Shifting Paradigms in *Pseudomonas aeruginosa* Biofilm Research

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Abstract Biofilms formed by *Pseudomonas aeruginosa* have long been recognized as a challenge in clinical settings. Cystic fibrosis, endocarditis, device-related infections, and ventilator-associated pneumonia are some of the diseases that are considerably complicated by the formation of bacterial biofilms, which are resistant to most current antimicrobial therapies. Due to intense research efforts, our understanding of the molecular events involved in *P. aeruginosa* biofilm formation, maintenance, and antimicrobial resistance has advanced significantly. Over the years, several dogmas regarding these multicellular structures have emerged. However, more recent data reveal a remarkable complexity of *P. aeruginosa* biofilms and force investigators to continually re-evaluate previous findings. This chapter provides examples in which paradigms regarding *P. aeruginosa* biofilms have been challenged, reflecting the need to critically re-assess what is emerging in this rapidly growing field. In this process, several avenues of research have been opened that will ultimately provide the foundation for the development of preventative measures and therapeutic strategies to successfully treat *P. aeruginosa* biofilm infections.

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1 Introduction

The rod-shaped motile Gram-negative bacterium *Pseudomonas aeruginosa* is an environmental organism that rarely causes disease in individuals with a healthy immune system. However, serious infection frequently develops in immunocompromised hosts and cystic fibrosis (CF) patients (Deretic et al. 1995; Govan and Deretic 1996; Ramsey and Wozniak 2005). *P. aeruginosa* is a predominant cause of opportunistic nosocomial infections, accounting for 10% of hospital-acquired infections, with case fatality due to bacteremia as high as 50% (Richards et al. 1999; Hancock and Speert 2000). In addition, its high intrinsic resistance to antibiotics, its remarkable ability to develop resistance during antimicrobial treatment, and its preferred biofilm-mode of growth considerably complicates therapies aimed at eradicating both acute and chronic *P. aeruginosa* infections (Hancock and Speert 2000; Mah and O'Toole 2001; Donlan and Costerton 2002; Hoffman et al. 2005).

Over the past decade, the study of biofilms has gained attention when it was appreciated that biofilms contribute substantially to infectious disease (Potera 1999). Cystic fibrosis, ventilator-associated pneumonia, device-related infections, and endocarditis are examples of diseases that are considerably complicated by *P. aeruginosa* biofilms. Colonization of a CF patient with *P. aeruginosa*, along with the emergence of alginate-producing mucoid variants, is considered a poor prognostic indicator, as these infections are impossible to eradicate with current therapeutic strategies (Govan and Deretic 1996; Ramsey and Wozniak 2005). Matrix-encased bacteria indicative of biofilms are found in the bronchioles of CF patients during later stages of the disease (Lam et al. 1980; Baltimore et al. 1989; Hoiby et al. 2001). In addition, quorum-sensing signals can be detected in the CF lung, which is yet another piece of evidence for the presence of *P. aeruginosa* biofilms (Singh et al. 2000). In the case of *P. aeruginosa*-induced endocarditis, symptoms are initially transient (Donlan and Costerton 2002). When biofilms are eventually identified as the cause of disease, treatment becomes tremendously difficult. Usually, immediate valve replacement and long-term administration of high-dose antibiotics are required. However, even these drastic measures are often futile, leading to significant morbidity and mortality (Gavin et al. 2003).

Due to their clinical importance, *P. aeruginosa* biofilms are one of the best-studied single-species biofilms. Over the years, certain themes regarding *P. aeruginosa* biofilms have emerged and been considered dogmatic. However, new advances in the field challenge many of the original findings. This has been the case with regard to the role of motility in *P. aeruginosa* biofilm formation, antimicrobial resistance strategies in biofilms, and the composition of the biofilm matrix. The studies reviewed in this chapter emphasize the importance of using caution when interpreting and evaluating data that is emerging in this rapidly expanding field of research.

2 Role of *P. aeruginosa* Motility in the Formation of Biofilms

The first evidence for a role of bacterial motility in the formation of *P. aeruginosa* biofilms was provided in 1998 by O'Toole and Kolter (1998). Using a static attachment model, it was shown that both flagellar and twitching motility are involved in the development of biofilms. In this study, wild type cells attached to the PVC surface and formed a monolayer of cells followed by the appearance of dispersed microcolonies. An isogenic flagellar mutant, on the other hand, did not attach to the substrate. This finding supported the hypothesis that flagella and/or motility are required for initial cell-surface interactions. In the same report, an isogenic mutant lacking type IV pili attached to the surface and formed a monolayer, but did not develop microcolonies, which indicated that type IV pili are required in the biofilm maturation process. Thus, the seminal study by O'Toole and Kolter (1998) provided compelling evidence that the *P. aeruginosa* motility status affects the initiation and maturation of biofilms.

More recent studies reveal that the findings described above may be somewhat generalized. For example, in contrast to O'Toole's (O'Toole and Kolter 1998) observation, Klausen et al. (2003a) discovered that flagellar mutants of *P. aeruginosa* can, in fact, form a biofilm. In this study, however, bacteria were grown as biofilms in flow chambers irrigated with a minimal medium containing citrate as carbon source rather than in a static model with glucose minimal medium (O'Toole and Kolter 1998). Confocal scanning laser microscopy (CSLM) revealed that under the conditions used by Klausen et al. (2003a), both the parental strain and the isogenic flagellar mutant formed biofilms, albeit with distinct structural differences. While the wild-type strain formed a flat "carpet-like" biofilm structure, the flagellar mutant biofilm was thicker and "hilly" (Klausen et al. 2003a). With regard to type IV pili, Klausen et al. (2003a) observed that both the wild type and the isogenic pilus mutant are able to form biofilms in the dynamic flow chamber system but display dramatic structural differences. Under the conditions used, wild type cells formed a uniform flat biofilm without microcolonies, whereas the pilus mutant formed a biofilm with distinct microcolonies. These observations appeared opposite those made by O'Toole (O'Toole and Kolter 1998) where the wild type cells formed microcolonies over time, which were absent in the type IV pili mutant. The discrepancies are likely due to the media used as other reports reveal that biofilms grown in the presence of glucose (Stewart et al. 1993; Davies et al. 1998) are different from those grown in the presence of citrate (Heydorn et al. 2000; Heydorn et al. 2002).

Another important question addresses the contribution of *P. aeruginosa* motility in the formation of the distinctive mushroom-like structures separated by water-filled channels that are frequently observed in biofilms (Lawrence et al. 1991; deBeer et al. 1994). Over the years, several plausible hypotheses have been proposed to explain how these structures are assembled. It has been suggested, for example, that bacterial cells are forced to differentiate as a result of nutrient-limiting conditions depending on their location within the biofilm (Wimpenny and Colasanti 1997;

Picioreanu et al. 1998). A second hypothesis implies that the differentiation of *P. aeruginosa* in the complex multicellular structures is mediated through the expression of specific genes, which directly control the spatial organization of cells (Stoodley et al. 2002). A third hypothesis proposes that cell-to-cell signaling is required (Davies et al. 1998). More recent data reveal that bacterial motility plays an important role in the development of the multicellular structures. For instance, a model by Shrout et al. (2006) suggests that nutritional conditions dictate the contributions of quorum sensing and swarming motility on biofilm formation and can result in structurally distinct biofilms. Moreover, there is compelling evidence that type IV pili-mediated motility is crucial for the proper development of the mushroom-like structures. In an inventive series of experiments, Klausen and colleagues (2003b) used differentially fluorescently labeled wild type and type IV pilin *P. aeruginosa* isolates to show that these multicellular structures are formed in a sequential process involving motile and nonmotile subpopulations of *P. aeruginosa.* In this model, nonmotile bacteria form the mushroom stalks, whereas the caps are assembled by migrating cells that ascend the stalks and aggregate on the tops via a type IV pili-driven mechanism. A subsequent study discovered that the motile and nonmotile subpopulations exhibit differential sensitivity to the membrane-active antimicrobial agents colistin and sodium dodecyl sulfate (SDS) (Haagensen et al. 2007).

Collectively, the data discussed above demonstrate that *P. aeruginosa* motility plays a central role in biofilm formation and development. However, the studies also clearly show that the experimental conditions employed (i.e., biofilm reactor, growth conditions) strongly dictate how and to what extent motility affects the biofilm. This implies that certain environmental parameters promote the expression of specific subsets of genes, which allow the bacterium to form a biofilm most suitable for the conditions encountered. This scenario provides a plausible explanation for the remarkable ability of *P. aeruginosa* to successfully form biofilms in an unusually wide range of ecological niches.

3 Resistance of *P. aeruginosa* Biofilms to Antimicrobial Treatment

One of the most perplexing aspects of biofilms is their remarkable resistance to antimicrobial agents. This phenomenon has been attributed to a variety of factors, including (1) impaired access of antibiotics due to the protective matrix encasing the biofilm (Costerton et al. 1999; Gilbert et al. 2002), (2) reduced growth rates of bacteria within hypoxic/anoxic zones of the biofilm (Gilbert et al. 1990), perhaps in conjunction with the inhibition of microbial activity within these zones (Worlitzsch et al. 2002; Yoon et al. 2002), and (3) induced and/or increased expression of particular resistance genes (Costerton et al. 1999; Gilbert et al. 2002). However, more recent studies challenge and/or expand these suppositions.

The idea of the exopolysaccharide as a protective shield from antimicrobials is appealing and was commonly accepted as a plausible explanation for the unusual resistance of *P. aeruginosa* biofilms to antimicrobials. However, newer evidence does not support this hypothesis. Several studies have shown that the diffusion of many antibiotics into the biofilm is not impeded (Gordon et al. 1988; Shigeta et al. 1997; Ishida et al. 1998). Quinolones, for instance, readily enter *P. aeruginosa* biofilms (Shigeta et al. 1997; Vrany et al. 1997; Ishida et al. 1998) and should thus be able to kill biofilm-growing bacteria. Interestingly, however, a successful breach of the exopolysaccharide barrier by a given antibiotic does not automatically result in eradication of *P. aeruginosa* in the biofilm. For example, Brooun et al. (2000) observed that the quinolone ofloxacin effectively penetrates biofilm-grown *P. aeruginosa* at clinically feasible concentrations (5 μg/ml). Upon administration, the antibiotic resulted in a drop in viable cells. However, a small percentage of cells were unaffected. Higher concentrations of the antibiotic did not have an effect, which was evidenced by the constant number of super-resistant cells at ofloxacin concentrations as high as 100 μg/ml. In addition, other groups reported significant differences in the bactericidal activity of various quinolones despite their ability to successfully penetrate the matrix (Vrany et al. 1997; Ishida et al. 1998). Interestingly, Ishida et al. (1998) found levofloxacin to be significantly more bactericidal than ciprofloxacin, whereas Vrany et al (1997) observed the opposite effect, which may be attributed to the experimental conditions used in the studies.

Another factor that needs to be considered with regard to the ability of antibiotics to cross the polysaccharide barrier is the rate of penetration. Jefferson (2005) proposed that as an antibiotic diffuses through the matrix, cells are exposed to different concentrations of the drug. Thus, the bacteria may have time to mount a protective response to the antibiotic. In support of this idea, Jefferson et al. (2005) showed that vancomycin binds quickly to cells on the biofilm surface but requires more than 1 h to bind to bacteria located within the deepest layer of the biofilm. While this particular study examined *Staphylococcus aureus* biofilms, it is quite possible that this mechanism translates to *P. aeruginosa.* In fact, there is evidence that subinhibitory (sub-MIC) levels of antibiotics affect *P. aeruginosa* biofilm formation. For example, Fonseca et al. (2004) observed that treatment of *P. aeruginosa* biofilms with sub-MIC concentrations of a piperacillin/tazobactam mixture affects biofilm morphology and results in a decrease of biofilm formation, an increase in sensitivity to oxidative stress, and a decrease in type IV pili-mediated twitching motility. Wozniak and Keyser (2004) observed that subinhibitory levels of macrolides also have an effect on biofilm architecture and result in reduced type IV pili-mediated twitching motility. Other studies have shown that aminoglycosides used at sub-MIC concentrations can actually induce *P. aeruginosa* biofilm formation, presumably as a consequence of stress (Hoffman et al. 2005). More recently, Linares et al. (2006) proposed a model that designates antibiotics as signaling molecules. The study demonstrated that exposure of *P. aeruginosa* biofilms to subinhibitory levels of particular antibiotics modulates the expression of virulence determinants including motility and type III secretion systems.

A second hypothesis attempting to explain the exceptionally high resistance of *P. aeruginosa* biofilms to antimicrobial killing involves slow growth of bacteria within the biofilm. However, this explanation may only be plausible with regard to antibiotics that kill rapidly dividing cells such as carbenicillin and other β-lactams. It does not account for resistance to antibiotics such as quinolones, which are able to successfully eradicate nongrowing cells. The observation that stationary-phase planktonic cells (which are in a state of slow growth) remain susceptible to antibiotics also argues against this hypothesis (Brooun et al. 2000). However, the latter finding has been challenged by Spoering and Lewis (2001), who reported that both biofilm-grown and stationary-phase planktonic cells are significantly more resistant to antibiotic killing than logarithmic phase planktonic cells. In addition, this study identified a super-resistant subpopulation of *P. aeruginosa*, which was impervious to the antibiotics used. Spoering and Lewis (2001) also provided evidence that the formation and/or maintenance of the super-resistant subpopulation is dependent on the density of the stationary-phase planktonic culture. The latter finding would help explain the discrepancy found in the literature regarding the antibiotic resistance status of stationary phase planktonic *P. aeruginosa* vs biofilm-grown cells: previous studies seeking to compare antibiotic resistance of biofilm-grown vs planktonic bacteria tended to dilute cells in order to analyze similar cell numbers from both populations, which would obviously have a detrimental effect on any density-dependent components involved in antibiotic resistance.

A very interesting report was recently published by Kaneko et al. (2007), who showed that slow-growing *P. aeruginosa* within a biofilm may actually be targeted by gallium, a transition metal. The study was based on the observation that many biological systems are unable to distinguish between gallium and iron (Chitambar and Narasiham 1991). Thus, gallium may be used in a Trojan horse approach to interfere with and limit iron metabolism. In theory, this would have a detrimental effect on bacteria, and there is evidence to support this hypothesis (Bullen et al. 2005). Kaneko et al. (2007) demonstrated that gallium is able to kill established *P. aeruginosa* in biofilms in a dose-dependent manner. Surprisingly, the slow-growing bacteria located in the center of the biofilm, which are usually unaffected by antibiotics, were particularly susceptible to the bactericidal effects of gallium. Another study found that in addition to gallium, cells located in the middle of the biofilm are also sensitive to killing by colistin and SDS (Haagensen et al. 2007).

Several recent studies have suggested that oxygen limitation may play an important role in *P. aeruginosa* biofilm development both in vitro and in vivo (Worlitzsch et al. 2002; Yoon et al. 2002; Walters et al. 2003). Based on these findings, others have reported that low levels of oxygen result in slow growth of cells within the biofilm and subsequent recalcitrance to antibiotic treatment (Walters et al. 2003; Borriello et al. 2004). However, a more recent study showed that growth of *P. aeruginosa* under anaerobic conditions did not reduce the ability of ceftazidime, meropenem, aztreonam, piperacillin, or piperacillin/tazobactam to inhibit planktonic growth (Field et al. 2005).

A popular explanation for antimicrobial resistance of *P. aeruginosa* is the induced and/or increased expression of particular resistance genes, which may confer a super-resistant phenotype. Interestingly, the phenomenon of super-resistant bacterial subpopulations is nothing new. In 1944, Bigger (Bigger 1944) reported that penicillin treatment of a population of Staphylococci did not sterilize the culture, but rather left a small subpopulation of bacteria that was impervious to the antibiotic. The cells within this subpopulation were defined as persisters. Regrowth of these persisters resulted in a population like the original one with respect to growth inhibition by penicillin and the generation of persisters. While this was a noteworthy discovery, its clinical significance was questioned. Thus, there is relatively little information regarding the molecular basis of persister cells. However, with the expanding study of biofilms, persisters have been met with a rekindled interest as they may offer a logical explanation for the remarkable antibiotic resistance of biofilms. In this context, Lewis (2007) has proposed a persister-based model for the resistance phenomenon in *P. aeruginosa* biofilms: upon administration of a bactericidal antibiotic to treat a biofilm-based infection, the entire population, with the exception of the persisters, is killed. If the antibiotic is withdrawn, the biofilm reforms and the cycle begins anew. This model is attractive as it accounts for the extraordinary resistance of biofilms to antibiotics as well as for the relapsing nature of biofilm infections. However, while the presence of persisters within biofilms has been established, much work is needed to decipher the unusual phenotype of these super-resistant cells. In this regard, several reports and computer modeling studies have provided mechanistic insights into the basis for antibiotic tolerance of biofilm-grown *P. aeruginosa* (Drenkard and Ausubel 2002; Mah et al. 2003; Hoffman et al. 2005; Szmolay et al. 2005; Chambless et al. 2006).

Together, the work discussed in this section reflects the intriguing complexity of the antimicrobial resistance phenomenon of *P. aeruginosa* biofilms. It appears that this opportunistic pathogen has evolved a diverse array of strategies that allow it to counteract the detrimental effects of various classes of antimicrobial agents. Moreover, it becomes clear that the development of therapeutic strategies that can successfully overcome the remarkable antimicrobial resistance of *P. aeruginosa* biofilms is certainly not trivial.

4 Composition of the *P. aeruginosa* Biofilm Matrix

It is now widely recognized that the structural integrity of biofilms depends on an extracellular matrix, which is produced by the bacterial cells constituting a given biofilm (Branda et al. 2005). Although the production of an extracellular substance is a commonality among biofilms, there is remarkable diversity in their individual composition. Table 1 summarizes existing data and features regarding components of the *P. aeruginosa* matrix. It is important to note that the relative ratios and abundance of these elements depends on a variety of factors, including strain background, the growth medium used, and the biofilm reactor system.

Table 1 Components of the *P. aeruginosa* biofilm matrix

Biofilm matrix component	Features	References
Alginate	Mannuronate and guluronate polymer	Linker and Jones 1966
	High–level synthesis associated with isolates from CF airway	Govan and Deretic 1996
	Widely conserved among *P. aeruginosa* strains	Goldberg et al. 1993; Wolfgang et al. 2003
	Expressed in *mucA* mutant *P. aeruginosa*	Martin et al. 1993; Govan and Deretic 1996
	Not essential for biofilm formation in non–mucoid strains but affects biofilm architecture and resistance phenotype	Hentzer et al. 2001; Nivens et al. 2001; Wozniak et al. 2003; Stapper et al. 2004
	Tightly regulated	Govan and Deretic 1996; Ramsey and Wozniak 2005
Psl	Mannose–rich	Friedman and Kolter 2004b; Matsukawa and Greenberg 2004
	Required for surface interactions	Friedman and Kolter 2004b; Jackson et al. 2004; Matsukawa and Greenberg 2004; Overhage et al. 2005
	Overproduced in variants from aged biofilms	Kirisits et al. 2005
	Maintains biofilm structure post–attachment	Ma et al. 2006
	Regulated by c–di–GMP	D'Argenio et al. 2002; Goodman et al. 2004; Hickman et al. 2005; Ventre et al. 2006
Pel	Glucose–rich	Friedman and Kolter 2004b
	Required for pellicle formation in some *P. aeruginosa* strains	Friedman and Kolter 2004a
	Overproduced in variants from aged biofilms	Kirisits et al. 2005
	Regulated by c–di–GMP	D'Argenio et al. 2002; Goodman et al. 2004; Hickman et al. 2005; Ventre et al. 2006
DNA	Major component of in vitro biofilm matrix	Sutherland 2001; Matsukawa and Greenberg 2004; Steinberger and Holden 2005
	Connects cells within the biofilm matrix	Allesen–Holm et al. 2006
	Localized release by a subpopulation of bacteria within the biofilm	Allesen–Holm et al. 2006
	DNase treatment prevents biofilm formation	Whitchurch et al. 2002
	Iron levels can mediate DNA release	Yang et al. 2007
CupA fimbriae	Chaperone–usher family adhesin	Vallet et al. 2001
Membrane vesicles	Derived from outer membrane. Package virulence factors and bind antibiotics.	Schooling and Beveridge 2006

Alginate is perhaps the most extensively studied *P. aeruginosa* exopolysaccharide. It forms a linear polymer consisting of β-D-mannuronic and α-l-guluronic acid residues (Linker and Jones 1966; Ramsey and Wozniak 2005) and is associated with mucoid *P. aeruginosa* isolates recovered from patients suffering from cystic fibrosis (Govan and Deretic 1996; Ramsey and Wozniak 2005; Hoiby 2006). Historically, alginate has been considered the sole component of the *P. aeruginosa* biofilm matrix. This is not surprising since early biofilm studies utilized mucoid *P. aeruginosa* strains. Additionally, many reports show that the presence of alginate as well as its modification strongly influences the physical properties of mucoid biofilms (Hentzer et al. 2001; Nivens et al. 2001; Stapper et al. 2004). However, the hypothesis that the matrix of *P. aeruginosa* biofilms consist of only alginate was challenged by the discovery that most mucoid strains of this opportunistic pathogen harbored one or more mutations that resulted in the constitutive production of the exopolysaccharide (Govan and Deretic 1996). Thus, extracellular matrices produced by non-mucoid *P. aeruginosa* were analyzed with regard to their chemical composition. Interestingly, the exopolysaccharide embedding biofilms formed by common laboratory strains such as PAO1 and PA14 contained little detectable alginate (Wozniak et al. 2003). Moreover, inactivation of genes required for alginate biosynthesis in these strains neither affected the formation nor the structure of the biofilms in vitro (Wozniak et al. 2003; Stapper et al. 2004). This led to the hypothesis that in *P. aeruginosa*-based lung infections in CF patients, biofilm formation may precede the appearance of mucoid isolates. In this context, it was also speculated that the transition of *P. aeruginosa* from a non-mucoid to a mucoid phenotype might involve a switch from a yet unidentified exopolysaccharide to alginate.

Subsequently, several laboratories concomitantly identified two genetic loci, *pel* and *psl*, which encoded putative biochemically distinct polysaccharides (Friedman and Kolter 2004a, 2004b; Jackson et al. 2004; Matsukawa and Greenberg 2004; Overhage et al. 2005). There is evidence that these exopolysaccharides are involved in both early- and late-stage biofilm development (Friedman and Kolter 2004b; Jackson et al. 2004; Matsukawa and Greenberg 2004; Vasseur et al. 2005; Ma et al. 2006). Overproduction of Psl and Pel results in distinct alterations in colony morphology, biofilm architecture, and auto aggregation properties (Friedman and Kolter 2004a; Kirisits et al. 2005; Ma et al. 2006). An exciting recent discovery is that both polysaccharides seem to be regulated by bis-(3′, 5′)-cyclic dimeric guanosine monophosphate (c-di-GMP), a ubiquitous second messenger molecule found in bacteria (D'Argenio et al. 2002; Goodman et al. 2004; Hickman et al. 2005; Ventre et al. 2006). Levels of c-di-GMP are regulated by the opposing activities of GGDEF diguanylate cyclase and EAL phosphodiesterase proteins. Interestingly, c-di-GMP signaling seems to play an important role in the control of biofilm development in other Gram-negative bacteria, including *Yersinia*, *Salmonella*, *Vibrio*, and *Escherichia coli* (Jenal and Malone 2006).

Aside from polysaccharides, the *P. aeruginosa* biofilm matrix contains a considerable amount of DNA (Sutherland 2001; Whitchurch et al. 2002; Matsukawa and Greenberg 2004; Steinberger and Holden 2005). While early biofilms (≤60 h) can be

dissociated by DNase treatment, older biofilms (≥84 h) seem to be recalcitrant to such treatment (Whitchurch et al. 2002). Allesen-Holm et al. (2006) discovered that the DNA found in the biofilm matrix is comprised of random chromosomal DNA, which is released by a subpopulation of cells within the biofilm, either through cell lysis or the generation of DNA-containing membrane vesicles (Schooling and Beveridge 2006). Presumably, this process is regulated via quorum sensing (Allesen-Holm et al. 2006) and can also be modulated by iron (Yang et al. 2007). Moreover, the extracellular DNA seems to localize in a time-dependent manner in the stalks of the mushroom-shaped microcolonies. Particularly high concentrations of DNA are found in the outer parts of the stalk, thus forming a border between the stalk- and the cap-forming *P. aeruginosa* subpopulations (Allesen-Holm et al. 2006). It is important to note, however, that in the context of CF, the source of DNA in the biofilm matrix is probably not solely of bacterial origin. In this context, Walker et al. (2005) suggest that DNA and actin derived from necrotic neutrophils become part of the biofilm matrix, which may ultimately enhance *P. aeruginosa* biofilm development in vivo. In any case, the presence of considerable amounts of DNA in the biofilm matrix provides a partial explanation for the therapeutic benefits of inhaled nebulized recombinant DNase I in the treatment of chronic lung infections in CF patients (Bollert et al. 1999; Ratjen et al. 2005).

5 Concluding Remarks

In the last decade, the study of bacterial biofilms and surface-associated communities has been met with rekindled interest. It is now recognized that many of the early findings were rather generalized and that biofilms are much more complex and dynamic than originally anticipated. This chapter reviews past and current *P. aeruginosa* biofilm research and provides insight into how older paradigms are challenged by newer and sometimes conflicting observations. The reports show how strain background as well as the choice of biofilm reactors and/or growth medium can substantially influence the outcome of a given experiment and reflect the ability of *P. aeruginosa* to successfully adapt to various environmental conditions. However, the broad spectrum of results obtained in these studies also reminds us that our understanding of *P. aeruginosa* biofilm formation, architecture, and resistance phenotype is rudimentary and that we have merely scratched its surface.

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