Bacterial Biofilms on Fungal Surfaces

13

Deborah A. Hogan, Matthew J. Wargo, and Nancy Beck

Abstract

Bacterial biofilm formation on fungi participates in the synergistic degradation of substrates, antagonism of fungal growth, bacterial utilization of fungi as nutrient sources, and the formation of more complex synergistic associations for the purposes of nutrient acquisition. While bacterial biofilm formation has been described in many systems, the molecular mechanisms that govern these interactions are not yet well understood. Analysis of physical interactions between *Pseudomonas aeruginosa* and the dimorphic opportunistic fungal pathogen *Candida albicans* has provided insights into factors involved in attachment and matrix production, and has demonstrated a role for the bacterial quorum sensing molecule, 3-oxo-C12-homoserine lactone, within bacterial-fungal biofilms. Subsequent to *P. aeruginosa* biofilm formation on the fungus, extracellular bacterial products contribute to the death of the fungal hyphae. Studies focused on the interactions between bacteria and fungi in the phytosphere have illustrated additional processes that contribute to bacterial biofilm formation on fungi including bacterial chemotaxis towards fungal cells, the cross-feeding of nutrients between interacting species, and the expression of specific genes upon contact with fungal cells. By understanding bacterial biofilm formation on fungi, we will gain insight into economically important interactions, such as those involved in the bacterial biocontrol of fungal plant pathogens. Furthermore, using tractable bacterial-fungal biofilm model systems, we may uncover important elements of bacterial biofilms on other *living* surfaces such as plant and animal tissues.

Overview: bacterial biofilms on fungal surfaces

From the perspective of the bacterium, there are a number of ways in which biofilm formation on fungi can be beneficial. First, bacterial colonization of a fungal surface may enable the bacteria to exploit the fungus as a source of nutrients (Hogan and Kolter, 2002). Bacteria may scavenge nutrients from the fungal cell wall, consume fungi-secreted products, or induce lysis of the fungal cells thereby liberating the intracellular contents for consumption by the local bacterial population. Second, in communities where bacteria and fungi are in competition for nutrients, biofilm formation on fungal cells could enhance bacterial antagonism of fungi by concentrating bacterially derived antifungal compounds. Third, biofilm formation on the surface of fungal hyphae would enable bacteria to "travel" with fungi as they extend into new areas in search of nutrients. Fourth, bacterial attachment to the hyphal surfaces may enhance synergistic actions of bacteria and fungi needed to breakdown complex substrates. Lastly, bacterial colonization of a fungal surface may be a first step in

more complex bacterial-fungal endosymbiont interactions such as those that are critical in the root rhizosphere (Dorr *et al.*, 1998). The wide array of different interactions between bacteria and fungi illustrates the potential importance of these relationships in many free-living and host-associated ecosystems.

In this chapter, we focus on the molecular factors involved in bacterial biofilm formation on fungi. To facilitate future research in this area, this chapter aims to highlight a number of different techniques and concepts that are important in the study of these specialized interspecies interactions. We mainly focus on biofilm formation by Pseudomonads on the surfaces of fungal hyphae, though the biofilm interactions between fungi and a handful of other Gram-positive and Gram-negative bacteria are also discussed. The chapter is divided into two sections. First, the interactions between two opportunistic pathogens (*Pseudomonas aeruginosa* and the fungus *Candida albicans*) are discussed. Second, we address interactions between bacteria and fungi in the rhizosphere and phyllosphere.

Pseudomonas aeruginosa biofilm formation on *Candida albicans*

Biofilm interactions between the Gram-negative bacterium *P. aeruginosa* and the fungus *C. albicans* may have relevance to the study of infections associated with cystic fibrosis (CF). Individuals with CF, a genetic disease that results from mutations in the CFTR transmembrane conductance regulator, are highly susceptible to chronic, progressive pulmonary infections that severely damage lung tissues and most often lead to respiratory failure in early adulthood (Rajan and Saiman, 2002). Several lines of evidence suggest that the microorganisms in CF sputum are in a biofilm-like state (Costerton *et al.*, 1999; Hoiby *et al.*, 2001; Singh *et al.*, 2000). While the predominant colonizer of the CF lung is *P. aeruginosa*, *C. albicans*, a dimorphic fungus, and *Aspergillus fumigatus*, another opportunistic fungal pathogen, are also commonly observed (Bakare *et al.*, 2003; Bauernfeind *et al.*, 1987; Bhargava *et al.*, 1989; Burns *et al.*, 1999; Cheng *et al.*, 1990; Haase *et al.*, 1991; Hughes and Kim, 1973; Navarro *et al.*, 2001). The effects of mixed bacterial-fungal infections on the host lung are not yet known.

In vitro analysis of the relationship between *P. aeruginosa* and *C. albicans* has shown that *P. aeruginosa* attaches to and forms biofilms on the surface of *C. albicans* (Hogan and Kolter, 2002). Within the *P. aeruginosa* biofilms, the fungal hyphae are killed. Studies using different *P. aeruginosa* and *C. albicans* strains have yielded data that support the hypothesis that *P. aeruginosa* biofilm formation is necessary, but not sufficient, for killing (Hogan and Kolter, 2002). Thus, the *P. aeruginosa*–*C. albicans* biofilm interaction may enable us to study some of the links between biofilm formation and virulence. Below we describe several aspects of the *P. aeruginosa*–*C. albicans* relationship including attachment, biofilm development, quorum sensing regulation, and *P. aeruginosa* killing of fungal cells. Each of these topics will be discussed separately.

Attachment

When *P. aeruginosa* and *C. albicans* are co-cultured in carbon-limited minimal medium at 37°C, the bacteria readily attach to the fungal filaments (Figure 13.1A) (Hogan and Kolter, 2002). Subsequently, the attached cells form a dense biofilm over the course of

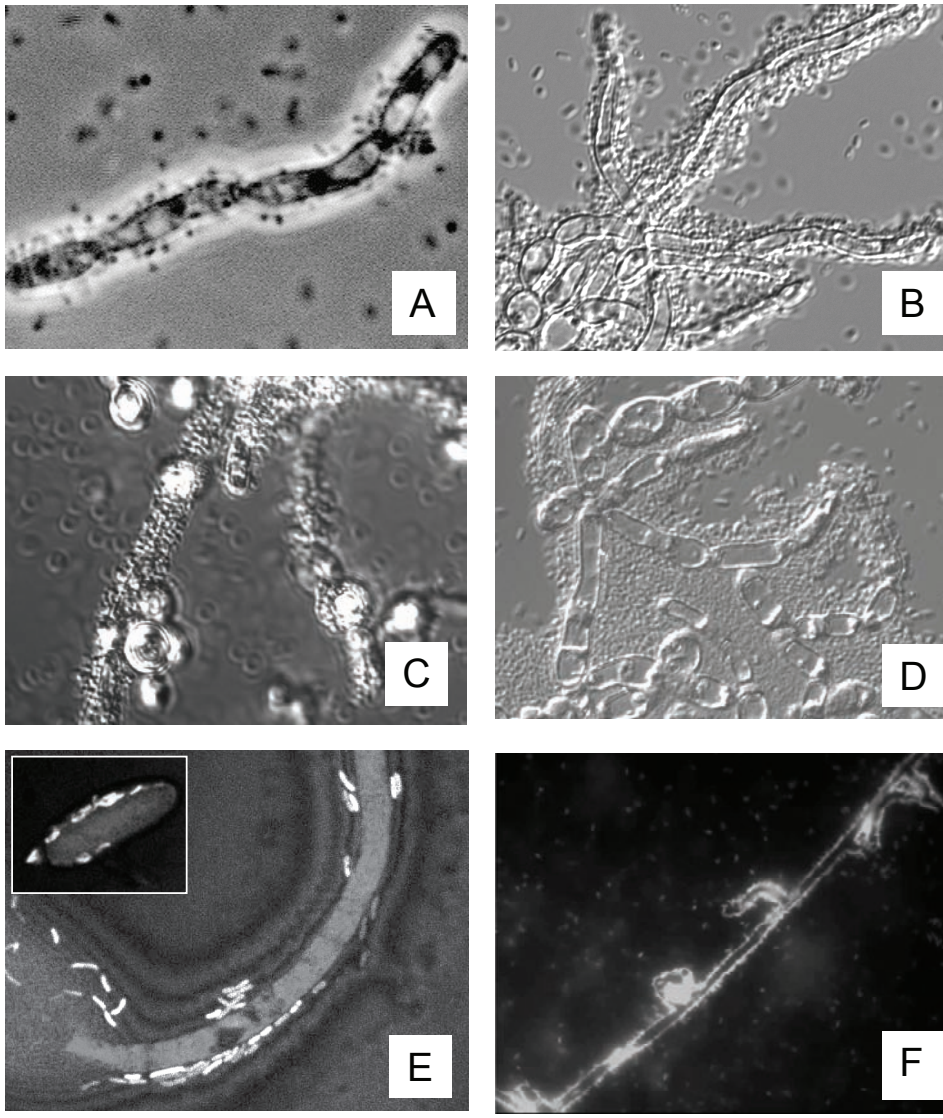

Figure 13.1 Attachment and biofilm formation on fungi by different *Pseudomonas* species. A-D Biofilm formation by *P*. *aeruginosa* PA14 on *C*. *albicans* hyphae. (A) *P*. *aeruginosa* initial colonization of the fungus as shown by phase contrast microscopy to demonstrate attachment by one bacterial pole. (B) *P*. *aeruginosa* biofilm formation at 24h is shown by differential interference contrast microscopy (DIC). (C-D) DIC images of *P*. *aeruginosa* biofilms to show colonization of *C*. *albicans* hyphae, but not yeast (C) and the development of dense bacterial biofilms between fungal hyphae (D). (E) Confocal laser scanning microscopy analyses of the attachment and colonization of *Fusarium oxysporum*. f.sp. *radicis lycopersici* (tagged with CFP) by *Pseudomonas fluorescens* WCS365 (tagged with E-GFP). A colonized hyphae (E) and a colonized spore (E-inset) are shown. (F) *Pseudomonas syringae* pv. syringae B728a, constitutively expressing gfp, colonizing the surface of an unlabeled *Neurospora* crassa hypha grown over on water-agar. Images 1A-D captured by N.B. and D.A.H. Figure 13.1E is courtesy of S. de Weert and G.V. Bloemberg. Figure 13.1F is courtesy of G. Wichmann and S. Lindow.

24–72 hours (Figure 13.1B) (Hogan and Kolter, 2002). There is a significant degree of specificity to the factors that are involved in the physical interactions between *P. aeruginosa* and *C. albicans*. While *P. aeruginosa* quickly colonizes *C. albicans* hyphae, it cannot attach to, nor form biofilms on, yeast-form *C. albicans* cells (Hogan and Kolter, 2002) (Figure 13.1C). *C. albicans* hyphal growth is stimulated by numerous factors including nutrient limitation, serum and certain amino acids; hyphae induced under any of these conditions support *P. aeruginosa* attachment. The reason for the differential attachment to hyphae and yeast is not yet known and could be due to the presence of epitopes that are unique to hyphae, or to factors that obstruct bacterial attachment on yeast cells. Numerous differences between the protein profiles, carbohydrate composition and surface charge of hyphae versus yeast have been reported (Chaffin *et al.*, 1998). Because *P. aeruginosa* is able to form biofilms on fungi other than *C. albicans*, such as *Aspergillus nidulans* and *Alternaria alternato* (Hogan unpublished data), it appears that either *P. aeruginosa* recognizes surface structures that are common across a wide range of ascomycete fungi or that *P. aeruginosa* can recognize multiple fungal surface structures. These two possibilities are not mutually exclusive.

The bacterial factors that participate in fungal attachment are under regulatory control. *P. aeruginosa* attachment is enhanced by unknown factors that are regulated by cell density-dependent acylhomoserine lactone signals and nutrient availability (Hogan and Kolter, 2002). Interestingly, some *P. aeruginosa* strains, including strain PAO1, do not readily attach to *C. albicans* hyphae when grown under the conditions that promote *P. aeruginosa* strain PA14 attachment (Hogan and Kolter, 2002). These data illustrate that *P. aeruginosa* strains have different bacterial surface characteristics or structures that will impact their ability to physically interact with different cell types. As has been described for bacterial attachment to abiotic surfaces such as glass or plastic (Marshall *et al.*, 1971) (see MacEachran and O'Toole, this volume) and to plant cells (Hendrickson *et al.*, 2001), *P. aeruginosa* PA14 attachment to the fungus occurs by one bacterial pole (Hogan and Kolter, 2002). Time-lapse microscopy shows that bacterial attachment to fungal hyphae is at first reversible with *P. aeruginosa* cells rapidly attaching to and detaching from the hyphal surface (data not shown). At some frequency, cells remain attached to the hyphal cell and initiate the formation of biofilms (Figure 13.1B–D).

Biofilm development

After individual *P. aeruginosa* cells have colonized the surfaces of the fungal hyphae, the formation of mature biofilms on the fungal surfaces is observed. The extent of the similarities between biofilm formation on abiotic surfaces and biofilm formation on fungi are not yet known. A number of *P. aeruginosa* mutants that are defective in *P. aeruginosa* attachment to plastic are also defective in biofilm formation on fungi. For example, flagellar mutants are delayed in biofilm formation on both abiotic surfaces and *C. albicans* hyphae; and *P. aeruginosa* mutants defective in the retractile type IV pili, which form weak, undifferentiated biofilms on plastic, produce biofilms on fungi that are thicker and less tightly packed compared to those formed by the wild type (Hogan and Kolter, 2002; O'Toole and Kolter, 1998). An extracellular matrix surrounds the bacterial and fungal cells within *P. aeruginosa* biofilms on *C. albicans* hyphae (Figure 13.1C), and over the course of 72 hours, the biofilms continue to grow to fill in the spaces between *C. albicans* filaments (Figure 13.1D).

Since both organisms are capable of producing an extracellular matrix, it is unclear if the matrix is of bacterial or fungal origin or if it is comprised of a mixture of the materials from both organisms (Chandra *et al*., 2001; Friedman and Kolter, 2004a; Friedman and Kolter, 2004b; Hawser *et al*., 1998; Jackson *et al*., 2004; Matsukawa and Greenberg, 2004) For more information on biofilm matrix, see Pamp *et al*., this volume.

P. aeruginosa antagonism towards *C. albicans*

Using both vital staining techniques and viable counts, it has been shown that the fungal hyphae within *P. aeruginosa* biofilms are killed within 24–48 hours of biofilm formation (Hogan and Kolter, 2002). To identify those factors that participate in this antagonistic interaction, the rate of fungal killing by different *P. aeruginosa* mutants were compared to the wild type using a quantitative, plate-count assay to monitor fungal viability of a constitutively filamentous *C. albicans tup1/tup1* mutant (Braun and Johnson, 1997; Hogan and Kolter, 2002). Under the nutrient-limiting conditions of the assay, biofilm formation is necessary for fungal killing. *P. aeruginosa* mutants that lack a functional flagellum, such as the *flgK* mutant, are delayed in biofilm formation on the fungal surface and are delayed in fungal killing. Mutants lacking certain global regulators, such as RpoN, or quorum sensing-related transcription factors, such as LasR and RhlR, were both defective in biofilm formation (see Atkinson *et al*., this volume) and decreased in their ability to kill *C. albicans* hyphae (Hogan and Kolter, 2002). Though these mutations in global regulators are pleiotropic, the correlation between the ability to form biofilms and fungal killing towards *C. albicans* is consistent with other data that link biofilm formation and virulence.

Virulence factors that have been implicated in human disease, such as the secreted phospholipase C, which degrades eukaryotic membrane lipids, also participate in biofilm-related fungal killing (Hogan and Kolter, 2002; Hollsing *et al*., 1987; Lanotte *et al*., 2003; Woods *et al*., 1997). The fact that biofilm formation enhances, and may be required for, killing suggests that these genes may be differentially regulated within biofilms on biotic surfaces, or that these secreted factors are more efficacious when they are produced in close proximity to the target cells. Because *P. aeruginosa* biofilm formation on *C. albicans* occurs much more readily under nutrient limiting conditions, it has been speculated that the bacteria are using the fungal hyphae as a source of nutrients and that biofilm formation allows for the synergistic action of degradative enzymes and for the capture of nutrients released upon fungal lysis.

Quorum sensing molecules in bacterial biofilms on fungi

When *P. aeruginosa* and *C. albicans* were co-cultured, the presence of the bacteria promoted growth as yeast-form cells despite conditions that would normally stimulate *C. albicans* hyphal growth. Because *P. aeruginosa* can neither colonize, nor kill yeast cells, this change in morphology allows *C. albicans* cells to survive in the presence of *P. aeruginosa*. Through a genetic screen, it was found that *P. aeruginosa* mutants defective in the production of 3-oxo-C12 homoserine lactone, a cell–cell signaling molecule, are unable to inhibit *C. albicans* filamentation (Hogan *et al*., 2004). The addition of 3OC12HSL alone to inducing medium at concentrations between 20–200 μM is sufficient to block hyphae formation with no effects on overall growth. The accumulation of 3OC12HSL in *P. aeruginosa* biofilms or in

colonies likely contributes to this interaction (Charlton *et al.*, 2000) as supernatants from planktonic cultures do not contain sufficient quantities of 3OC12HSL to affect C. *albicans* morphology. These findings indicate the possibility that there are chemical interactions within biofilms that are difficult or impossible to detect in planktonic co-cultures (Hogan *et al.*, 2004).

Pseudomonas spp. biofilm interactions with fungi in association with plants

In soils and in association with plant surfaces, there are many opportunities for physical interactions between bacteria and fungi. Fungi-associated bacterial communities appear to be distinct from the microflora in bulk soil in terms of species composition and relative abundance with *Pseudomonas* and *Burkholderia* frequently being detected as fungal colonizers (Frey-Klett *et al.*, 2005; Rangel-Castro *et al.*, 2002; Timonen and Hurek, 2006). These bacterial–fungal interactions likely play very important roles in microbial community ecology. Some bacterial species can serve as biocontrol agents that protect plants from pathogenic fungi (Whipps, 2001). For example, *P. fluorescens* can effectively protect tomato plants against infection by *Fusarium oxysporum* and its ability to colonize both the roots and fungal cells (Figure 13.1E) likely aids in this interaction (Bolwerk *et al.*, 2003). Recent unpublished work by G. Wichmann and S. Lindow has found that *Pseudomonas syringae* readily colonizes *Neurospora crassa* hyphae under a variety of conditions. Like *P. aeruginosa* attachment to C. *albicans* (described above) initial colonization of fungal hyphae by *P. syringae* involves polar attachment to the fungal surface followed by biofilm development (Figure 13.1F) (G. Wichmann and S. Lindow, unpublished data). Two different pathovars, *P. syringae* pv. syringae B728a and pv. tomato DC3000 are able to colonize the fungal hyphae, and contact between *P. syringae* and *N. crassa* correlates with fungal cell death. As *P. syringae* is commonly found on plant surfaces where it interacts with numerous microbial species including yeasts and filamentous fungi (Lindow and Brandl, 2003), the antagonistic interactions demonstrated between *P. syringae* and *N. crassa* may reflect a role for bacterial biofilm formation in competition with fungi in the phyllosphere. Other bacterial-fungal interactions that occur in association with plants are those within mycorrhizas. Mycorrhizas are symbiotic associations between fungi and plants for the purposes of nutrient acquisition; these associations also often contain helper bacteria that promote the formation of these beneficial structures (Aspray *et al.*, 2006; Garbaye, 1994). Like the antagonistic interactions described above, the formation of mycorrhiza also involves a number of physical interactions between bacteria and fungi.

As described for *P. aeruginosa* interactions with C. *albicans* above, the interactions can be broken down into several stages including detection of the fungal host, attachment to the fungal cells, and the response of the bacterium to growth on a biotic, fungal surface. Examples of chemotaxis towards fungal products, colonization of fungal surfaces, and induction of antifungal activities are discussed in detail below.

Bacterial detection of the fungus

There are many types of motility that may enable bacteria, such as *Pseudomonas* spp. and *Burkholderia* spp. to reach the fungus. Studies by Martinez-Granero *et al.* have found that the

rhizosphere, which contains abundant fungal hyphae, selects for *P. fluorescens* phenotypic variants that are highly motile suggesting that motility plays a critical role in accessing and exploiting this environment (Martinez-Granero *et al.*, 2006). Several examples illustrate that soil Pseudomonads exhibit chemotaxis towards plant and fungal exudates thereby allowing bacteria to congregate around populations of fungi in soils (Chet *et al.*, 1971; Lugtenberg *et al.*, 2001). Specific compounds that have been shown to elicit chemotaxis are fusaric acid, a secreted mycotoxin (de Weert *et al.*, 2004) and trehalose, a sugar commonly found in fungi (Thevelein, 1984; P. Frey-Klett, personal communication). In some cases, the viability of the fungus affects bacterial attachment. In one study, *Pseudomonas fluorescens* was shown to attach fifty-two times better to the live hyphae of a specific *Glomus* sp. than to dead hyphae, while *Bacillus cereus* was seen to attach better to the dead *Glomus* spp. hyphae (Toljander *et al.*, 2006). *Bacillus* has also been shown to attach preferentially to damaged hyphae in an *in vivo* analysis (Artursson and Jansson, 2003). The factors that contribute to the differentiation between live and dead hyphae are not yet known. Selective colonization of live hyphae may reflect a selection for bacteria capable of exploiting fungal metabolites, while attachment to only dead hyphae may imply the role of some bacteria in succession within the community. Furthermore, in some instances, bacteria colonization of only dead hyphae may suggest that some fungi have active mechanisms to resist bacterial colonization.

Bacterial attachment and biofilm formation

Recent studies have begun to examine the molecular features of soil bacteria and fungi that allow for bacterial colonization of the fungal surface. Different bacteria can recognize a number of fungal species in a variety of morphological states including hyphae, spores, and fruiting bodies (Levy *et al.*, 2003; Malajczuk *et al.*, 1977; Rangel-Castro *et al.*, 2002) as well as on and in mycorrhizas (Frey-Klett *et al.*, 1997). Furthermore, fungal-associated bacteria can be selective in terms of which fungal species they attach to, and in the particular region of the fungus to be colonized. For example, a variety of unidentified, but morphologically diverse, bacteria preferentially colonize the mycorrhiza instead of the radial hyphae network of fungi associated with pine roots (Nurmiaho-Lassila *et al.*, 1997). Electron microscopic analysis of *Burkholderia* sp. colonization of an arbuscular mycorrhizal fungus, *Gigaspora decipiens*, shows the involvement of fibrillar structures of unknown composition (Levy *et al.*, 2003). Dorr *et al.* (1998) showed that an *Azoracus* sp. uses type IV pili, and that pili-mediated interactions were critical for adhesion and establishment of mycorrhizal associations with the fungus. As these relationships are studied further, the extent of biofilm maturation on the fungal surface will be examined and the role of other previously identified biofilm development genes will be explored.

Bacterial response within fungal biofilms

One of the most exciting aspects of research on bacterial biofilm formation on fungi is the analysis of how microbial physiologies are affected during these intimate and dynamic interactions between bacterium and fungus. Using an *in vivo* expression technology (IVET) approach, Lee and Cooksey (2000) identified four *Pseudomonas putida* genes that are specifically induced during the growth of this biocontrol bacterium on the surface of the

fungus *Phytophthora parasitica*. The genes associated with fungal colonization included an uncharacterized transcription factor, an ABC transporter, and a porin (Lee and Cooksey, 2000). Separate studies have shown that trehalose may prove to be an important molecule during bacterial interactions with fungi. While trehalose utilization is not common among bacteria, many *Pseudomonas* spp. can grow on this fungally derived substrate. It has been proposed that the ability for *Pseudomonas* spp. to utilize trehalose may be an important part of bacterial growth in association with fungi such as the edible ectomycorrhizal fungus, *Cantharellus cibarius*, without causing fungal cell damage (Rangel-Castro *et al*., 2002). A different *P. fluorescens* sp. has been shown to induce its trehalose utilization genes when exposed to fungal culture supernatant, and the presence of trehalose enhances inhibition of *Pythium debaryanum* in a radial growth assay (Gaballa *et al*., 1997; Rincon *et al*., 2005). There is evidence that other uncharacterized chemical and physical signals likely also participate in the bacterial response to growth on fungi. Close association between *Burkholderia* sp. and fungi has been shown to promote mycorrhiza formation (Aspray *et al*., 2006), and Azoarcus sp., which uses type IV pili to attach to the fungal surface, only develops the intracellular structures necessary for efficient nitrogen fixation when the bacterial and fungal species are grown together (Dorr *et al*., 1998).

Summary and future directions

The study of bacterial biofilm formation on fungi could lead in a number of exciting directions. First, the identification of factors involved in the attachment and colonization of specific bacterial–fungal pairs could greatly aid in the development of more effective strains for biocontrol applications or degradation of complex substrates. The identification of the attachment factors themselves, along with an understanding of their regulation in accordance with environmental conditions may also provide insight into those environments where bacterial-fungal interactions are important. A second interesting area of research is the response of the fungus to bacterial biofilm formation on its surface. The identification of fungal factors that can block or disrupt bacterial biofilm formation, or that can kill bacterial biofilm cells could have important practical applications. Bacterial biofilms on implanted medical devices are highly problematic and costly in the clinic, and the high levels of antibiotic resistance exhibited by biofilm bacteria is thought to contribute to the inability to treat some biofilm-associated human infections. Lastly, the molecular analysis of bacterial factors that are important for beneficial and antagonistic biofilm formation on microscopic eukaryotes, including fungi, may provide new insight into those genes and factors that participate in the colonization of other eukaryotic cells including those belonging to plant and animals.

Acknowledgments

This work was funded by the Pew Biomedical Scholars Program (D.A.H) and the National Institutes of Health P20-RR018787 from the IDeA Program of the National Center for Research Resources (D.A.H.), T32 AI07519 (M.J.W.), and T32 DK007301 (N.B.). We would like to thank Dr. G. Bloemberg and Dr. S. de Weert, University of Leiden, for contributing images of *P. fluorescens* on *Fusarium* cells. Special thanks to G. Wichmann and S. Lindow, Univ. of California, Berkeley, for their contribution of unpublished findings.

References

Artursson, V., and Jansson, J.K. (2003). Use of bromodeoxyuridine immunocapture to identify active bacteria associated with arbuscular mycorrhizal hyphae. Appl. Environ. Microbiol. *69*, 6208–6215.

Aspray, T.J., Eirian Jones, E., Whipps, J.M., and Bending, G.D. (2006). Importance of mycorrhization helper bacteria cell density and metabolite localization for the *Pinus sylvestris-Lactarius rufus* symbiosis. FEMS Microbiol. Ecol. *56*, 25–33.

Bakare, N., Rickerts, V., Bargon, J., and Just-Nubling, G. (2003). Prevalence of *Aspergillus fumigatus* and other fungal species in the sputum of adult patients with cystic fibrosis. Mycoses *46*, 19–23.

Bauernfeind, A., Bertele, R.M., Harms, K., Horl, G., Jungwirth, R., Petermuller, C., Przyklenk, B., and Weisslein-Pfister, C. (1987). Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis. Infection *15*, 270–277.

Bhargava, V., Tomashefski, J.F., Jr., Stern, R.C., and Abramowsky, C.R. (1989). The pathology of fungal infection and colonization in patients with cystic fibrosis. Hum. Pathol. *20*, 977–986.

Bolwerk, A., Lagopodi, A.L., Wijf es, A.H.M., Lamers, G.E.M., Chin-A-Woeng, T.F.C., Lugtenberg, B.J.J., and Bloemberg, G.V. (2003). Interactions in the tomato rhizosphere of two *Pseudomonas* biocontrol strains with the phytopathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Mol. Plant Microbe Interact. *16*, 983–993.

Braun, B.R., and Johnson, A.D. (1997). Control of filament formation in *Candida albicans* by the transcriptional repressor TUP1. Science *277*, 105–109.

Burns, J.L., Van Dalfsen, J.M., Shawar, R.M., Otto, K.L., Garber, R.L., Quan, J.M., Montgomery, A.B., Albers, G.M., Ramsey, B.W., and Smith, A.L. (1999). Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. J. Infect. Dis. *179*, 1190–1196.

Chaffin, W.L., Lopez-Ribot, J.L., Casanova, M., Gozalbo, D., and Martinez, J.P. (1998). Cell wall and secreted proteins of *Candida albicans*: identification, function, and expression. Microbiol. Mol. Biol. Rev. *62*, 130–180.

Chandra, J., Kuhn, D.M., Mukherjee, P.K., Hoyer, L.L., McCormick, T., and Ghannoum, M.A. (2001). Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. J. Bacteriol. *183*, 5385–5394.

Charlton, T.S., de Nys, R., Netting, A., Kumar, M., Givskov, M., and Kjellerberg, S. (2000). A novel and sensitive method for the quantification of *N*-3-oxoacyl homoserine lactones using gas chromatography-mass spectrometry: application to a model bacterial biofilm. Environ. Microbiol. *2*, 530–541.

Cheng, S.H., Gregory, R.J., Marshall, J., Paul, S., Souza, D.W., White, G.A., O'Riordan, C.R., and Smith, A.E. (1990). Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. Cell *63*, 827–834.

Chet, I., Fogel, S., and Mitchell, R. (1971). Chemical detection of microbial prey by bacterial predators. J. Bacteriol. *106*, 863–867.

Costerton, J.W., Stewart, P.S., and Greenberg, E.P. (1999). Bacterial biofilms: a common cause of persistent infections. Science *284*, 1318–1322.

de Weert, S., Kuiper, I., Lagendijk, E.L., Lamers, G.E.M., and Lugtenberg, B.J.J. (2004). Role of chemotaxis towards fusaric acid in colonization of hyphae of *Fusarium oxysporum* f. sp. *radicis-lycopersici* by *Pseudomonas fluorescens* WCS365. Mol. Plant Microbe Interact. *16*, 1185–1191.

Dorr, J., Hurek, T., and Reinhold-Hurek, B. (1998). Type IV pili are involved in plant-microbe and fungus-microbe interactions. Mol. Microbiol. *30*, 7–17.

Frey-Klett, P., Chavatte, M., Clausse, M.L., Courrier, S., Le Roux, C., Raaijmakers, J., Martinotti, M.G., Pierrat, J.C., and Garbaye, J. (2005). Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. New Phytol. *165*, 317–328.

Frey-Klett, P., Pierrat, J.C., and Garbaye, J. (1997). Location and survival of mycorrhiza helper *Pseudomonas fluorescens* during establishment of ectomycorrhizal symbiosis between *Laccaria bicolor* and Douglas Fir. Appl. Environ. Microbiol. *63*, 139–144.

Friedman, L., and Kolter, R. (2004a). Genes involved in matrix formation in *Pseudomonas aeruginosa* PA14 biofilms. Mol. Microbiol. *51*, 675–690.

Friedman, L., and Kolter, R. (2004b). Two genetic loci produce distinct carbohydrate-rich structural components of the *Pseudomonas aeruginosa* biofilm matrix. J. Bacteriol. *186*, 4457–4465.

Gaballa, A., Abeysinghe, P.D., Urich, G., Matthijs, S., De Greve, H., Cornelis, P., and Koedam, N. (1997). Trehalose induces antagonism towards *Pythium debaryanum* in *Pseudomonas fluorescens* ATCC 17400. Appl. Environ. Microbiol. *63*, 4340–4345.

Garbaye, J. (1994). Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol. *128*, 197–210.

Haase, G., Skopnik, H., Groten, T., Kusenbach, G., and Posselt, H.G. (1991). Long-term fungal cultures from sputum of patients with cystic fibrosis. Mycoses *34*, 373–376.

Hawser, S.P., Baillie, G.S., and Douglas, L.J. (1998). Production of extracellular matrix by *Candida albicans* biofilms. J. Med. Microbiol. *47*, 253–256.

Hendrickson, E.L., Plotnikova, J., Mahajan-Miklos, S., Rahme, L.G., and Ausubel, F.M. (2001). Differential roles of the *Pseudomonas aeruginosa* PA14 *rpoN* gene in pathogenicity in plants, nematodes, insects, and mice. J. Bacteriol. *183*, 7126–7134.

Hogan, D.A., and Kolter, R. (2002). *Pseudomonas-Candida* interactions: An ecological role for virulence factors. Science *296*, 2229–2232.

Hogan, D.A., Vik, A., and Kolter, R. (2004). A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. Mol. Microbiol. *54*, 1212–1223.

Hoiby, N., Krogh Johansen, H., Moser, C., Song, Z., Ciofu, O., and Kharazmi, A. (2001). *Pseudomonas aeruginosa* and the in vitro and in vivo biofilm mode of growth. Microbes Infect. *3*, 23–35.

Hollsing, A.E., Granstrom, M., Vasil, M.L., Wretlind, B., and Strandvik, B. (1987). Prospective study of serum antibodies to *Pseudomonas aeruginosa* exoproteins in cystic fibrosis. J. Clin. Microbiol. *25*, 1868–1874.

Hughes, W.T., and Kim, H.K. (1973). Mycoflora in cystic fibrosis: some ecologic aspects of *Pseudomonas aeruginosa* and *Candida albicans*. Mycopathol. Mycol. Appl. *50*, 261–269.

Jackson, K.D., Starkey, M., Kremer, S., Parsek, M.R., and Wozniak, D.J. (2004). Identification of *psl*, a locus encoding a potential exopolysaccharide that is essential for *Pseudomonas aeruginosa* PAO1 biofilm formation. J. Bacteriol. *186*, 4466–4475.

Lanotte, P., Mereghetti, L., Lejeune, B., Massicot, P., and Quentin, R. (2003). *Pseudomonas aeruginosa* and cystic fibrosis: correlation between exoenzyme production and patient's clinical state. Pediatr. Pulmonol. *36*, 405–412.

Lee, S.W., and Cooksey, D.A. (2000). Genes expressed in *Pseudomonas putida* during colonization of a plant-pathogenic fungus. Appl. Environ. Microbiol. *66*, 2764–2772.

Levy, A., Chang, B.J., Abbott, L.K., Kuo, J., Harnett, G., and Inglis, T.J. (2003). Invasion of spores of the arbuscular mycorrhizal fungus *Gigaspora decipiens* by *Burkholderia* spp. Appl. Environ. Microbiol. *69*, 6250–6256.

Lindow, S.E., and Brandl, M.T. (2003). Microbiology of the phyllosphere. Appl. Environ. Microbiol. *69*, 1875–1883.

Lugtenberg, B.J., Dekkers, L., and Bloemberg, G.V. (2001). Molecular determinants of rhizosphere colonization by *Pseudomonas*. Annu. Rev. Phytopathol. *39*, 461–490.

Malajczuk, N., Nesbitt, H.J., and Glenn, A.R. (1977). A light and electron microscope study of the interaction of soil bacteria with *Phytophthora cinnamomi* Rands. Can. J. Microbiol. *23*, 1518–1525.

Marshall, K.C., Stout, R., and Mitchell, R. (1971). Selective sorption of bacteria from seawater. Can. J. Microbiol. *17*, 1413–1416.

Martinez-Granero, F., Rivilla, R., and Martin, M. (2006). Rhizosphere selection of highly motile phenotypic variants of *Pseudomonas fluorescens* with enhanced competitive colonization ability. Appl. Environ. Microbiol. *72*, 3429–3434.

Matsukawa, M., and Greenberg, E.P. (2004). Putative exopolysaccharide synthesis genes influence *Pseudomonas aeruginosa* biofilm development. J. Bacteriol. *186*, 4449–4456.

Navarro, J., Rainisio, M., Harms, H.K., Hodson, M.E., Koch, C., Mastella, G., Strandvik, B., and McKenzie, S.G. (2001). Factors associated with poor pulmonary function: cross-sectional analysis of data from the ERCF. European Epidemiologic Registry of Cystic Fibrosis. Eur. Respir. J. *18*, 298–305.

Nurmiaho-Lassila, E.L., Timonen, S., Haahtela, K., and Sen, R. (1997). Bacterial colonization patterns of intact *Pinus sylvestris* mycorrhizospheres in dry pine forest soil: an electron microscopy study. Can. J. Microbiol. *43*, 1017–1035.

O'Toole, G.A., and Kolter, R. (1998). Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. Mol. Microbiol. *30*, 295–304.

Rajan, S., and Saiman, L. (2002). Pulmonary infections in patients with cystic fibrosis. Semin. Respir. Infect. *17*, 47–56.

Rangel-Castro, J.I., Levenfors, J.J., and Danell, E. (2002). Physiological and genetic characterization of fluorescent *Pseudomonas* associated with *Cantharellus cibarius*. Can. J. Microbiol. *48*, 739–748.

Rincon, A., Ruiz-Diez, B., Garcia-Fraile, S., Garcia, J.A., Fernandez-Pascual, M., Pueyo, J.J., and de Felipe, M.R. (2005). Colonisation of *Pinus halepensis* roots by *Pseudomonas fluorescens* and interaction with the ectomycorrhizal fungus *Suillus granulatus*. FEMS Microbiol. Ecol. *51*, 303–311.

Singh, P.K., Schaefer, A.L., Parsek, M.R., Moninger, T.O., Welsh, M.J., and Greenberg, E.P. (2000). Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature *407*, 762–764.

Thevelein, J.M. (1984). Regulation of trehalose mobilization in fungi. Microbiol. Rev. *48*, 42–59.

Timonen, S., and Hurek, T. (2006). Characterization of culturable bacterial populations associating with *Pinus sylvestris*: *Suillus bovinus* mycorrhizospheres. Can. J. Microbiol. *52*, 769–778.

Toljander, J.F., Artursson, V., Paul, L.R., Jansson, J.K., and Finlay, R.D. (2006). Attachment of different soil bacteria to arbuscular mycorrhizal fungal extraradical hyphae is determined by hyphal vitality and fungal species. FEMS Microbiol. Lett. *254*, 34–40.

Whipps, J.M. (2001). Microbial interactions and biocontrol in the rhizosphere. J. Exp. Bot. *52*, 487–511.

Woods, D.E., Lam, J.S., Paranchych, W., Speert, D.P., Campbell, M., and Godfrey, A.J. (1997). Correlation of *Pseudomonas aeruginosa* virulence factors from clinical and environmental isolates with pathogenicity in the neutropenic mouse. Can. J. Microbiol. *43*, 541–551.