphere, is particularly amenable to studies of microbial ecology and the information gained should lead to more effective means of plant protection.

Addresses
*University of California, Department of Plant and Microbial Biology, 111 Koshland Hall, Berkeley, California 94720, USA
†e-mail: icelab@socrates.berkeley.edu
‡The Netherlands Institute of Ecology (KNAW-NIOO), Centre for Terrestrial Ecology, Heteren, The Netherlands;
e-mail: leveau@cto.nioo.knaw.nl

Current Opinion in Biotechnology 2002, **13**:238–243

0958-1669/02/$ – see front matter

DOI 10.1016/S0958-1669(02)00313-0

Abbreviations
GFP green fluorescent protein
UV ultraviolet

Introduction

Aerial plant parts harbor hundreds of species of bacteria, yeast, and fungi. Bacteria are by far the most numerous colonists, often being found at upwards of 10^7 cells/cm^2 of leaf surface. When one considers that a large fraction of the earth's surface is covered with plants, that leaf surfaces often represent a substantial multiple of the soil surface area, and that leaves and flowers often have complex topographical features on which colonization can occur, the potential population size of microbial associates of plants is indeed impressive. The aerial habitat influenced by plants is termed the phyllosphere and inhabitants are called epiphytes. Much of the interest in phyllosphere microbiology has been driven by the need to better understand the behavior and control of the plant pathogens that are prominent members of this community. Their spread, colonization, survival and pathogenicity mechanisms have been the subject of much research. Plant productivity can be affected by bacteria that incite frost injury [1], whereas others produce phytohormones that have the potential to affect plant development and productivity [2]. Much less is understood about the identity or properties of the numerous non-pathogenic microbes that inhabit the phyllosphere; such colonists apparently play important roles in modulating population sizes of deleterious microbes, and some are being exploited as biological control agents for disease and frost control. New molecular and microscopic tools are being developed to better understand both the identity and behavior of epiphytes as well as the nature of the plant surfaces that they inhabit. Such information will be important for better understanding the process of plant disease and for developing and implementing new methods of control, for example, by interfering with growth, survival or other behaviors of harmful epiphytic microbes. In this review we will emphasize the recent advances made in understanding the epiphytic biology of bacteria since publication of the last reviews on this topic [3–6]. After addressing new studies that focus on the biology of phytopathogens and mechanisms and practice of their biological control, we will illustrate how other fundamental studies of epiphytic bacteria promise to provide the basis for a more comprehensive understanding of the microbial ecology of the phyllosphere.

Biological control of plant disease and frost injury

Potentially devastating diseases such as fire blight of pear and apple are typical of most bacterial diseases in that inoculum of the pathogen, *Erwinia amylovora*, develops on susceptible plant tissues (flowers in the case of fire blight). Detailed study of the ecology of the pathogen as well as potential antagonists has led to non-chemical means of disease control, thus reducing the need for the frequent applications of antibiotics such as streptomycin and oxytetracycline normally used for disease control. Recent work has shown that prior colonization of the stigmatic surface of flowers with non-pathogenic bacteria such as *Pseudomonas fluorescens* strain A506 and *Pantoea agglomerans* C9-1 can greatly inhibit colonization by the pathogen, leading to substantial reductions in disease [7–10] (Figure 1). Lyophilized preparations of *P. fluorescens* strain A506 are now commercially available for spray application to flowers in the early spring for disease control (Blightban A506®). These antagonists were also shown to move readily from inoculated to non-inoculated flowers, thereby facilitating biocontrol in flowers that otherwise would support relatively few other indigenous bacteria [8,9,11,12]. Non-chemical management of fire blight disease is perhaps the most advanced example of biological disease control, and draws directly from detailed studies of the ecology of both the pathogen and antagonists. 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 [13–18]. They illustrate the considerable potential for further development of useful biological control organisms for diverse diseases.

Epiphytic bacterial species with ice nucleation activity (Ice$^+$ bacteria) such as *Pseudomonas syringae* contribute to frost injury of many frost-sensitive plant species by reducing their ability to supercool and avoid damaging ice formation [1,19]. Because the nucleation temperature of these plants increases with increasing population sizes of Ice$^+$ bacteria, pre-emptive 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 [1,9]. This model system has received perhaps the most attention as a vehicle to examine interactions of bacteria on plants. Recombinant Ice$^-$ bacteria, the first microorganisms released into the open environment in field experiments, were used to illustrate the specificity with which competitive exclusion of Ice$^+$ bacteria occurred [1,20]. Management of frost injury by reducing Ice$^+$ bacterial populations has become an important new method of frost control. In fact, the adoption of Blightban A506® for disease control by pear and apple growers has been bolstered by the fact that it competitively excludes Ice$^+$ bacteria as well as *E. amylovora* on plants [9].

Microbial food safety

There is a growing recognition that human pathogenic bacteria can be colonists of food plants. With a trend towards consumption of more fresh (uncooked) fruits and vegetables, and world-wide distribution of such products from diverse production areas with different sanitation schemes, consumers will be at higher risk of exposure to such pathogens. Although most studies of *Escherichia coli* and *Salmonella* contamination of plants are anecdotal reports of their occurrence, there is a growing body of information on features of the plant and/or the environment that dictate their growth and/or survival on plants [21–23]. The extensive information available on the behavior of plant pathogens and other indigenous bacteria on plants (discussed in part below) will undoubtedly aid in developing effective procedures to minimize contamination of plants with human pathogens.

Studies of the epiphytic biology of plant pathogenic bacteria

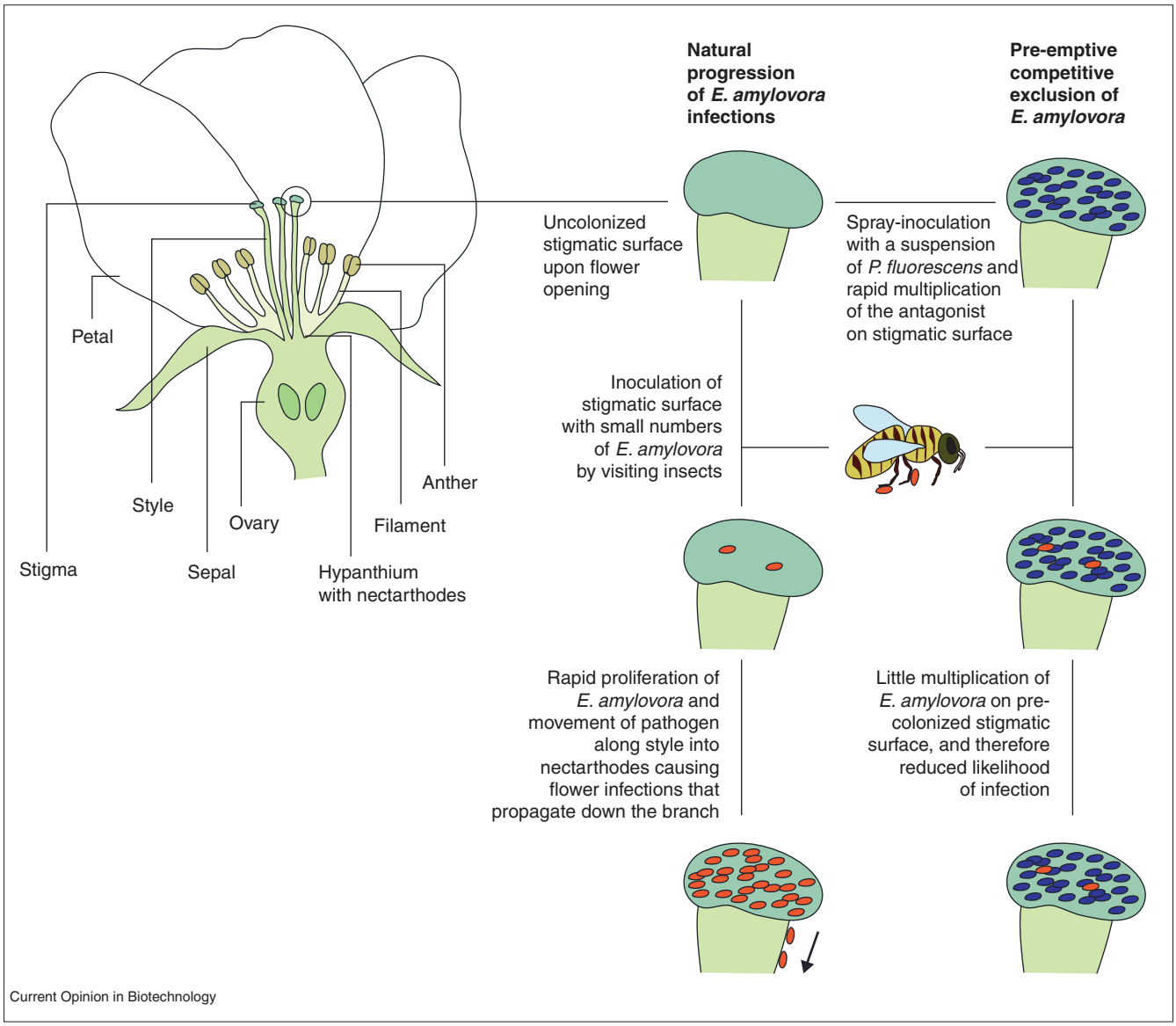
Because plant pathogenic bacteria cause important economic losses, the processes that mediate their epiphytic existence on plants has received much attention. Of particular importance is the question as to whether traits that confer virulence are also required for epiphytic fitness. That such a relation would exist is suggested by recent studies that addressed the location of *P. syringae* and non-pathogenic bacteria on leaves. Although pathogenicity was not required for growth of bacteria in the phyllosphere under conditions of high relative humidity, pathogenicity was involved in the ability to access and/or multiply in certain protected sites in the phyllosphere and in growth on dry leaves [24]. Such results support speculation that there is a broad-spectrum of epiphytic bacteria. Bacteria range from those that employ solely a tolerance strategy of existence during stressful conditions on leaves (such as non-pathogenic bacteria) to those that can also employ an avoidance strategy and establish populations in the internal leaf regions (such as some phytopathogens) [4,5]. Such a phenomenon may also explain why the variation in population size of epiphytic bacteria among leaves changes rapidly upon imposition of stressful conditions; leaves may vary greatly in their ability to shelter bacteria from environmental stresses [25]. The term 'epiphyte', by implying a strictly surface location for plant-associated bacteria, may be misleading in the case of certain plant pathogens that might also establish internal populations. The 'phyllo-sphere' might thus be somewhat more three-dimensional than one would at first conceive.

Specific genes have been associated with epiphytic fitness in *P. syringae*. Mutants in *hrcC* and *hrpJ* (genes encoding components of the type III secretion system for delivery of virulence effector proteins into plants) as well as *gacS* (global regulator) and *pilD* (type IV pili) all exhibited reduced epiphytic fitness under field conditions [26–28]. Although mutations in *hrcC* and *hrpJ* affect growth of *P. syringae* within the plant, and thus may reduce its ability to avoid stresses on plants, the virulence of *gacS* and *pilD* mutants was similar to that of the parental strains. For *gacS* and *pilD*, the loss of epiphytic fitness was postulated to result from, respectively, a reduced production of a protective alginate capsule and diminished cell–cell aggregation on leaves that may shield some cells from stressful conditions [27,28]. Clearly, the behavior of more mutants with altered expression of fitness traits will be required before we can achieve a comprehensive view of the process of epiphytic colonization.

Understanding phyllosphere bacterial ecology and the habitats the leaf provides to its bacterial residents

Molecular tools have proven exceptionally useful in describing the composition and interactions of members of phyllosphere communities as well as the nature of the habitat that they occupy. Like most other habitats, the identity of microbes in the phyllosphere has until recently been limited to those that could be cultured. Although a great diversity of culturable bacteria has been described in the phyllosphere [6,29], the pioneering study of Yang *et al.* [30••] has shown that phyllosphere microbial communities are more complex than previously thought and that many members have not yet been cultured. This study of 16S rRNA sequences revealed that a majority of sequences were from species not previously recognized as phyllosphere bacteria [30••]. Although these results are perhaps not surprising given that such findings have been made in other habitats, it does suggest that there are many phyllosphere inhabitants that have never been investigated and which may harbor unique traits enabling them to thrive on leaves. Given that the leaf surface is considered to be a hostile location for bacterial colonization owing to frequent changes in water availability, incident irradiation, and low nutrient availability (see below), such strains may serve as

Figure 1

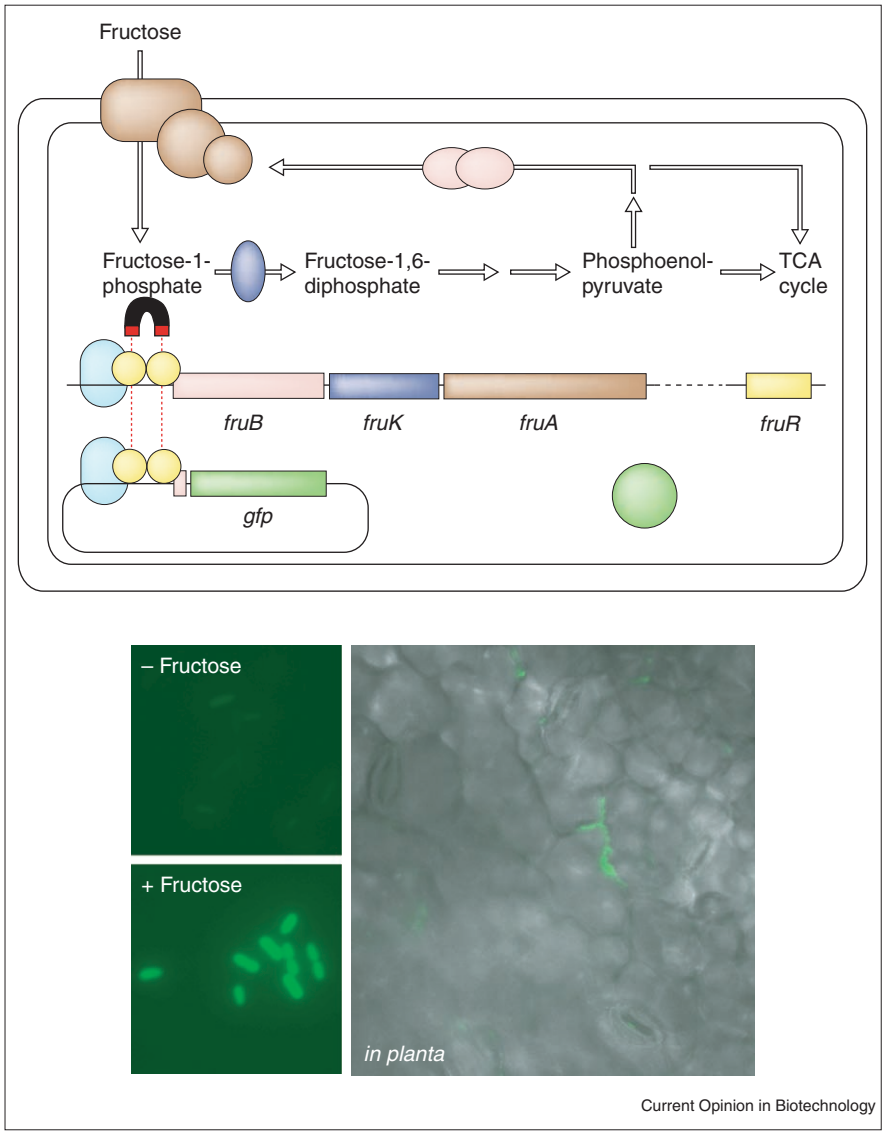


The process of pre-emptive competitive exclusion of the pathogen causing fire blight of pear and apple, *E. amylovora,* from flowers. Although flowers emerge from buds nearly axenic, they are rapidly colonized by immigrant bacteria and populations reach as high as 10^6 cells/flower, primarily on the nutrient-rich stigmatic surface. As *E. amylovora* is often one of the initial immigrants to flowers, being vectored by visiting insects and bees, it is often a dominant member of the flower's microflora. Movement of this large stigmatic inoculum along the surface of the pistil to the hypanthium by water allows infection to occur. Spray inoculation of flowers upon opening with suspensions of *P. fluorescens* A506 (10^8 cells/mL) establishes initial populations of the antagonist of about 10^3 cells/flower. Rapid multiplication of strain A506 to 10^6 cells/flower effectively prevents multiplication of the small numbers of *E. amylovora* that might subsequently be vectored to flowers, thereby preventing infection. Note that stigma, bacteria, and bee are not drawn to scale.

sources of genes encoding stress tolerance traits that may be of considerable biotechnological value.

Several reports have documented remarkably high rates of plasmid transfer among phyllosphere bacteria. The transfer of plasmid RP1 from donor to recipient *P. syringae* cells on leaves occurred in frequencies as high as 40% after inoculation onto bean leaves [31]. Surprisingly, the rates of transfer were equally high on plants exposed to high relative humidities and low relative humidities, whereas the metabolic activity of the cells was lower at low relative humidities [31]. In an ingenious experiment, plasmid transfer from a *Pseudomonas putida* strain could be visualized on leaves by green fluorescence of recipient cells due to the derepression of a green fluorescent protein (*gfp*) reporter gene. As many as 33% of the recipient population acquired a derivative of a TOL plasmid [32•]. There was no relationship between the metabolic activity of cells and

Figure 2



Fructose metabolism in enteric bacteria and its exploitation in whole-cell bioreporters. Fructose is transported into the cell as fructose-1-phosphate by the phosphoenolpyruvate:fructose phosphotransferase system encoded by *fruB* and *fruA*. Further conversion to fructose-1,6-diphosphate is catalyzed by the *fruK* gene product. The *fruB*, *fruK* and *fruA* genes are organized in a single operon that is negatively regulated by the product of the *fruR* gene. In the absence of fructose, the FruR protein prevents RNA polymerase from transcribing the *fruBKA* operon by direct binding to upstream DNA sequences. When fructose is present, fructose-1-phosphate acts as an inducer of *fru* expression by lowering the DNA-binding affinity of FruR. By introduction of a fusion of the FruR-binding region to the gene for green fluorescent protein (*gfp*), the cell is forced to communicate its engagement in fructose metabolism by the accumulation of this fluorescent protein. We have exploited this system to explore plant leaf surfaces for the availability of fructose [38••]. *Erwinia herbicola* cells harboring this gene fusion exhibit no detectable fluorescence in the absence of fructose, but high levels in the presence of this sugar in culture. The patchy distribution of fluorescent cells on leaves suggests that sugars are only locally abundant.

conjugal efficiency and the 30-fold higher rate of plasmid transfer on leaves compared with membrane surfaces was ascribed to the aggregation of cells that occurred between epidermal cells, thus facilitating exchange [32•]. 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 [33]. The abundance of phage reported on plants suggests that transduction may also be prevalent [34,35]. Given that the communities of bacteria on plants undergo substantial compositional changes during a growing season [35] and that epiphytic bacterial species harbor a diversity of plasmids [36], the potential for extensive mixing of genes in these communities seems large. Together, these observations indicate that compared with other habitats such as the soil, rates of plasmid transfer on leaves are very high and such high rates of horizontal gene movement may make the genetic and phenotypic stability of inocula introduced onto plants unpredictable with time. It also suggests that leaf surfaces are hot spots for horizontal dissemination of genetic information and therefore are important breeding grounds for microbial diversity.

Molecular biosensors have revealed a great deal about the chemical and physical nature of the phyllosphere at the spatial scales of relevance to microbes. Although chemical analysis showed that about 0.2–10 μg of sugars (enough to support the growth of 10^7 to 10^8 cells/leaf) could be washed from uncolonized bean leaves, a portion of this sugar remained on leaves after bacterial colonization, suggesting that nutrient resources were patchy and that some nutrients were spatially sequestered from epiphytes [37]. Data in support of this conjecture were obtained using whole-cell bacterial biosensors responsive to fructose and sucrose, which consisted of *Erwinia herbicola* cells harboring fructose/sucrose-responsive promoters fused to a *gfp*

reporter gene [38••] (Figure 2). Although nearly all bioreporter cells were engaged in consumption of fructose (as evidenced by GFP fluorescence) within 1 h after inoculation, this fraction dropped to less than 1% within 24 h, suggesting a highly heterogeneous availability of nutrients to individual cells [38••]. The use of short half-life variants of the *gfp* reporter gene in these studies provided unparalleled information on the process of nutrient consumption on plants. A similar variability in available Fe^{+3} on leaves was observed using an iron biosensor strain of *P. syringae* [39]. Variation in sucrose abundance on leaves was also reported [40]. Such heterogeneity in the phyllosphere environment places constraints on the patterns of competition and other interactions that can occur among phyllosphere bacteria. Microbes can be exposed to high fluxes of ultraviolet (UV) irradiation on leaves, and most bacteria recovered from leaves exhibit high levels of UV tolerance [41]. In *P. syringae* this tolerance was associated with UV-inducible plasmid-borne *rulAB* genes conferring mutagenic DNA repair in most strains, as naturally occurring or induced *rulA* mutants exhibited less UV survival in culture and on plants [42,43,44•,45]. Clearly, epiphytes have evolved effective mechanisms for coping with UV damage. The presence of such adaptive traits on plasmids may be one means by which epiphytes maintain such elements and other conditionally beneficial genes [46]. The further examination of traits harbored on plasmids should shed light on traits important for an epiphytic lifestyle.

Microscopic examinations of colonized leaves have revealed that many epiphytes occur in large aggregates on plant surfaces [47,48•]. While large numbers of solitary bacterial cells occur on plants, a few large masses of apparently mixed bacterial species can also be found. Initial results suggest that, although uncommon, such aggregates could constitute between 10 and 40% of the total bacterial population on certain plant species [48•]. Given the new appreciation for cell-density-dependent gene expression and the different behavior of bacterial cells in biofilms that has been demonstrated in other habitats, such biofilms on leaves have great implications for not only the behavior of epiphytic bacteria, but also for plant disease management. If cell–cell signaling via small molecules proves to be an important factor in regulating genes involved in epiphytic fitness, as in other habitats, then many new avenues for managing bacterial colonization of plants might be developed.

Conclusions

The phyllosphere is both scientifically and economically an important habitat in which to study microbial ecology. Because of the importance of many phyllosphere microbial inhabitants to plant health, there will probably be many practical applications that result from a better understanding of the interactions of microbes with the plant and with themselves. While the microbiology of roots has received quite a lot of attention, the microbiology of aerial plant parts is much less well-studied, although it is arguably of even more importance than the soil environment. The phyllosphere also has many features that make it a far better habitat in which to study microbial ecology than most other habitats. Microbes can be directly observed on leaves, enabling the use of powerful new microscopic techniques to measure microbial identity, activity, and gene expression. Plants can be readily genetically altered to change habitat conditions to test models of microbial behavior. Phyllosphere communities can be readily manipulated and can be made as simple or complex as needed by simple inoculation. Important microbial processes such as immigration and models such as island biogeography can be readily explored in plant systems. Thus, phyllosphere microbiology has much to offer to the field of microbial ecology and promises more effective and less environmentally damaging means of plant protection.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lindow SE: **Control of epiphytic ice nucleation-active bacteria for management of plant frost injury.** In *Biological Ice Nucleation and its Applications*. Edited by Lee RE, Warren GJ, Gusta LV. St Paul: APS Press; 1995:239-256.
2. Brandl MT, Quinones B, Lindow SE: **Heterogeneous transcription of an indoleacetic acid biosynthetic gene in *Erwinia herbicola* on plant surfaces.** *Proc Natl Acad Sci USA* 2001, **98**:3454-3459.
3. Andrews JH, Harris RF: **The ecology and biogeography of microorganisms on plant surfaces.** *Ann Rev Phytopathol* 2000, **38**:145-180.
4. Beattie GA, Lindow SE: **The secret life of foliar bacterial pathogens on leaves.** *Ann Rev Phytopathol* 1995, **33**:145-172.
5. Beattie GA, Lindow SE: **Bacterial colonization of leaves: a spectrum of strategies.** *Phytopathology* 1999, **89**:353-359.
6. Hirano SS, Upper CD: **Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*: a pathogen, ice nucleus, and epiphyte.** *Microbiol Mol Biol Rev* 2000, **64**:624-653.
7. Johnson KB, Stockwell VO: **Management of fire blight: a case study in microbial ecology.** *Ann Rev Phytopathol* 1998, **36**:227-248.
8. Johnson KB, Stockwell VO, Sawyer TL, Sugar D: **Assessment of environmental factors influencing growth and spread of *Pantoea agglomerans* on and among blossoms of pear and apple.** *Phytopathology* 2000, **90**:1285-1294.
9. Lindow SE, McGourty G, Elkins R: **Interactions of antibiotics with *Pseudomonas fluorescens* strain A506 in the control of fire blight and frost injury to pear.** *Phytopathology* 1996, **86**:841-848.
10. Mercier J, Lindow SE: **Field performance of antagonistic bacteria identified in a novel laboratory assay for biological control of fire blight of pear.** *Biological Control* 2001, **22**:66-71.
11. Nuclo RL, Johnson KB, Stockwell VO, Sugar D: **Secondary colonization of pear blossoms by two bacterial antagonists of the fire blight pathogen.** *Plant Dis* 1998, **82**:661-668.
12. Stockwell VO, McLaughlin RJ, Henkels MD, Loper JE, Sugar D, Roberts RG: **Epiphytic colonization of pear stigmas and hypanthia by bacteria during primary bloom.** *Phytopathology* 1999, **89**:1162-1168.
13. Braun-Kiewnick A, Jacobsen BJ, Sands DC: **Biological control of *Pseudomonas syringae* pv. *syringae*, the causal agent of basal kernel blight of barley, by antagonistic *Pantoea agglomerans.*** *Phytopathology* 2000, **90**:368-375.
14. Stromberg KD, Kinkel LL, Leonard KJ: **Relationship between phyllosphere population sizes of *Xanthomonas translucens* pv. *translucens* and bacterial leaf streak severity on wheat seedlings.** *Phytopathology* 1999, **89**:131-135.

15. Stromberg KD, Kinkel LL, Leonard KJ: **Interactions between *Xanthomonas translucens* pv. *translucens*, the causal agent of bacterial leaf streak of wheat, and bacterial epiphytes in the wheat phyllosphere.** *Biological Control* 2000, **17**:61-72.

16. Voelksch B, May R: **Biological control of *Pseudomonas syringae* pv. *glycinea* by epiphytic bacteria under field conditions.** *Microb Ecol* 2001, **41**:132-139.

17. Yuen GY, Steadman JR, Lindgren DT, Schaff D, Jochum C: **Bean rust biological control using bacterial agents.** *Crop Prot* 2001, **20**:395-402.

18. Zhang Z, Yuen GY: **Biological control of *Bipolaris sorokiniana* on tall fescue by *Stenotrophomonas maltophilia* strain C3.** *Phytopathology* 1999, **89**:817-822.

19. Karamanoli K, Vokou D, Menkissoglu U, Constantinidou HI: **Bacterial colonization of phyllosphere of Mediterranean aromatic plants.** *J Chem Ecol* 2000, **26**:2035-2048.

20. Skirvin RM, Kohler E, Steiner H, Ayers D, Laughnan A, Norton MA, Warmund M: **The use of genetically engineered bacteria to control frost on strawberries and potatoes. Whatever happened to all of that research?** *Sci Hortic* 2000, **84**:179-189.

21. Guo X, Chen J, Brackett RE, Beuchat LR: **Survival of salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening.** *Appl Environ Microbiol* 2001, **67**:4760-4764.

22. Takeuchi K, Matute CM, Hassan AN, Frank JF: **Comparison of the attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Pseudomonas fluorescens* to lettuce leaves.** *J Food Prot* 2000, **63**:1433-1437.

23. Takeuchi K, Hassan AN, Frank JF: **Penetration of *Escherichia coli* O157:H7 into lettuce as influenced by modified atmosphere and temperature.** *J Food Prot* 2001, **64**:1820-1823.

24. Wilson M, Hirano SS, Lindow SE: **Location and survival of leaf-associated bacteria in relation to pathogenicity and potential for growth within the leaf.** *Appl Environ Microbiol* 1999, **65**:1435-1443.

25. Kinkel LL, Wilson M, Lindow SE: **Plant species and plant incubation conditions influence variability in epiphytic bacterial population size.** *Microb Ecol* 2000, **39**:1-11.

26. Hirano SS, Charkowski AO, Collmer A, Willis DK, Upper CD: **Role of the Hrp type III protein secretion system in growth of *Pseudomonas syringae* pv. *syringae* B728a on host plants in the field.** *Proc Natl Acad Sci USA* 1999, **96**:9851-9856.

27. Hirano SS, Ostertag EM, Savage SA, Baker LS, Willis DK, Upper CD: **Contribution of the regulatory gene *lemA* to field fitness of *Pseudomonas syringae* pv. *syringae*.** *Appl Environ Microbiol* 1997, **63**:4304-4312.

28. Roine E, Raineri DM, Romantschuk M, Wilson M, Nunn DN: **Characterization of type IV pilus genes in *Pseudomonas syringae* pv. *tomato* DC3000.** *Mol Plant Microbe Interact* 1998, **11**:1048-1056.

29. Jurkevitch EJ, Shapira G: **Structure and colonization dynamics of epiphytic bacterial communities and of selected component strains on tomato (*Lycopersicon esculentum*) leaves.** *Microb Ecol* 2000, **40**:300-308.

30. •• Yang C-H, Crowley DE, Borneman J, Keen NT: **Microbial phyllosphere populations are more complex than previously realized.** *Proc Natl Acad Sci USA* 2001, **98**:3889-3894.

The first use of culture-independent methods for describing bacterial communities on the phyllosphere reveal that many species not previously described on plants were common on plants harvested from southern California in the fall. This work suggests that there may be many previously unidentified bacterial species with interesting and potentially useful traits enabling them to tolerate the stressful environment thought to exist on plants.

31. Bjorklof K, Nurmiaho-Lassila EL, Klinger N, Haahtela K, Romantschuk M: **Colonization strategies and conjugal gene transfer of inoculated *Pseudomonas syringae* on the leaf surface.** *J Appl Microbiol* 2000, **89**:423-432.

32. •• Normander B, Christensen BB, Molin S, Kroer N: **Effect of bacterial distribution and activity on conjugal gene transfer on the phylloplane of the bush bean (*Phaseolus vulgaris*).** *Appl Environ Microbiol* 1998, **64**:1902-1909.

The use of a *gfp* marker gene allows the process of plasmid transfer to be visualized on the leaf surface. The close spatial coincidence of bacteria on leaves is apparent and helps explain why this factor governs gene exchange to a greater extent than a higher metabolic activity.

33. Lilley AK, Bailey MJ: **The acquisition of indigenous plasmids by a genetically marked pseudomonad population colonizing the sugar beet phytosphere is related to local environment conditions.** *Appl Environ Microbiol* 1997, **63**:1577-1583.

34. Ashelford KE, Fry JC, Bailey MJ, Jeffries AR, Day MJ: **Characterization of six bacteriophages of *Serratia liquefaciens* CP6 isolated from the sugar beet phytosphere.** *Appl Environ Microbiol* 1999, **65**:1959-1965.

35. Ellis RJ, Thompson IP, Bailey MJ: **Temporal fluctuations in the pseudomonad population associated with sugar beet leaves.** *FEMS Microbiol Ecol* 1999, **28**:345-356.

36. Lilley AK, Bailey MJ, Day MJ, Fry JC: **Diversity of mercury resistance plasmids obtained by exogenous isolation from the bacteria of sugar beet in three successive years.** *FEMS Microbiol Ecol* 1996, **20**:211-227.

37. Mercier J, Lindow SE: **Role of leaf surface sugars in colonization of plants by bacterial epiphytes.** *Appl Environ Microbiol* 2000, **66**:369-374.

38. •• Leveau JHJ, Lindow SE: **Appetite of an epiphyte: quantitative monitoring of bacterial sugar consumption in the phyllosphere.** *Proc Natl Acad Sci USA* 2001, **98**:3446-3453.

The use of short half-life variants of a *gfp* reporter gene in the development of a whole-cell fructose biosensor reveals a great heterogeneity of sugar availability on plants. Together with a fluorescence *in situ* hybridization procedure using probes to 16S rRNA genes to reveal the metabolic state of cells on leaves, this study provides great insight into the process of colonization of leaves.

39. Joyner DC, Lindow SE: **Heterogeneity of iron bioavailability on plants assessed with a whole-cell GFP-based bacterial biosensor.** *Microbiology* 2000, **146**:2435-2445.

40. Miller WG, Brandl MT, Quiñones B, Lindow SE: **Biological sensor for sucrose availability: relative sensitivities of various reporter genes.** *Appl Environ Microbiol* 2001, **67**:1308-1317.

41. Jacobs JL, Sundin GW: **Effect of solar UV-B radiation on a phyllosphere bacterial community.** *Appl Environ Microbiol* 2001, **67**:5488-5496.

42. Kim JJ, Sundin GW: **Construction and analysis of photolyase mutants of *Pseudomonas aeruginosa* and *Pseudomonas syringae*: contribution of photoreactivation, nucleotide excision repair, and mutagenic DNA repair to cell survival and mutability following exposure to UV-B radiation.** *Appl Environ Microbiol* 2001, **67**:1405-1411.

43. Sundin GW: **Sequence diversity of *rulA* among natural isolates of *Pseudomonas syringae* and effect on function of *rulAB*-mediated UV radiation tolerance.** *Appl Environ Microbiol* 2000, **66**:5167-5173.

44. •• Sundin GW: **Functional analysis of the *Pseudomonas syringae* *rulAB* determinant in tolerance to ultraviolet B (290 to 320 nm) radiation and distribution of *rulAB* among *P. syringae* pathovars.** *Environ Microbiol* 1999, **1**:75-87.

The major role of plasmid-encoded *rul*AB genes in allowing epiphytic strains of *P. syringae* to tolerate UV irradiation typical of that on leaves is demonstrated using mutant analysis and complementation. The common presence of this UV tolerance locus on a ubiquitous plasmid in *P. syringae* provided evidence that such determinants may help maintain these plasmids in the bacterial population. Furthermore, *rul*AB determinants in *P. syringae* strains may distinguish them from the many plant-associated bacteria that are not good phyllosphere inhabitants.

45. Sundin GW, Jacobs JL: **Ultraviolet radiation (UVR) sensitivity analysis and UVR survival strategies of a bacterial community from the phyllosphere of field-grown peanut (*Arachis hypogeae* L.).** *Microb Ecol* 1999, **38**:27-38.

46. Sesma A, Sundin GW, Murillo J: **Phylogeny of the replication regions of pPT23A-like plasmids from *Pseudomonas syringae*.** *Microbiology* 2000, **146**:2375-2384.

47. Morris CE, Monier J-M, Jacques M-A: **Methods for observing microbial biofilms directly on leaf surfaces and recovering them for isolation of culturable microorganisms.** *Appl Environ Microbiol* 1997, **63**:1570-1576.

48. • Morris CE, Monier J-M, Jacques M-A: **A technique to quantify the population size and composition of the biofilm component in communities of bacteria in the phyllosphere.** *Appl Environ Microbiol* 1998, **64**:4789-4795.

A clear illustration that bacterial communities on field-grown leaves can include large aggregates, often of mixed species. The magnitude of the size of such aggregates are illustrated well here for the first time.