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Bacterial Biofilms: Development, Dispersal, and Therapeutic Strategies in the Dawn of the Postantibiotic Era

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Biofilm formation constitutes an alternative lifestyle in which microorganisms adopt a multicellular behavior that facilitates and/or prolongs survival in diverse environmental niches. Biofilms form on biotic and abiotic surfaces both in the environment and in the healthcare setting. In hospital wards, the formation of biofilms on vents and medical equipment enables pathogens to persist as reservoirs that can readily spread to patients. Inside the host, biofilms allow pathogens to subvert innate immune defenses and are thus associated with long-term persistence. Here we provide a general review of the steps leading to biofilm formation on surfaces and within eukaryotic cells, highlighting several medically important pathogens, and discuss recent advances on novel strategies aimed at biofilm prevention and/or dissolution.

Biofilm formation enables single-cell organisms to assume a temporary multicellular lifestyle, in which "group behavior" facilitates survival in adverse environments. What was once defined as the formation of a community of microorganisms attached to a surface has come to be recognized as a complex developmental process that is multifaceted and dynamic in nature. The transition from planktonic growth to biofilm occurs in response to environmental changes, and involves multiple regulatory networks, which translate signals to concerted gene expression changes thereby mediating the spatial and temporal reorganization of the bacterial cell (Pratt and Kolter 1998; O'Toole et al. 2000; Prigent-Combaret et al. 2001; Parsek and Singh 2003; Lenz et al. 2008; Monds and O'Toole 2009). This cellular reprogramming alters the expression of surface molecules, nutrient utilization, and virulence factors and equips bacteria with an arsenal of properties that enable their survival in unfavorable

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conditions (Whiteley et al. 2001; Schembri et al. 2003; Stanley et al. 2003; Bagge et al. 2004; Beloin et al. 2004; Vuong et al. 2004; Lenz et al. 2008; Zhang and Mah 2008; Klebensberger et al. 2009).

Within the biofilm, bacteria are cocooned in a self-produced extracellular matrix, which accounts for ~90% of the biomass (Flemming and Wingender 2010). The matrix is composed of extracellular polymeric substances (EPS) that, along with carbohydrate-binding proteins (Tielker et al. 2005; Branda et al. 2006; Diggle et al. 2006), pili, flagella, other adhesive fibers (Zogaj et al. 2001; Pinkner et al. 2006; Cegelski et al. 2009), and extracellular DNA (eDNA) (Whitchurch et al. 2002; Palchevskiy and Finkel 2006; Qin et al. 2007; Yang et al. 2007; Thomas et al. 2008, 2009; Guiton et al. 2009; Vilain et al. 2009), act as a stabilizing scaffold for the three-dimensional biofilm structure (Fig. 1). In the matrix, nutrients are trapped for metabolic utilizations by the resident bacteria and water is efficiently retained through H-bond interactions with hydrophilic polysaccharides (Conrad et al. 2003; Flemming and Wingender 2010). Enzymes secreted by the bacteria modify EPS composition in response to changes in nutrient availability (Sauer et al. 2004; Gjermansen et al. 2005), thereby tailoring biofilm architecture to the specific environment (Sauer et al. 2004; Ma et al. 2009). Thus, the structural components of the matrix give rise to a highly hydrated, robust structure with high tensile strength that keeps bacteria in close proximity, enabling intimate cell-to-cell interactions and DNA exchange (Flemming and Wingender 2010; Koo et al. 2010), while protecting the biomass from desiccation, predation, oxidizing molecules, radiation, and other damaging agents (Walters et al. 2003; Jefferson et al. 2005; Mai-Prochnow et al. 2008; Flemming and Wingender 2010). The resilient nature of biofilms is also partly attributed to the presence of environmental gradients within the biomass, which give rise to community "division of labor" with subpopulations of bacteria showing differential gene expression in response to local nutrient and oxygen availability (Lewis 2005; Domka et al. 2007). Studies have shown the presence of metabolically inactive nondividing persister cells within biofilms, which are tolerant to a number of antibiotics despite the fact that they are genetically identical to the rest of the bacterial population (Lewis 2005, 2008). These are believed to be responsible for the reseeding of biofilms on cessation of antibiotic treatment in the clinical setting (Lewis 2005, 2008).

Inside the host, the matrix protects biofilm bacteria from exposure to innate immune defenses (such as opsonization and phagocytosis) and antibiotic treatments (Jesaitis et al. 2003; Walters et al. 2003; Jefferson et al. 2005; Leid et al. 2005; Cerca et al. 2006, 2007). Interbacterial interactions can promote the spread of drug-resistance markers and other virulence factors (Vuong et al. 2004). As a result, biofilm-forming pathogens persist, establishing chronic and recalcitrant infections such as upper respiratory infections (*Pseudomonas aeruginosa*) (Koch and Hoiby 1993; Govan and Deretic 1996), urinary tract infections (UTIs) (uropathogenic *Escherichia coli* [UPEC], *Klebsiella pneumoniae*) (Foxman 2010), periodontitis (mixed biofilms of *Streptococcus mutans* and other bacteria) (Kuramitsu and Wang 2011), catheter-induced and other device-associated infections (*E. coli, Enterococcus faecalis*, and others) (Venditti et al. 1993; Ferrieres et al. 2007; Jacobsen et al. 2008; Fey 2010). Especially in immunocompromised patients, the manifestation of infections by opportunistic biofilm-forming pathogens can be devastating, leading to severe symptoms and, in many instances, death.

Here we review the processes leading to the formation of extracellular and intracellular biofilms, highlighting several medically important pathogens. Given the prevalence and recalcitrance of biofilm-related infections, we also provide a synopsis of the most recent advances in the development of novel antibiofilm strategies.

EXTRACELLULAR BIOFILM FORMATION

Bacterial Adherence on Surfaces—What Does It Take to Stick and Stick Around?

Bacterial aggregation and subsequent biofilm maturation consists of reversible and irreversible stages and involves numerous conserved

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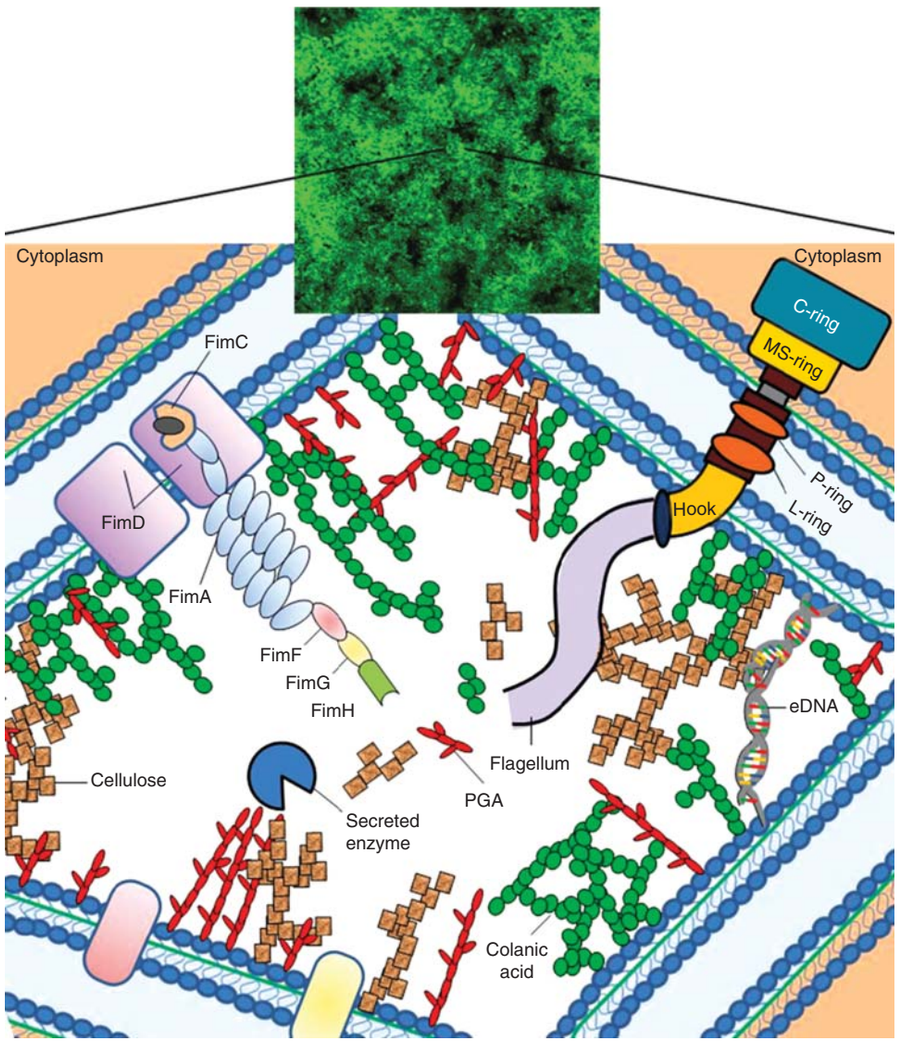

Figure 1. Schematic of the extracellular matrix composition in *E. coli*. Structural components include the EPS molecules colonic acid, cellulose, and PGA (polyglucosamine), which enable intercellular interactions, keeping bacteria in close proximity with each other. eDNA also serves as a connecting agent, as well as a nutritional source. Extracellular organelles such as flagella and CUP (chaperone usher pathway) pili enable bacterial aggregation strengthening the biofilm lattice. Secreted enzymes modify EPS components in response to environmental changes.

and/or species-specific factors. The first step involves the introduction of bacteria to a surface, a process which is at least in part stochastic, driven by Brownian motion and gravitational forces, and influenced by surrounding hydrodynamic forces (Donlan 2002; Beloin et al. 2008). Within a niche, bacteria encounter attractive or repelling forces that vary depending on nutrient levels, pH, ionic strength, and temperature. Medium properties, along with bacterial cell-surface composition affect velocity and direction toward or away from the contact surface

(Donlan 2002). Motile bacteria have a competitive advantage, utilizing flagella to overcome hydrodynamic and repulsive forces. The importance of flagellar motility for initial attachment has been documented for several pathogens, including *P. aeruginosa*, *Vibrio cholerae, Listeria monocytogenes*, and *E. coli* (O'Toole and Kolter 1998; Pratt and Kolter 1998; Watnick and Kolter 1999; Klausen et al. 2003a,b; Lemon et al. 2007; Toutain et al. 2007). In some bacterial species, chemotaxis also plays a role in directing attachment in response to nutrient composition; mutations in the CheR1 methyltransferase have been shown to alter the amino acid response of *P. aeruginosa* and impair attachment and biofilm maturation (Schmidt et al. 2011). Previous studies showed that chemotaxis is dispensable in *E. coli* (Pratt and Kolter 1998); however, recent investigations have revealed that disruption of the methyl-accepting chemotaxis protein II (*tar*), imparts biofilm defects in UPEC (Hadjifrangiskou et al. 2012).

Upon intercepting the surface, adherence is mediated by additional extracellular adhesive appendages and secreted adhesins. However, the decision to "stick" is not absolute; initial attachment is dynamic and reversible, during which bacteria can detach and rejoin the planktonic population if perturbed by hydrodynamic forces (sloughing bacteria off the surface), repulsive forces (Dunne 2002), or in response to nutrient availability (Banin et al. 2005; Anderson et al. 2008; Wu and Outten 2009).

Irreversible attachment is attained by bacteria that can weather shear forces and maintain a steadfast grip on the surface. UPEC and other *E. coli* pathotypes rely heavily on type 1 pili (Mulvey et al. 1998; Pratt and Kolter 1998; Martinez et al. 2000; Hung et al. 2002; Anderson et al. 2003; Beloin et al. 2008), which are multisubunit adhesive organelles assembled by the chaperone usher pathway (CUP) (Waksman and Hultgren 2009). UPEC harbor numerous CUP pili systems, which are differentially expressed and are presumed to facilitate adherence in a niche-specific manner (Welch et al. 2002; Chen et al. 2006, 2009; Hadjifrangiskou et al. 2011; Spurbeck et al. 2011). Adherence is mediated by the FimH adhesin at the tip of type 1 pili, which recognizes mannosylated moieties (Thankavel et al. 1997; Martinez et al. 2000; Zhou et al. 2001; Hung et al. 2002; Bouckaert et al. 2005; Eto et al. 2007; Nilsson et al. 2007; Wellens et al. 2008; Thumbikat et al. 2009). FimH is thought to play a critical role in UPEC pathogenesis; it mediates binding and invasion to human bladder epithelial cells, binds to human uroplakin, and is critical in a murine preclinical model of cystitis, which mimics human disease (Mulvey et al. 1998; Martinez et al. 2000; Kau et al. 2005; Bishop et al. 2007; Eto et al. 2007; Garofalo et al. 2007; Rosen et al. 2007; Wright et al. 2007; Chen et al. 2009). FimH is found under positive selection in UPEC, consistent with its role as a virulence factor in human disease (Sokurenko et al. 1994, 1995, 1998, 2004; Chen et al. 2006, 2009; Weissman et al. 2007; Wright et al. 2007) and has been said to have fulfilled Koch's postulates (Connell et al. 1996; Snyder et al. 2006). In addition to type 1 pili, curli fibers and Antigen 43 have been shown to mediate attachment and interbacterial interactions on abiotic surfaces (Henderson et al. 1997; Hasman et al. 1999; Danese et al. 2000a; Kjaergaard et al. 2000; Ulett et al. 2007; Cegelski et al. 2009). Curli also facilitates binding to the eukaryotic extracellular matrix components laminin, fibronectin, and plasminogen (Vidal et al. 1998; Cookson et al. 2002; Uhlich et al. 2006).

P. aeruginosa, an important pathogen and avid biofilm former, also uses several attachment organelles to irreversibly adhere to a surface. Besides flagella, *P. aeruginosa* uses type IV pili-mediated twitching motility to wade through the liquid interface and contact the surface, maintain adherence, and move across the attachment plane (O'Toole and Kolter 1998; Klausen et al. 2003a,b). Similar to UPEC, *P. aeruginosa* express numerous CUP fimbriae, of which CupA is involved in surface adherence and autoaggregation (Vallet et al. 2001; Klebensberger et al. 2009).

In contrast to *Pseudomonas* and UPEC, the Gram-positive *Enterococci* are nonmotile and, up until recently, were thought to possess no adhesive pili. Over the years, investigations identified a panel of enterococcal adhesins that

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mediate adherence to eukaryotic extracellular matrix components. Examples include SagA, Acm (*E. faecium*), and Ace (*E. faecalis*), which bind collagen (Mohamed et al. 2006), and the surface protein Esp, which has been shown to promote biofilm formation on abiotic surfaces in *esp*-expressing *E. faecalis* strains (Toledo-Arana et al. 2001). Recent studies elucidated the presence of *Enterococcal* biofilm pili (Ebp) in *E. faecalis* and showed their contribution to biofilm formation, endocarditis, and urinary tract infection (Ton-That and Schneewind 2003; Ton-That et al. 2004; Nallapareddy et al. 2006; Kemp et al. 2007; Guiton et al. 2009; Kline et al. 2010).

Biofilm Maturation—Keeping It Together

Surface contact triggers responses that lead to gene expression changes, up-regulating factors favoring sessility, such as those implicated in the formation of the extracellular matrix (Prigent-Combaret and Lejeune 1999; Otto and Silhavy 2002; Inagaki et al. 2005; Morici et al. 2007; Beloin et al. 2008; Bhomkar et al. 2010). In the case of *E. coli*, relatively little is known about matrix constituents. Cellulose was first identified as an important component of commensal *E. coli* pellicle biofilms, and was later shown to be coexpressed with curli in UPEC and gastrointestinal *E. coli* isolates (Zogaj et al. 2001, 2003; Romling 2002; Bokranz et al. 2005; Kai-Larsen et al. 2010). Curli are amyloid fibers that are critical for the formation of pellicle biofilms, as curli inhibitors (curlicides) inhibit pellicle formation and curli mutants cannot form pellicles (Cegelski et al. 2009). Additional studies showed that polyglucosamine (PGA) and colanic acid contribute to biofilm architecture (Prigent-Combaret and Lejeune 1999; Danese et al. 2000b; Prigent-Combaret et al. 2001; Wang et al. 2004; Agladze et al. 2005), with PGA being prevalent among clinical isolates, including UPEC (Cerca et al. 2007). More detailed analyses are required for a complete characterization of the extracellular matrix in pathogenic *E. coli.*

Extracellular matrix composition has been more extensively investigated in *P. aeruginosa*, and has been shown to vary depending on environmental conditions (Harmsen et al. 2010). Two primary EPS components are Pel and Psl (Friedman and Kolter 2004a,b; Jackson et al. 2004; Matsukawa and Greenberg 2004; Vasseur et al. 2005; Ma et al. 2006). Psl augments *Pseudomonas* attachment to mucin and airway epithelial cells (Ma et al. 2006), whereas increased expression of *pel* in small colony variants isolated from cystic fibrosis patients has been associated with *P. aeruginosa* persistence in lung airways (Starkey et al. 2009). Recently, Borlee and colleagues identified CdrA, a large secreted adhesin, which is expressed in the biofilm in response to high levels of the universal signal 3,5-cyclic diguanylic acid (c-di-GMP) and binds Psl, stabilizing biofilm structures (Borlee et al. 2010). Alginate, another *P. aeruginosa* EPS component, has been associated with increased resistance to antibiotic treatments and host immune defenses during chronic infection (Govan and Deretic 1996; Hatch and Schiller 1998; Hentzer et al. 2001; Leid et al. 2005). As is the case with Pel and Psl, alginate production is subject to regulation by fluctuating levels of c-di-GMP. Recent studies have shown that a surface-bound diguanylate cyclase MucR positively activates alginate synthesis, presumably through high local concentrations of c-di-GMP (Hay et al. 2009). In addition to EPS, several studies have shown that eDNA is critical for cell-to-cell connections and stabilization of *Pseudomonas* biofilms (Whitchurch et al. 2002; Yang et al. 2007). Young *Pseudomonas* biofilms are more sensitive to DNase treatment compared with mature biofilms, indicating a stabilizing role for eDNA during the initial biofilm stages when EPS components are not as abundant (Whitchurch et al. 2002). As the biofilm matures, eDNA amounts increase through lysis of a bacterial subpopulation in response to the *P. aeruginosa* quinolone signal (Pqs) quorum sensing system (Allesen-Holm et al. 2006). Allesen-Holm et al. showed that eDNA is organized in distinct patterns and localizes in the stalk portion of the mushroom-shaped biofilms (Allesen-Holm et al. 2006). This localization may act as a scaffold for the formation of the mushroom structure, as type IV pili show high eDNA binding affinity, inducing the accumulation of

migrating bacteria toward areas of high eDNA concentration (Barken et al. 2008).

The contribution of eDNA to biofilm architecture has also been reported for *E. faecalis*, making it one of the few known *E. faecalis* matrix components. Thomas et al. first reported that eDNA is critical for *E. faecalis* biofilms and identified that the secreted enzymes GelE (zinc metalloprotease) and SprE (serine protease) influence biofilm formation by affecting cellular autolysis and DNA release (Thomas et al. 2008, 2009). In a separate study, Mohamed et al. reported that a mutant lacking the Atn autolysin had 30% reduction in biofilm (Mohamed et al. 2004). Guiton and colleagues later established that Atn plays a role in the temporal regulation of DNA release at specific stages during biofilm formation (Guiton et al. 2009).

Escape from the Matrix—Dispersing Mechanisms

Within the mature biofilm there is a bustling community that actively exchanges and shares products that play a pivotal role in maintaining biofilm architecture and providing a favorable living environment for the resident bacteria. However, as biofilms mature, dispersal becomes an option. Besides passive dispersal, brought about by shear stresses, bacteria have evolved ways to perceive environmental changes and gauge whether it is still beneficial to reside within the biofilm or whether it is time to resume a planktonic lifestyle. Biofilm dispersal can be the result of several cues, such as alterations in nutrient availability, oxygen fluctuations and increase of toxic products, or other stress-inducing conditions (Sauer et al. 2004; Karatan and Watnick 2009; Hong et al. 2010; Rowe et al. 2010). In UPEC, increase in extracellular iron induces biofilm dispersal (Rowe et al. 2010), whereas *P. aeruginosa* biofilms disperse in response to increased amounts of various carbon and nitrogen sources (Sauer et al. 2004; Karatan and Watnick 2009). Several sensory systems monitor the levels of small molecules, as a proxy to environmental changes, and alter gene expression accordingly, promoting dispersal (Hammer and Bassler 2003; Kaplan 2010). Among other signals, the universal c-di-GMP has been extensively implicated in the shift between sessility and motility in bacteria, including *P. aeruginosa* and *E. coli*. Typically, an increase in c-di-GMP favors sessility, whereas reduced c-di-GMP leads to up-regulation of motility (Morgan et al. 2006; Pruss et al. 2006; Barraud et al. 2009; Wood et al. 2010). Ma et al. recently reported that a c-di-GMP binding protein, BdcA, is at least partly responsible for the reduction of available c-di-GMP in biofilm communities, down-regulation of EPS, and up-regulation of swimming and swarming motility; a phenomenon that the investigators showed also occurs in *Pseudomonas* species and *Rhizobium mellioti* (Ma et al. 2011a,b).

EPS-degrading enzymes, such as alginate lyase in *P. aeruginosa,* also contribute to bacterial detachment from the matrix (Boyd and Chakrabarty 1994). In *E. coli*, the CsrA protein was shown to repress PGA synthesis, also aiding in dispersion (Wang et al. 2005). Besides down-regulating EPS, surfactant molecules are produced, reducing surface-bacterial interactions; for example, although controlled rhamnolipid production contributes to channel formation within mature *P. aeruginosa* biofilms, an increase in rhamnolipid levels aids bacterial dispersal (Boles et al. 2005; Dong et al. 2008; Harmsen et al. 2010). In addition, studies have identified flagellated subpopulations within *P. aeruginosa* biofilms, which emigrate from the biofilm, creating microcolonies with a central void (Purevdorj-Gage et al. 2005; Harmsen et al. 2010). Voids within the biofilm are also created by cell death, serving as an additional dispersal mechanism that frees resident live bacteria, as shown by studies in *P. aeruginosa* (Webb et al. 2003). Dispersing bacteria have the capacity to reinitiate the process of biofilm formation, on encountering a suitable environment.

Studies using *Bacillus subtilis* as a model organism revealed another sophisticated dispersal mechanism that may be widespread among bacteria. *B. subtilis* forms robust biofilms, which lose their integrity after 5–8 d; Kolodkin-Gal and colleagues found that biofilm disassembly is facilitated by a mixture of D-amino acids

(D-leucine, D-methionine, D-tyrosine, and D-tryptophan) that are produced during the stationary phase of growth and get incorporated into the peptide side chains of peptidoglycan in place of the terminal D-alanine (Lam et al. 2009; Kolodkin-Gal et al. 2010). This D-amino acid incorporation interferes with the anchoring of adhesive fibers on the cell surface, leading to fiber dissociation and loss of bacterial adherence, without influencing bacterial growth or expression of matrix components (Kolodkin-Gal et al. 2010). Exogenous addition of the D-amino acid mixture or the individual D-amino acids disrupted preformed biofilms of *B. subtilis* and other bacterial species (Kolodkin-Gal et al. 2010). Further studies revealed that D-amino acids work together with norspermidine, another factor produced by *B. subtilus*, to cause biofilm disassembly (Kolodkin-Gal et al. 2012). Thus, D-amino acid/norsperimidine treatment may hold promising potential in preventing or eradicating biofilms.

THE LIFE WITHIN—INTRACELLULAR BIOFILMS

Accumulating evidence indicates that many bacterial pathogens previously considered as strictly extracellular can persist inside the host by adapting an intracellular lifestyle that involves the formation of bacterial communities with biofilm-like properties. These intracellular bacterial communities (IBCs) were first documented for UPEC, using a murine model of infection (Mulvey et al. 1998; Anderson et al. 2003; Justice et al. 2004). UPEC use type 1 pili to bind mannosylated receptors on the superficial umbrella bladder cells (Zhou et al. 2001; Hung et al. 2002; Bouckaert et al. 2005; Eto et al. 2007; Wellens et al. 2008; Thumbikat et al. 2009), triggering events that lead to bacterial internalization. Although internalized UPEC are expelled in a TLR-4-dependent process (Bishop et al. 2007), some bacteria avoid the exocytic process and escape into the host-cell cytoplasm, where they replicate into IBCs (Anderson et al. 2003; Justice et al. 2004).

IBCs progress through several developmental stages that show distinct morphological characteristics (Fig. 2) (Justice et al. 2004). During the first 6 h following bladder inoculation, UPEC divide rapidly (doubling time of ~30–35 min) resulting in small clusters of loosely associated rods (early IBCs), morphing into coccoid-shaped bacteria, with an average length of 0.7 μm that begin packing into a tight biomass. Then, between 6 and 8 h, the growth rate drops dramatically, resulting in doubling times >60 min. At this stage, bacteria are tightly packed together forming a highly organized sphere inside the cell that comprises the mature middle-stage IBC (Fig. 2). The number of IBCs can range between 3 and 700 IBCs in an infected bladder; each IBC is clonal and composed of $\sim 10^4$–10^5 bacteria (Anderson et al. 2003; Schwartz et al. 2011). IBC bacteria are surrounded by numerous fibers that emanate from the bacterial surface, resembling an extracellular matrix and encasing bacteria in individualized compartments (Anderson et al. 2004). Polysaccharides, such as the sialic acid capsule, are also present throughout the IBC and function, in part, to protect the bacteria from neutrophil attack (Anderson et al. 2010). Similar to extracellular biofilms, IBCs are heterogeneous, composed of subpopulations with different gene expression patterns (Anderson et al. 2004).

As IBCs enlarge, the bacterial mass pushes against the host-cell membrane creating a pod-like protrusion on the surface of the infected cell (Anderson et al. 2003). Eventually, UPEC at the IBC periphery detach as single rods or filaments, and flux out of the infected cell into the bladder lumen where they can reinitiate the process by binding and invading naive epithelial cells (Justice et al. 2004). The cell division inhibitor SulA has been shown to be important for filamentation and dispersal of UPEC from the biomass and, thus, establishment of next-generation IBCs (Justice et al. 2006). UPEC filaments have been shown to be a common feature in the urines of patients with UTI, but not in otherwise healthy controls (Rosen et al. 2007). Further, UPEC isolated from the urine of patients with a UTI have been shown to form IBCs when inoculated into the bladders of six different strains of mice, indicating that IBCs are important for human infection (Garofalo

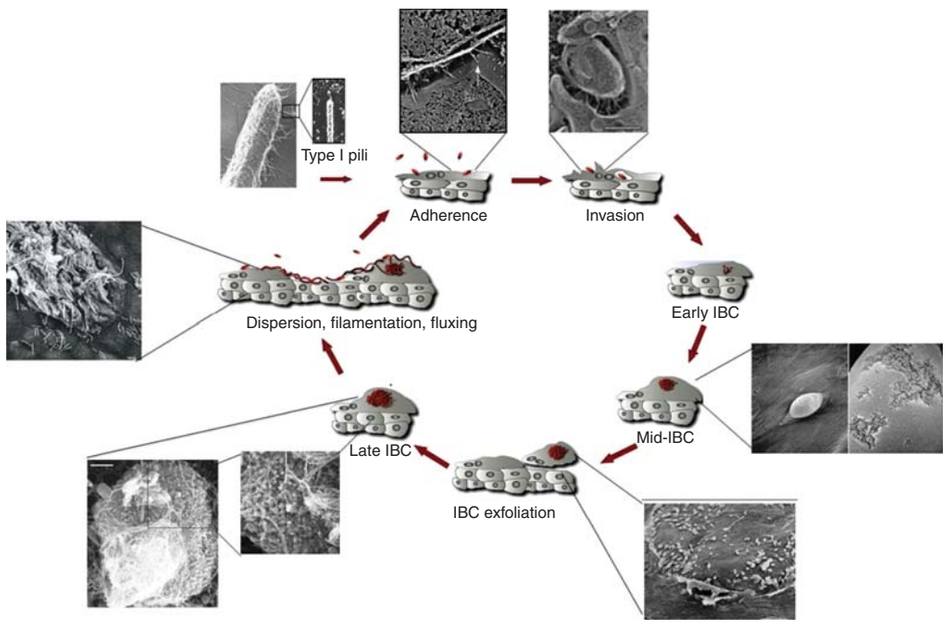

Figure 2. Schematic of the IBC developmental cascade in UPEC (uropathogenic *Escherichia coli*), accompanied by SEM (scanning electron microscopy) images depicting the distinct morphological changes from attachment and invasion to filamentation and dispersal. (SEM images from Anderson et al. 2003, Hultgren Lab.)

et al. 2007). IBC formation is restricted by severe molecular bottlenecks, and higher IBC numbers during acute infection are associated with founding the development of chronic cystitis, indicating the importance of the intracellular pathway in UTI pathogenesis (Hannan et al. 2010; Schwartz et al. 2011).

The IBC cycle is FimH dependent, as interruption of type 1 pili expression after the invasion step, disrupts normal IBC development and leads to UPEC attenuation (Wright et al. 2007). The QseBC two-component system is one of the factors influencing type 1 pili, curli expression, and IBC formation. Recent studies showed that deletion of the QseC sensor results in overactivation of the cognate response regulator QseB, which leads to virulence gene downregulation by interfering with core metabolic processes (Kostakioti et al. 2009; Hadjifrangiskou et al. 2011). These studies also showed that the UPEC intracellular pathway requires completion of the TCA cycle (Hadjifrangiskou et al. 2011). Microarray and qPCR analyses probing the expression patterns of UPEC within IBCs revealed that iron-acquisition systems are highly up-regulated, indicating the importance of these systems for intracellular biofilm formation (Reigstad et al. 2007). Henderson et al. later showed that the same iron-acquisition systems are prevalent among UPEC isolates (Henderson et al. 2009).

Intracellular communities have also been reported for *K. pneumoniae*, which accounts for up to 5% of community-acquired UTIs, and is more prevalent in diabetic patients and in the nosocomial setting (Lye et al. 1992; Hansen et al. 1998). Similar to UPEC, type 1 pili mediate *K. pneumoniae* invasion and IBC formation, albeit with differences in pili expression kinetics and numbers of formed IBCs and filaments (Rosen et al. 2008a,b).

The ability to occupy an intracellular niche and persist inside the host by transitioning from single cell to a multicellular community is not confined to uropathogens. Using cell lines and animal models of acute lung infection, Garcia-

Medina and colleagues showed that following infection, *P. aeruginosa* can form clusters within the airway cells that matured to a podlike structure, similar in morphology to UPEC and *Klebsiella* IBCs (Garcia-Medina et al. 2005). Bacteria within the *Pseudomonas* pod structure showed regional variation in their expression patterns similar to what was reported for UPEC, which is a typical characteristic of extracellular biofilms (Garcia-Medina et al. 2005).

The ability to form intracellular biofilms may be an evolutionary adaptation that facilitates bacterial persistence to a level extending even beyond that attained by extracellular biofilms. For example, during UTI, hordes of neutrophils infiltrate the bladder migrating toward the infected superficial umbrella cells, but are unable to effectively penetrate the IBC or engulf dispersing filamentous bacteria (Justice et al. 2008; Horvath et al. 2010). The ability of IBCs to repel neutrophil penetration is lost in K1 capsule mutants (Anderson et al. 2010). Moreover, Blango and Mulvey showed that 17 different antibiotics capable of killing the virulent cystitis isolate UTI89 in vitro or in tissue culture were unable to eliminate UTI89 from bladder tissue during infection (Blango and Mulvey 2010). These findings indicate that IBC formation is a mechanism that enables rapid bacterial expansion within the host and contributes to bacterial persistence.

BIOFILM INHIBITION: TREATMENT STRATEGIES IN THE POSTANTIBIOTIC ERA

Antibiotics are currently the preferred treatment strategy for bacterial infections. Conventional antibiotics work by either preventing bacterial cell division (bacteriostatic) or killing the cell (bactericidal). Although over the years antibiotics have proven critical in eliminating bacterial pathogens, overwhelming evidence indicates that they extensively damage the host microbiota, creating an environment where opportunistic pathogens can prevail, and they increase the selective pressure toward antibiotic resistance (Dethlefsen et al. 2008; Dethlefsen and Relman 2010; Ubeda et al. 2010). Moreover, although prophylactic antibiotic administration preceding surgery is highly successful in reducing infection rate, it has little or no protective effects in surgical procedures involving implants or prostheses (Secinti et al. 2011). In most cases, the best treatment for foreign body-associated biofilm infections is to remove the infected device. However, in cases like implantable prostheses, pacemakers, and cardiac implants, device removal is difficult (Fey 2010).

Biofilm bacteria are particularly recalcitrant to antibiotic treatments not only owing to increased transmission of resistance markers within the biofilm community, but also because of diffusion limitations posed by the extracellular matrix, antibiotic inactivation by high metal ion concentration and low pH, and the presence of metabolically inactive persister cells that survive treatment (Mack et al. 2004; Lewis 2005; Costerton et al. 2007; Lewis 2008). Combined, these attributes make biofilm bacteria up to 1000-fold more tolerant and/or resistant to antibiotics than planktonic cells (Hoiby et al. 2010). Thus, the need for more effective biofilm dissolution treatments becomes imperative. Below we present some of the most recent advances in strategies designed to thwart biofilm formation by killing the bacteria or targeting different biofilm developmental stages (also summarized in Fig. 3).

Bactericidal Strategies

Phage Therapy

Phage therapy is a promising alternative to antibiotic treatments (Donlan 2009); phages are abundant and can be easily isolated from a wide range of environments, they are usually specific to narrow host ranges (thus not likely to perturb the host microbiota), and their self-replicating mode permits low dosage (Burrowes et al. 2011). Moreover, the high phage mutation rate facilitates adaptation as the corresponding bacterial hosts accumulate mutations to persist in a given environment. Phage therapy takes advantage of lytic phages that do not enter a prophage state and thus rarely contain or transfer virulence genes, although they result in rapid destruction of the bacterial cell. Many phages have been shown to encode EPS-degrading

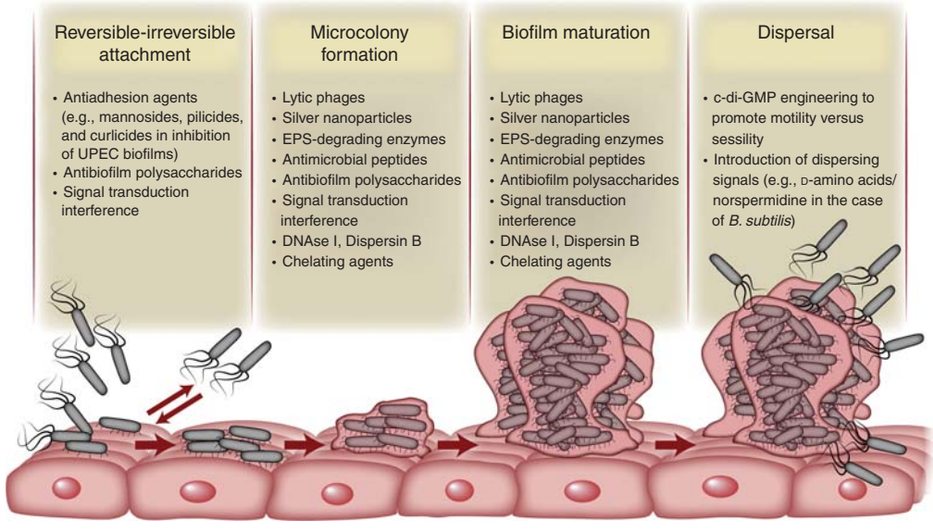

Figure 3. Schematic outlining the stages in biofilm development and listing the strategies aimed at inhibiting and/or disrupting biofilm formation at specific stages.

enzymes (Hughes et al. 1998a,b; Sutherland et al. 2004), or propagate on stationary-phase bacteria, making them more likely to persist within the biofilm (Burrowes et al. 2011).

Silver Nanoparticles

Impregnation of medical devices with antimicrobial agents has been the most commonly used approach for preventing device-associated biofilms (Fey 2010). One of the frequently used agents is silver, which has been used as an antiinfective for hundreds of years and has been extensively used to sterilize wound infections during World War I (Rupp et al. 2005; Chen and Schluesener 2008). The positively charged silver ions facilitate electrostatic attractions between the metal and the negatively charged bacterial membrane, augmenting uptake and antimicrobial activity (Kim et al. 2007). The lethality of silver for bacteria is partly owing to thiol-group reactions that inactivate enzymes (Chen and Schluesener 2008). As a result, silver treatment inhibits DNA replication, expression of ribosomal and other cellular proteins, and interferes with the bacterial electron transport chain (Bragg and Rainnie 1974; Feng et al. 2000; Yamanaka et al. 2005).

The potential toxicity of silver in humans led to its dwindling use for some time. However, the popularity of silver has been revived with the advent of nanotechnology (Chen and Schluesener 2008). Nanoparticles are typically no greater than 100 nm in size and their biocidal effectiveness is suggested to be owing to a combination of their small size and high surface-to-volume ratio, which enable intimate interactions with microbial membranes (Morones et al. 2005; Allaker 2010). Silver nanoparticles have been shown to inhibit *P. aeruginosa* and *Staphylococcus epidermidis* biofilms by >95%; studies in rabbits showed that nanoparticle silver ion-coated implants inhibited *Staphylococcus aureus* biofilm formation without causing silver accumulation in host tissues, even 28 d after impregnation (Kalishwaralal et al. 2010; Secinti et al. 2011).

Antimicrobial Peptides

Antimicrobial peptides are produced by the innate immune response system and have been

proposed as attractive candidates for the development of novel types of antibiotics (Yang et al. 2002). However, their activity spectrum and mechanism of action need to be more precisely defined before they can be considered as possible therapeutic strategies (Pompilio et al. 2011). Cathelicidins constitute one of the most important classes of antimicrobial peptides. Recent work indicated that SMAP-29, BMAP-28, and BMAP-27 significantly reduced biofilm formation by multidrug-resistant (MDR) *P. aeruginosa* strains isolated from patients with CF (cystic fibrosis), and killed bacteria within preformed biofilms (Pompilio et al. 2011). This study compared the bactericidal activity of cathelicidins to that of tobramycin, the frontline antibiotic used to treat *P. aeruginosa* airway infections in CF patients. Pompilio et al. found that in contrast to tobramycin, the active cathelicidin peptides showed faster kinetics, exerting a rapid bactericidal activity regardless of the species tested. Although the extent of bacterial killing was overall higher with tobramycin, this study shows that cathelicidins may hold potential as antibiofilm agents in the case of MDR strains (Pompilio et al. 2011).

Lytic peptides are another group of antimicrobial peptides assessed for their inhibitory effects on biofilm formation. Lytic peptides bind the LPS (lipopolysaccharide) moieties of the bacterial cell membrane, disrupting membrane stability. Studies in *Staphylococcus aureus* have shown that the lytic peptide PTP-7 prevented in vitro biofilm formation and was also capable of diffusing into the deep layer of preformed biofilm, killing 99.9% of biofilm bacteria. This peptide retained activity under highly acidic environments and in the presence of excess of metals, conditions that mimic the *S. aureus* biofilm environment (Kharidia and Liang 2011).

Antiadhesion Agents

Mannosides, Pilicides, and Curlicides

Attachment constitutes the first step in virtually all types of biofilm formation, thus numerous studies have focused on ablating bacterial adherence. In UPEC, efforts have concentrated on the development of compounds that interfere with the adhesive properties or assembly of type 1 pili, because they are the prevalent means for UPEC adherence during biofilm formation in vitro and within the host. The X-ray crystal structures of the FimH adhesin bound to mannose have been used to rationally design molecules, termed mannosides, which fit the FimH mannose binding pocket and competitively inhibit FimH binding to its host receptor (Fader and Davis 1980; Schaeffer et al. 1980; Hung et al. 2002; Bouckaert et al. 2005; Sperling et al. 2006; Wellens et al. 2008; Klein et al. 2010). Recent advances in this field led to the development of monomeric biphenyl mannosides with largely enhanced potency, relative to previously reported FimH inhibitors (Han et al. 2010). These optimized mannosides prevented UPEC biofilm formation in vitro and were shown to disrupt preformed biofilms (Cusumano et al. 2011). In vivo studies indicated that mannoside administration as a prophylactic measure for UTIs interfered with UPEC adherence and invasion, reducing IBC formation and attenuating UPEC during the acute infection stages (Cusumano et al. 2011). Moreover, mannosides enhanced the antimicrobial effects of trimethoprim-sulfamethoxazole (TMP-SMZ) preventing infection by PBC-1, a UPEC isolate that was resistant to TMP-SMZ treatment in the clinical setting (Cusumano et al. 2011). Given that the intracellular niche protects UPEC from antibiotic treatment (Blango and Mulvey 2010), the conferred PBC-1 susceptibility on dual mannoside/TMP-SMZ treatment is likely owing to bacterial sequestration in the extracellular environment, where antibiotic concentrations are higher relative to the intracellular compartment (Patel and Welling 1980; Cusumano et al. 2011). Mannosides were also efficient as a therapeutic strategy for chronic UTIs, significantly reducing the bladder bacterial load of orally treated mice within 6 h (Cusumano et al. 2011). Thus, if translated to the clinic mannosides, they hold great potential for the elimination of complicated UTIs associated with antibiotic-resistant UPEC strains.

In parallel, efforts have been made to inhibit assembly of type 1 pili and other CUP

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pili, through the use of pilicides, which are compounds rationally designed to interfere with export of the corresponding pilin subunits. Pilicides were shown to inhibit UPEC biofilm formation in vitro by 50%, at concentrations as low as 3 μM (Pinkner et al. 2006; Berg et al. 2008; Chorell et al. 2010, 2011). Similar compounds have been shown to be effective against curli ("curlicides"), inhibiting in vitro curli biogenesis, biofilm formation, and potentiating UPEC clearance from the urinary tract (Cegelski et al. 2009).

Polysaccharides

Exopolysaccharides mediate cell-to-surface and cell-to-cell interactions that are critical for biofilm formation and stabilization. Mutants unable to synthesize or export such polysaccharides are typically deficient in adherence and biofilm formation and thus are highly sensitive to killing by antibiotics and host immune defenses (Rendueles et al. 2012). However, recent evidence indicates that some bacterial exopolysaccharides inhibit or destabilize biofilm formation by other species. For example, in the case of *P. aeruginosa*, Qin and colleagues showed that Pel and Psl-containing culture supernatants disrupted preformed *S. epidermidis* and *S. aureus* biofilms without inhibiting bacterial growth (Qin et al. 2009). Moreover, the presence of *P. aeruginosa* inhibited *S. epidermidis* biofilm formation in dual-species in vitro biofilm experiments (Pihl et al. 2010). Polysaccharides with nonbiocidal antibiofilm properties have also been isolated from cell-free biofilm extracts of several species (Rendueles et al. 2012). Their antibiofilm properties are believed to lie on their ability to (a) alter the physical characteristics of bacterial cells or abiotic surfaces; (b) act as signaling molecules that impact the gene expression patterns of susceptible bacteria; or (c) competitively inhibit multivalent carbohydrate–protein interactions, thereby interfering with adhesion. Most antibiofilm polysaccharides show a broad spectrum of biofilm inhibition, whereas some are capable of dispersing preformed biofilms. Given their nonbiocidal mode of action, as well as their biocompatibility and biodegradability, antibiofilm polysaccharides could be a promising strategy suitable for the treatment and prevention of biofilm-related infections. Furthermore, several studies suggest that antibiofilm polysaccharides could be valuable as an adjuvant, because they enhance antibiotic functions when administered together. Other potential applications could be coating surfaces of indwelling medical devices or even using antibiofilm polysaccharide-producing bacteria in probiotics to outcompete pathogens (Rendueles et al. 2012).

Signal Transduction Interference

Many studies have focused on inhibiting biofilm initiation by interfering with bacterial signaling cascades, given that two-component systems constitute a central means of intercepting and translating environmental changes (Lyon et al. 2000; Okada et al. 2007; Cegelski et al. 2008; Rasko et al. 2008; Watanabe et al. 2008; Njoroge and Sperandio 2009). Inhibition of signal transduction systems poses an attractive means of antivirulence therapy, because interference with signaling does not kill the bacteria but rather deprograms optimal gene expression and ablates virulence without applying pressure for selection of resistance. Among the systems that appear to be attractive drug target candidates are the QseBC two-component system that is common among biofilm-forming Gram-negative pathogens (Clarke et al. 2006; Bearson and Bearson 2008; Rasko et al. 2008; Kostakioti et al. 2009; Khajanchi et al. 2011; Wang et al. 2011). Previous studies have investigated the potential of inhibiting QseC kinase activity and showed efficacy in reducing enterohemorrhagic *Escherichia coli* (EHEC) virulence (Rasko et al. 2008). Studies in UPEC and EHEC have shown that deletion of QseC results in the overactivation of the QseB response regulator owing to the specific phosphatase activity of QseC required for QseB deactivation. Thus, targeting QseC phosphatase activity would be an optimized strategy to decouple normal gene expression in QseC-bearing pathogens (Kostakioti et al. 2009; Hadjifrangiskou et al. 2011).

In *E. faecalis*, studies have targeted the FsrC/FsrA TCS. FsrC/FsrA controls the expression of

fsrBDC and *gelE-sprE*, leading to increased production of gelatinase and serine protease, both of which are required for appropriate production of eDNA (Qin et al. 2000; Thomas et al. 2008, 2009). High-throughput screening of compounds that inhibited gelatinase and the gelatinase biosynthesis-activating pheromone (GBAP), identified a peptide antibiotic, Siamycin I, as an inhibitor of GBAP signaling by FsrC/FsrA (Nakayama et al. 2007; Gotoh et al. 2010).

"Antimatrix" Agents

Besides ablating adherence, bacterial aggregation can be targeted by disrupting components of the extracellular matrix. Several investigations exploited the potential of inhibiting enzymes involved in the synthesis or modification of cell wall-associated or secreted EPS components and other matrix constituents. These studies use the direct use of naturally occurring or engineered enzymes, use bacteriophages (phage therapy) as a vehicle of enzyme delivery and expression, or take advantage of metal chelators as a means to disrupt matrix integrity.

Enzymes

N-acetyl-D-glucosamine-1-phosphate acetyltransferase (GlmU), which is involved in the biosynthesis of activated UDP-GlcNAc, an essential peptidoglycan and lipopolysaccharide (LPS) precursor in Gram-positive and Gram-negative pathogens, respectively, is among the enzymes targeted for matrix disruption (Burton et al. 2006). Burton et al. tested the effects of GlmU inhibitors, including *N*-ethyl maleimide (NEM), and the NEM analogs *N*-phenyl maleimide, *N*,*N*′-(1,2-phenylene)dimaleimide (oPDM), and *N*-(1-pyrenyl)maleimide (PyrM), on catheter-associated uropathogen biofilms. All NEM analogs showed antibiofilm activity against clinical isolates of *E. coli*, *P. aeruginosa, K. pneumoniae, S. epidermidis*, and *E. faecalis* (Burton et al. 2006). The same study showed that coating silicone catheters with a mixture of oPDM and the cationic polypeptide protamine sulfate (PS) enhanced the antibiofilm activity of PS against *P. aeruginosa* and *S. epidermidis*, demonstrating that this dual administration could be used as a broad-spectrum anti-infective coating for medical devices (Burton et al. 2006).

The enzymes DNase I and Dispersin B have also recently gained attention as potential antibiofilm agents, particularly against Gram-positive pathogens. The effects of DNase I lie on its ability to digest the eDNA found within the biofilm structure (Qin et al. 2007; Zhu et al. 2007; Guiton et al. 2009). DNase treatment prevented *Staphylococcus* and *Enterococcus* biofilm formation (Guiton et al. 2009; Mann et al. 2009) and dispersed preformed biofilms in vitro (Guiton et al. 2009). A recombinant form of DNase I, pulmozyme, is used in certain cases to treat patients with CF (Shak et al. 1990; Fey 2010). Dispersin B is a glycoside hydrolase produced by *Actinobacillus actinomycetemcomitans* that cleaves β 1–6 *N*-acetylglucosamine polymers (PNAG) in the bacterial peptidoglycan layer (Fey 2010). Dispersin-B treatment has been shown to be effective against *S. aureus* and *S. epidermidis* biofilms and other PNAG-containing bacteria (Izano et al. 2008; Kaplan 2010).

Engineering Dispersin B into a phage that replicates in stationary-phase cells led to complete disruption of preformed *E. coli* biofilms in vitro, an effect that was more dramatic than administration of either nonengineered phage or Dispersin B alone, likely because Dispersin B-mediated degradation of EPS allowed the phage to access the deeper layers of the biofilm structure (Lu and Collins 2007).

Chelating Agents

Metal cations, such as calcium, magnesium, and iron have been implicated in maintaining matrix integrity (Patrauchan et al. 2005; Raad et al. 2008). Consistent with this observation, chelating agents have been shown to destabilize biofilm architecture besides interfering with bacterial membrane stability (Donlan 2011). For example, sodium citrate inhibited biofilm formation by several *Staphylococci* species in vitro (Shanks et al. 2006). In addition, tetrasodium-EDTA eradicated biofilms in an in vitro biofilm model and on explanted hemodialysis catheters

(Kite et al. 2004; Percival et al. 2005), whereas disodium-EDTA, in combination with tigecyclin or gentamicin, reduced biofilm formation by *Staphylococcus* species and *P. aeruginosa* on Hickman catheter segments in vitro (Bookstaver et al. 2009). Raad et al. showed efficacy of a combination of minocycline and disodium-EDTA against biofilms in vitro or on explanted catheter tips, as well as in the treatment of catheter-related bloodstream infections in three different patient studies (Raad et al. 1997, 2003). A minocycline-EDTA solution was also successfully used to prevent indwelling implantable-port infections in children with cancer; no port infections or other adverse effects were observed in patients whose ports were flushed with the monocycline-EDTA solution, whereas 21% of the patients in the untreated control group developed infection (Chatzinikolaou et al. 2003). Moreover, reduction of catheter-related bloodstream infections was observed in hemodialysis patients after treating catheters with minocycline-EDTA (Bleyer et al. 2005; Feely et al. 2007).

Manipulating Dispersal Signals to Disassemble Biofilms

Given that planktonic cells are more susceptible to treatments, a novel treatment strategy in which a signal for biofilm dispersion is combined with administration of an antimicrobial agent for killing the dispersed organisms could be successful. As we discussed earlier, one of the signals most associated with bacterial dispersal is c-di-GMP. In a recent study, Ma et al. exploited the potential of engineering a protein that causes biofilms to disperse (Ma et al. 2011b). Using a knockout library of previously uncharacterized genes known to be influenced by impaired autoinducer-2 secretion, the investigators identified BdcA as a protein that enhances biofilm dispersal, by sequestering c-di-GMP and reducing its local concentration (Ma et al. 2011b). Subsequent analyses showed that mutating the BdcA E50 residue to V or Q, resulted in higher induction of motility by increasing the c-di-GMP binding affinity of BdcA (Ma et al. 2011b). Given that BdcA is conserved in several pathogens, this analysis provides new tools, which in combination with novel delivery strategies, such as phage therapy, could facilitate active biofilm dispersal as a therapeutic approach.

D-Amino Acids and Norspermidine

The idea of manipulating natural dispersion factors to combat biofilms has also been exploited for Gram-positive organisms. Losick and colleagues showed that exogenous addition of the D-amino acids produced by dispersing *B. subtilis* disrupted preformed biofilms and were also effective in preventing biofilm formation by *S. aureus* and *P. aeruginosa* (Kolodkin-Gal et al. 2010). It is likely that D-amino acids promote biofilm disassembly by disrupting adhesive fiber interactions (Cava et al. 2010). Indeed, studies by Hochbaum et al. showed that D-amino acids inhibit *S. aureus* biofilm formation by preventing protein localization to the cell surface (Hochbaum et al. 2011). Given that D-amino acids are produced by many bacterial species, they may provide a general strategy for biofilm disassembly (Kolodkin-Gal et al. 2010) and thus might be useful in medical and industrial antibiofilm applications. A second biofilm-disassembly molecule was recently discovered in *B. subtilus*; norspermidine works in a manner complimentary to D-amino acids by targeting the exopolysaccharide (Kolodkin-Gal et al. 2012). As is the case for D-amino acids, the biofilm-inhibiting properties of norspermidine were not limited to *B. subtilus*, but biofilm inhibition was also observed in the case of *S. aureus* and *E. coli* pellicle biofilm. Thus, norspermidine and other polyamines synthesized to bind to specific exopolysaccharides could be exploited in conjunction with D-amino acids as a novel antibiofilm approach (Kolodkin-Gal et al. 2012).

CONCLUDING REMARKS

Biofilm formation enables bacterial pathogens to colonize a wide variety of host niches and persist in harsh environments, making their eradication particularly difficult. Biofilm characteristics determine whether, to what extent,

and which antimicrobial treatments may be effective. The age and composition of the biofilm are the major factors influencing the susceptibility of the resident microorganisms. As the biofilm matures, increased EPS accumulation, combined with the nutrient and oxygen gradients that affect cell metabolism and growth rates, result in reduced entry and activity of antimicrobial agents making biofilm-forming pathogens progressively more resistant to antibiotic regimens. Thus, novel strategies, designed to block a specific biofilm step without killing the bacteria, such as the use of antiadhesion agents, or using natural, bacterially produced signals to promote bacterial dispersal, are exciting avenues for exploration and ultimately the development of fast-acting, potent, and bioavailable treatment strategies.

REFERENCES

Agladze K, Wang X, Romeo T. 2005. Spatial periodicity of *Escherichia coli* K-12 biofilm microstructure initiates during a reversible, polar attachment phase of development and requires the polysaccharide adhesin PGA. *J Bacteriol* **187:** 8237–8246.

Allaker RP. 2010. The use of nanoparticles to control oral biofilm formation. *J Dent Res* **89:** 1175–1186.

Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. 2003. Intracellular bacterial biofilm-like pods in urinary tract infections. *Science* **301:** 105–107.

Anderson GG, Martin SM, Hultgren SJ. 2004. Host subversion by formation of intracellular bacterial communities in the urinary tract. *Microbes Infect* **6:** 1094–1101.

Allesen-Holm M, Barken KB, Yang L, Klausen M, Webb JS, Kjelleberg S, Molin S, Givskov M, Tolker-Nielsen T. 2006. A characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms. *Mol Microbiol* **59:** 1114–1128.

Anderson GG, Moreau-Marquis S, Stanton BA, O'Toole GA. 2008. In vitro analysis of tobramycin-treated *Pseudomonas aeruginosa* biofilms on cystic fibrosis-derived airway epithelial cells. *Infect Immun* **76:** 1423–1433.

Anderson GG, Goller CC, Justice S, Hultgren SJ, Seed PC. 2010. Polysaccharide capsule and sialic acid-mediated regulation promote biofilm-like intracellular bacterial communities during cystitis. *Infect Immun* **78:** 963–975.

Bagge N, Hentzer M, Andersen JB, Ciofu O, Givskov M, Hoiby N. 2004. Dynamics and spatial distribution of β-lactamase expression in *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother* **48:** 1168–1174.

Banin E, Vasil ML, Greenberg EP. 2005. Iron and *Pseudomonas aeruginosa* biofilm formation. *Proc Natl Acad Sci* **102:** 11076–11081.

Barken KB, Pamp SJ, Yang L, Gjermansen M, Bertrand JJ, Klausen M, Givskov M, Whitchurch CB, Engel JN, Tolker-Nielsen T. 2008. Roles of type IV pili, flagellum-mediated motility and extracellular DNA in the formation of mature multicellular structures in *Pseudomonas aeruginosa* biofilms. *Environ Microbiol* **10:** 2331–2343.

Barraud N, Schleheck D, Klebensberger J, Webb JS, Hassett DJ, Rice SA, Kjelleberg S. 2009. Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic di-GMP levels, and enhanced dispersal. *J Bacteriol* **191:** 7333–7342.

Bearson BL, Bearson SM. 2008. The role of the QseC quorum-sensing sensor kinase in colonization and norepinephrine-enhanced motility of *Salmonella enterica* serovar *Typhimurium*. *Microb Pathog* **44:** 271–278.

Beloin C, Roux A, Ghigo JM. 2008. *Escherichia coli* biofilms. *Curr Top Microbiol Immunol* **322:** 249–289.

Beloin C, Valle J, Latour-Lambert P, Faure P, Kzreminski M, Balestrino D, Haagensen JA, Molin S, Prensier G, Arbeille B, et al. 2004. Global impact of mature biofilm lifestyle on *Escherichia coli* K-12 gene expression. *Mol Microbiol* **51:** 659–674.

Berg V, Das P, Chorell E, Hedenstrom M, Pinkner JS, Hultgren SJ, Almqvist F. 2008. Carboxylic acid isosteres improve the activity of ring-fused 2-pyridones that inhibit pilus biogenesis in *E. coli*. *Bioorg Med Chem Lett* **18:** 3536–3540.

Bhomkar P, Materi W, Semenchenko V, Wishart DS. 2010. Transcriptional response of *E. coli* upon FimH-mediated fimbrial adhesion. *Gene Regul Syst Bio* **4:** 1–17.

Bishop BL, Duncan MJ, Song J, Li G, Zaas D, Abraham SN. 2007. Cyclic AMP-regulated exocytosis of *Escherichia coli* from infected bladder epithelial cells. *Nat Med* **13:** 625–630.

Blango MG, Mulvey MA. 2010. Persistence of uropathogenic *Escherichia coli* in the face of multiple antibiotics. *Antimicrob Agents Chemother* **54:** 1855–1863.

Bleyer AJ, Mason L, Russell G, Raad II, Sherertz RJ. 2005. A randomized, controlled trial of a new vascular catheter flush solution (minocycline-EDTA) in temporary hemodialysis access. *Infect Control Hosp Epidemiol* **26:** 520–524.

Bokranz W, Wang X, Tschape H, Romling U. 2005. Expression of cellulose and curli fimbriae by *Escherichia coli* isolated from the gastrointestinal tract. *J Med Microbiol* **54:** 1171–1182.

Boles BR, Thoendel M, Singh PK. 2005. Rhamnolipids mediate detachment of *Pseudomonas aeruginosa* from biofilms. *Mol Microbiol* **57:** 1210–1223.

Bookstaver PB, Williamson JC, Tucker BK, Raad II, Sherertz RJ. 2009. Activity of novel antibiotic lock solutions in a model against isolates of catheter-related bloodstream infections. *Ann Pharmacother* **43:** 210–219.

Borlee BR, Goldman AD, Murakami K, Samudrala R, Wozniak DJ, Parsek MR. 2010. *Pseudomonas aeruginosa* uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix. *Mol Microbiol* **75:** 827–842.

Bouckaert J, Berglund J, Schembri M, De Genst E, Cools L, Wuhrer M, Hung CS, Pinkner J, Slattegard R, Zavialov A, et al. 2005. Receptor binding studies disclose a novel class of high-affinity inhibitors of the *Escherichia coli* FimH adhesin. *Mol Microbiol* **55:** 441–455.

Boyd A, Chakrabarty AM. 1994. Role of alginate lyase in cell detachment of *Pseudomonas aeruginosa*. *Appl Environ Microbiol* **60:** 2355–2359.

Bragg PD, Rainnie DJ. 1974. The effect of silver ions on the respiratory chain of *Escherichia coli*. *Can J Microbiol* **20:** 883–889.

Branda SS, Chu F, Kearns DB, Losick R, Kolter R. 2006. A major protein component of the *Bacillus subtilis* biofilm matrix. *Mol Microbiol* **59:** 1229–1238.

Burrowes B, Harper DR, Anderson J, McConville M, Enright MC. 2011. Bacteriophage therapy: Potential uses in the control of antibiotic-resistant pathogens. *Expert Rev Anti Infect Ther* **9:** 775–785.

Burton E, Gawande PV, Yakandawala N, LoVetri K, Zhanel GG, Romeo T, Friesen AD, Madhyastha S. 2006. Antibiofilm activity of GlmU enzyme inhibitors against catheter-associated uropathogens. *Antimicrob Agents Chemother* **50:** 1835–1840.

Cava F, Lam H, de Pedro MA, Waldor MK. 2010. Emerging knowledge of regulatory roles of D-amino acids in bacteria. *Cell Mol Life Sci* **68:** 817–831.

Cegelski L, Marshall GR, Eldridge GR, Hultgren SJ. 2008. The biology and future prospects of antivirulence therapies. *Nat Rev Microbiol* **6:** 17–27.

Cegelski L, Pinkner JS, Hammer ND, Cusumano CK, Hung CS, Chorell E, Aberg V, Walker JN, Seed PC, Almqvist F, et al. 2009. Small-molecule inhibitors target *Escherichia coli* amyloid biogenesis and biofilm formation. *Nat Chem Biol* **5:** 913–919.

Cerca N, Jefferson KK, Oliveira R, Pier GB, Azeredo J. 2006. Comparative antibody-mediated phagocytosis of *Staphylococcus epidermidis* cells grown in a biofilm or in the planktonic state. *Infect Immun* **74:** 4849–4855.

Cerca N, Maira-Litran T, Jefferson KK, Grout M, Goldmann DA, Pier GB. 2007. Protection against *Escherichia coli* infection by antibody to the *Staphylococcus aureus* poly-*N*-acetylglucosamine surface polysaccharide. *Proc Natl Acad Sci* **104:** 7528–7533.

Chatzinikolaou I, Zipf TF, Hanna H, Umphrey J, Roberts WM, Sherertz R, Hachem R, Raad I. 2003. Minocycline-ethylenediaminetetraacetate lock solution for the prevention of implantable port infections in children with cancer. *Clin Infect Dis* **36:** 116–119.

Chen X, Schluesener HJ. 2008. Nanosilver: A nanoproduct in medical application. *Toxicol Lett* **176:** 1–12.

Chen SL, Hung CS, Xu J, Reigstad CS, Magrini V, Sabo A, Blasiar D, Bieri T, Meyer RR, Ozersky P, et al. 2006. Identification of genes subject to positive selection in uropathogenic strains of *Escherichia coli*: A comparative genomics approach. *Proc Natl Acad Sci* **103:** 5977–5982.

Chen SL, Hung CS, Pinkner JS, Walker JN, Cusumano CK, Li Z, Bouckaert J, Gordon JI, Hultgren SJ. 2009. Positive selection identifies an in vivo role for FimH during urinary tract infection in addition to mannose binding. *Proc Natl Acad Sci* **106:** 22439–22444.

Chorell E, Pinkner JS, Phan G, Edvinsson S, Buelens F, Remaut H, Waksman G, Hultgren SJ, Almqvist F. 2010. Design and synthesis of C-2 substituted thiazolo and dihydrothiazolo ring-fused 2-pyridones: Pilicides with increased antivirulence activity. *J Med Chem* **53:** 5690–5695.

Chorell E, Bengtsson C, Sainte-Luce Banchelin T, Das P, Uvell H, Sinha AK, Pinkner JS, Hultgren SJ, Almqvist F. 2011. Synthesis and application of a bromomethyl substituted scaffold to be used for efficient optimization of anti-virulence activity. *Eur J Med Chem* **46:** 1103–1116.

Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V. 2006. The QseC sensor kinase: A bacterial adrenergic receptor. *Proc Natl Acad Sci* **103:** 10420–10425.

Connell I, Agace W, Klemm P, Schembri M, Marild S, Svanborg C. 1996. Type 1 fimbrial expression enhances *Escherichia coli* virulence for the urinary tract. *Proc Natl Acad Sci* **93:** 9827–9832.

Conrad A, Suutari MK, Keinanen MM, Cadoret A, Faure P, Mansuy-Huault L, Block JC. 2003. Fatty acids of lipid fractions in extracellular polymeric substances of activated sludge flocs. *Lipids* **38:** 1093–1105.

Cookson AL, Cooley WA, Woodward MJ. 2002. The role of type 1 and curli fimbriae of Shiga toxin-producing *Escherichia coli* in adherence to abiotic surfaces. *Int J Med Microbiol* **292:** 195–205.

Costerton JW, Montanaro L, Arciola CR. 2007. Bacterial communications in implant infections: A target for an intelligence war. *Int J Artif Organs* **30:** 757–763.

Cusumano CK, Pinkner J, Han Z, Greene SE, Ford B, Crowley JR, Henderson JP, Janetka JW, Hultgren S. 2011. Treatment and prevention of UTI with orally active mannoside FimH inhibitors. *Sci Transl Med* **3:** 109ra115.

Danese PN, Pratt LA, Dove SL, Kolter R. 2000a. The outer membrane protein, antigen 43, mediates cell-to-cell interactions within *Escherichia coli* biofilms. *Mol Microbiol* **37:** 424–432.

Danese PN, Pratt LA, Kolter R. 2000b. Exopolysaccharide production is required for development of *Escherichia coli* K-12 biofilm architecture. *J Bacteriol* **182:** 3593–3596.

Dethlefsen L, Relman DA. 2010. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci* **108** (Suppl 1)**:** 4554–4561.

Dethlefsen L, Huse S, Sogin ML, Relman DA. 2008. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. *PLoS Biol* **6:** e280.

Diggle SP, Stacey RE, Dodd C, Camara M, Williams P, Winzer K. 2006. The galactophilic lectin, LecA, contributes to biofilm development in *Pseudomonas aeruginosa*. *Environ Microbiol* **8:** 1095–1104.

Domka J, Lee J, Bansal T, Wood TK. 2007. Temporal gene-expression in *Escherichia coli* K-12 biofilms. *Environ Microbiol* **9:** 332–346.

Dong YH, Zhang XF, An SW, Xu JL, Zhang LH. 2008. A novel two-component system BqsS-BqsR modulates quorum sensing-dependent biofilm decay in *Pseudomonas aeruginosa*. *Commun Integr Biol* **1:** 88–96.

Donlan RM. 2002. Biofilms: Microbial life on surfaces. *Emerg Infect Dis* **8:** 881–890.

Donlan RM. 2009. Preventing biofilms of clinically relevant organisms using bacteriophage. *Trends Microbiol* **17:** 66–72.

Donlan RM. 2011. Biofilm elimination on intravascular catheters: Important considerations for the infectious disease practitioner. *Clin Infect Dis* **52:** 1038–1045.

Dunne WM Jr. 2002. Bacterial adhesion: Seen any good biofilms lately? *Clin Microbiol Rev* **15:** 155–166.

Eto DS, Jones TA, Sundsbak JL, Mulvey MA. 2007. Integrin-mediated host cell invasion by type 1-piliated uropathogenic *Escherichia coli*. *PLoS Pathog* **3:** e100.

Fader RC, Davis CP. 1980. Effect of piliation on *Klebsiella pneumoniae* infection in rat bladders. *Infect Immun* **30:** 554–561.

Feely T, Copley A, Bleyer AJ. 2007. Catheter lock solutions to prevent bloodstream infections in high-risk hemodialysis patients. *Am J Nephrol* **27:** 24–29.

Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO. 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J Biomed Mater Res* **52:** 662–668.

Ferrieres L, Hancock V, Klemm P. 2007. Specific selection for virulent urinary tract infectious *Escherichia coli* strains during catheter-associated biofilm formation. *FEMS Immunol Med Microbiol* **51:** 212–219.

Fey PD. 2010. Modality of bacterial growth presents unique targets: How do we treat biofilm-mediated infections? *Curr Opin Microbiol* **13:** 610–615.

Flemming HC, Wingender J. 2010. The biofilm matrix. *Nat Rev Microbiol* **8:** 623–633.

Foxman B. 2010. The epidemiology of urinary tract infection. *Nat Rev Urol* **7:** 653–660.

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

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

Garcia-Medina R, Dunne WM, Singh PK, Brody SL. 2005. *Pseudomonas aeruginosa* acquires biofilm-like properties within airway epithelial cells. *Infect Immun* **73:** 8298–8305.

Garofalo CK, Hooton TM, Martin SM, Stamm WE, Palermo JJ, Gordon JI, Hultgren SJ. 2007. *Escherichia coli* from urine of female patients with urinary tract infections is competent for intracellular bacterial community formation. *Infect Immun* **75:** 52–60.

Gjermansen M, Ragas P, Sternberg C, Molin S, Tolker-Nielsen T. 2005. Characterization of starvation-induced dispersion in *Pseudomonas putida* biofilms. *Environ Microbiol* **7:** 894–906.

Gotoh Y, Eguchi Y, Watanabe T, Okamoto S, Doi A, Utsumi R. 2010. Two-component signal transduction as potential drug targets in pathogenic bacteria. *Curr Opin Microbiol* **13:** 232–239.

Govan JR, Deretic V. 1996. Microbial pathogenesis in cystic fibrosis: Mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev* **60:** 539–574.

Guiton PS, Hung CS, Kline KA, Roth R, Kau AL, Hayes E, Heuser J, Dodson KW, Caparon MG, Hultgren SJ. 2009. Contribution of autolysin and Sortase A during *Enterococcus faecalis* DNA-dependent biofilm development. *Infect Immun* **77:** 3626–3638.

Hadjifrangiskou M, Kostakioti M, Chen SL, Henderson JP, Greene SE, Hultgren SJ. 2011. A central metabolic circuit controlled by QseC in pathogenic *Escherichia coli*. *Mol Microbiol* **80:** 1516–1529.

Hadjifrangiskou M, Gu AP, Pinkner JS, Kostakioti M, Zhang EW, Greene SE, Hultgren SJ. 2012. Transposon mutagenesis identifies uropathogenic *Escherichia coli* biofilm factors. *J Bacteriol* **194:** 6195–6205.

Hammer BK, Bassler BL. 2003. Quorum sensing controls biofilm formation in *Vibrio cholerae*. *Mol Microbiol* **50:** 101–104.

Han Z, Pinkner JS, Ford B, Obermann R, Nolan W, Wildman SA, Hobbs D, Ellenberger T, Cusumano CK, Hultgren SJ, et al. 2010. Structure-based drug design and optimization of mannoside bacterial FimH antagonists. *J Med Chem* **53:** 4779–4792.

Hannan TJ, Mysorekar IU, Hung CS, Isaacson-Schmid ML, Hultgren SJ. 2010. Early severe inflammatory responses to uropathogenic *E. coli* predispose to chronic and recurrent urinary tract infection. *PLoS Pathog* **6:** e1001042.

Hansen DS, Gottschau A, Kolmos HJ. 1998. Epidemiology of *Klebsiella bacteraemia*: A case control study using *Escherichia coli bacteraemia* as control. *J Hosp Infect* **38:** 119–132.

Harmsen M, Yang L, Pamp SJ, Tolker-Nielsen T. 2010. An update on *Pseudomonas aeruginosa* biofilm formation, tolerance, and dispersal. *FEMS Immunol Med Microbiol* **59:** 253–268.

Hasman H, Chakraborty T, Klemm P. 1999. Antigen-43-mediated autoaggregation of *Escherichia coli* is blocked by fimbriation. *J Bacteriol* **181:** 4834–4841.

Hatch RA, Schiller NL. 1998. Alginate lyase promotes diffusion of aminoglycosides through the extracellular polysaccharide of mucoid *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* **42:** 974–977.

Hay ID, Remminghorst U, Rehm BH. 2009. MucR, a novel membrane-associated regulator of alginate biosynthesis in *Pseudomonas aeruginosa*. *Appl Environ Microbiol* **75:** 1110–1120.

Henderson IR, Meehan M, Owen P. 1997. Antigen 43, a phase-variable bipartite outer membrane protein, determines colony morphology and autoaggregation in *Escherichia coli* K-12. *FEMS Microbiol Lett* **149:** 115–120.

Henderson JP, Crowley JR, Pinkner JS, Walker JN, Tsukayama P, Stamm WE, Hooton TM, Hultgren SJ. 2009. Quantitative metabolomics reveals an epigenetic blueprint for iron acquisition in uropathogenic *Escherichia coli*. *PLoS Pathog* **5:** e1000305.

Hentzer M, Teitzel GM, Balzer GJ, Heydorn A, Molin S, Givskov M, Parsek MR. 2001. Alginate overproduction affects *Pseudomonas aeruginosa* biofilm structure and function. *J Bacteriol* **183:** 5395–5401.

Hochbaum AI, Kolodkin-Gal I, Foulston L, Kolter R, Aizenberg J, Losick R. 2011. Inhibitory effects of D-amino acids on *Staphylococcus aureus* biofilm development. *J Bacteriol* **193:** 5616–5622.

Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. 2010. Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents* **35:** 322–332.

Hong SH, Lee J, Wood TK. 2010. Engineering global regulator Hha of *Escherichia coli* to control biofilm dispersal. *Microb Biotechnol* **3:** 717–728.

Horvath DJ Jr, Li B, Casper T, Partida-Sanchez S, Hunstad DA, Hultgren SJ, Justice SS. 2010. Morphological plasticity promotes resistance to phagocyte killing of uropathogenic *Escherichia coli*. *Microbes Infect* **13:** 426–437.

Hughes KA, Sutherland IW, Clark J, Jones MV. 1998a. Bacteriophage and associated polysaccharide depolymerases—Novel tools for study of bacterial biofilms. *J Appl Microbiol* **85:** 583–590.

Hughes KA, Sutherland IW, Jones MV. 1998b. Biofilm susceptibility to bacteriophage attack: The role of phageborne polysaccharide depolymerase. *Microbiology* **144 (Pt 11):** 3039–3047.

Hung CS, Bouckaert J, Hung D, Pinkner J, Widberg C, DeFusco A, Auguste CG, Strouse R, Langermann S, Waksman G, et al. 2002. Structural basis of tropism of *Escherichia coli* to the bladder during urinary tract infection. *Mol Microbiol* **44:** 903–915.

Inagaki S, Kuramitsu HK, Sharma A. 2005. Contact-dependent regulation of a *Tannerella forsythia* virulence factor, BspA, in biofilms. *FEMS Microbiol Lett* **249:** 291–296.

Izano EA, Amarante MA, Kher WB, Kaplan JB. 2008. Differential roles of poly-N-acetylglucosamine surface polysaccharide and extracellular DNA in *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. *Appl Environ Microbiol* **74:** 470–476.

Jackson KD, Starkey M, Kremer S, Parsek MR, Wozniak DJ. 2004. Identification of *psl*, a locus encoding a potential exopolysaccharide that is essential for *Pseudomonas aeruginosa* PAO1 biofilm formation. *J Bacteriol* **186:** 4466–4475.

Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME. 2008. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clin Microbiol Rev* **21:** 26–59.

Jefferson KK, Goldmann DA, Pier GB. 2005. Use of confocal microscopy to analyze the rate of vancomycin penetration through *Staphylococcus aureus* biofilms. *Antimicrob Agents Chemother* **49:** 2467–2473.

Jesaitis AJ, Franklin MJ, Berglund D, Sasaki M, Lord CI, Bleazard JB, Duffy JE, Beyenal H, Lewandowski Z. 2003. Compromised host defense on *Pseudomonas aeruginosa* biofilms: Characterization of neutrophil and biofilm interactions. *J Immunol* **171:** 4329–4339.

Justice SS, Hung C, Theriot JA, Fletcher DA, Anderson GG, Footer MJ, Hultgren SJ. 2004. Differentiation and developmental pathways of uropathogenic *Escherichia coli* in urinary tract pathogenesis. *Proc Natl Acad Sci* **101:** 1333–1338.

Justice SS, Hunstad DA, Seed PC, Hultgren SJ. 2006. Filamentation by *Escherichia coli* subverts innate defenses during urinary tract infection. *Proc Natl Acad Sci* **103:** 19884–19889.

Justice SS, Hunstad DA, Cegelski L, Hultgren SJ. 2008. Morphological plasticity as a bacterial survival strategy. *Nat Rev Microbiol* **6:** 162–168.

Kai-Larsen Y, Luthje P, Chromek M, Peters V, Wang X, Holm A, Kadas L, Hedlund KO, Johansson J, Chapman MR, et al. 2010. Uropathogenic *Escherichia coli* modulates immune responses and its curli fimbriae interact with the antimicrobial peptide LL-37. *PLoS Pathog* **6:** e1001010.

Kalishwaralal K, BarathManiKanth S, Pandian SR, Deepak V, Gurunathan S. 2010. Silver nanoparticles impede the biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. *Colloids Surf B Biointerfaces* **79:** 340–344.

Kaplan JB. 2010. Biofilm dispersal: Mechanisms, clinical implications, and potential therapeutic uses. *J Dent Res* **89:** 205–218.

Karatan E, Watnick P. 2009. Signals, regulatory networks, and materials that build and break bacterial biofilms. *Microbiol Mol Biol Rev* **73:** 310–347.

Kau AL, Hunstad DA, Hultgren SJ. 2005. Interaction of uropathogenic *Escherichia coli* with host uroepithelium. *Curr Opin Microbiol* **8:** 54–59.

Kemp KD, Singh KV, Nallapareddy SR, Murray BE. 2007. Relative contributions of *Enterococcus faecalis* OG1RF sortase-encoding genes, srtA and bps (srtC), to biofilm formation and a murine model of urinary tract infection. *Infect Immun* **75:** 5399–5404.

Khajanchi BK, Kozlova EV, Sha J, Popov VL, Chopra AK. 2011. Two-component QseBC signaling system regulates in vitro and in vivo virulence of *Aeromonas hydrophila*. *Microbiology* **158:** 259–271.

Kharidia R, Liang JF. 2011. The activity of a small lytic peptide PTP-7 on *Staphylococcus aureus* biofilms. *J Microbiol* **49:** 663–668.

Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, et al. 2007. Antimicrobial effects of silver nanoparticles. *Nanomedicine* **3:** 95–101.

Kite P, Eastwood K, Sugden S, Percival SL. 2004. Use of in vivo-generated biofilms from hemodialysis catheters to test the efficacy of a novel antimicrobial catheter lock for biofilm eradication in vitro. *J Clin Microbiol* **42:** 3073–3076.

Kjaergaard K, Schembri MA, Ramos C, Molin S, Klemm P. 2000. Antigen 43 facilitates formation of multispecies biofilms. *Environ Microbiol* **2:** 695–702.

Klausen M, Aaes-Jorgensen A, Molin S, Tolker-Nielsen T. 2003a. Involvement of bacterial migration in the development of complex multicellular structures in *Pseudomonas aeruginosa* biofilms. *Mol Microbiol* **50:** 61–68.

Klausen M, Heydorn A, Ragas P, Lambertsen L, Aaes-Jorgensen A, Molin S, Tolker-Nielsen T. 2003b. Biofilm formation by *Pseudomonas aeruginosa* wild type, flagella and type IV pili mutants. *Mol Microbiol* **48:** 1511–1524.

Klebensberger J, Birkenmaier A, Geffers R, Kjelleberg S, Philipp B. 2009. SiaA and SiaD are essential for inducing autoaggregation as a specific response to detergent stress in *Pseudomonas aeruginosa*. *Environ Microbiol* **11:** 3073–3086.

Klein T, Abgottspon D, Wittwer M, Rabbani S, Herold J, Jiang X, Kleeb S, Luthi C, Scharenberg M, Bezencon J, et al. 2010. FimH antagonists for the oral treatment of urinary tract infections: From design and synthesis to in vitro and in vivo evaluation. *J Med Chem* **53:** 8627–8641.

Kline KA, Dodson KW, Caparon MG, Hultgren SJ. 2010. A tale of two pili: Assembly and function of pili in bacteria. *Trends Microbiol* **18:** 224–232.

Koch C, Hoiby N. 1993. Pathogenesis of cystic fibrosis. *Lancet* **341:** 1065–1069.

Kolodkin-Gal I, Romero D, Cao S, Clardy J, Kolter R, Losick R. 2010. D-amino acids trigger biofilm disassembly. *Science* **328:** 627–629.

Kolodkin-Gal I, Cao S, Chai L, Bottcher T, Kolter R, Clardy J, Losick R. 2012. A self-produced trigger for biofilm disassembly that targets exopolysaccharide. *Cell* **149:** 684–692.

Koo H, Xiao J, Klein MI, Jeon JG. 2010. Exopolysaccharides produced by *Streptococcus mutans* glucosyltransferases modulate the establishment of microcolonies within multispecies biofilms. *J Bacteriol* **192:** 3024–3032.

Kostakioti M, Hadjifrangiskou M, Pinkner JS, Hultgren SJ. 2009. QseC-mediated dephosphorylation of QseB is required for expression of genes associated with virulence in uropathogenic *Escherichia coli*. *Mol Microbiol* **73:** 1020–1031.

Kuramitsu HK, Wang BY. 2011. The whole is greater than the sum of its parts: Dental plaque bacterial interactions can affect the virulence properties of cariogenic *Streptococcus mutans*. *Am J Dent* **24:** 153–154.

Lam H, Oh DC, Cava F, Takacs CN, Clardy J, de Pedro MA, Waldor MK. 2009. D-amino acids govern stationary phase cell wall remodeling in bacteria. *Science* **325:** 1552–1555.

Leid JG, Willson CJ, Shirtliff ME, Hassett DJ, Parsek MR, Jeffers AK. 2005. The exopolysaccharide alginate protects *Pseudomonas aeruginosa* biofilm bacteria from IFN-γ-mediated macrophage killing. *J Immunol* **175:** 7512–7518.

Lemon KP, Higgins DE, Kolter R. 2007. Flagellar motility is critical for *Listeria monocytogenes* biofilm formation. *J Bacteriol* **189:** 4418–4424.

Lenz AP, Williamson KS, Pitts B, Stewart PS, Franklin MJ. 2008. Localized gene expression in *Pseudomonas aeruginosa* biofilms. *Appl Environ Microbiol* **74:** 4463–4471.

Lewis K. 2005. Persister cells and the riddle of biofilm survival. *Biochemistry (Mosc)* **70:** 267–274.

Lewis K. 2008. Multidrug tolerance of biofilms and persister cells. *Curr Top Microbiol Immunol* **322:** 107–131.

Lu TK, Collins JJ. 2007. Dispersing biofilms with engineered enzymatic bacteriophage. *Proc Natl Acad Sci* **104:** 11197–11202.

Lye WC, Chan RK, Lee EJ, Kumarasinghe G. 1992. Urinary tract infections in patients with diabetes mellitus. *J Infect* **24:** 169–174.

Lyon GJ, Mayville P, Muir TW, Novick RP. 2000. Rational design of a global inhibitor of the virulence response in *Staphylococcus aureus*, based in part on localization of the site of inhibition to the receptor-histidine kinase, AgrC. *Proc Natl Acad Sci* **97:** 13330–13335.

Ma L, Jackson KD, Landry RM, Parsek MR, Wozniak DJ. 2006. Analysis of *Pseudomonas aeruginosa* conditional psl variants reveals roles for the psl polysaccharide in adhesion and maintaining biofilm structure postattachment. *J Bacteriol* **188:** 8213–8221.

Ma L, Conover M, Lu H, Parsek MR, Bayles K, Wozniak DJ. 2009. Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix. *PLoS Pathog* **5:** e1000354.

Ma Q, Guishan Z, Wood TK. 2011a. *Escherichia coli* BdcA controls biofilm dispersal in *Pseudomonas aeruginosa* and *Rhizobium meliloti*. *BMC Res Notes* **4:** 447.

Ma Q, Yang Z, Pu M, Peti W, Wood TK. 2011b. Engineering a novel c-di-GMP-binding protein for biofilm dispersal. *Environ Microbiol* **13:** 631–642.

Mack D, Becker P, Chatterjee I, Dobinsky S, Knobloch JK, Peters G, Rohde H, Herrmann M. 2004. Mechanisms of biofilm formation in *Staphylococcus epidermidis* and *Staphylococcus aureus*: Functional molecules, regulatory circuits, and adaptive responses. *Int J Med Microbiol* **294:** 203–212.

Mai-Prochnow A, Lucas-Elio P, Egan S, Thomas T, Webb JS, Sanchez-Amat A, Kjelleberg S. 2008. Hydrogen peroxide linked to lysine oxidase activity facilitates biofilm differentiation and dispersal in several gram-negative bacteria. *J Bacteriol* **190:** 5493–5501.

Mann EE, Rice KC, Boles BR, Endres JL, Ranjit D, Chandramohan L, Tsang LH, Smeltzer MS, Horswill AR, Bayles KW. 2009. Modulation of eDNA release and degradation affects *Staphylococcus aureus* biofilm maturation. *PLoS ONE* **4:** e5822.

Martinez JJ, Mulvey MA, Schilling JD, Pinkner JS, Hultgren SJ. 2000. Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. *Embo J* **19:** 2803–2812.

Matsukawa M, Greenberg EP. 2004. Putative exopolysaccharide synthesis genes influence *Pseudomonas aeruginosa* biofilm development. *J Bacteriol* **186:** 4449–4456.

Mohamed JA, Huang W, Nallapareddy SR, Teng F, Murray BE. 2004. Influence of origin of isolates, especially endocarditis isolates, and various genes on biofilm formation by *Enterococcus faecalis*. *Infect Immun* **72:** 3658–3663.

Mohamed JA, Teng F, Nallapareddy SR, Murray BE. 2006. Pleiotrophic effects of 2 *Enterococcus faecalis* sagA-like genes, *salA* and *salB*, which encode proteins that are antigenic during human infection, on biofilm formation and binding to collagen type I and fibronectin. *J Infect Dis* **193:** 231–240.

Monds RD, O'Toole GA. 2009. The developmental model of microbial biofilms: Ten years of a paradigm up for review. *Trends Microbiol* **17:** 73–87.

Morgan R, Kohn S, Hwang SH, Hassett DJ, Sauer K. 2006. BdlA, a chemotaxis regulator essential for biofilm dispersion in *Pseudomonas aeruginosa*. *J Bacteriol* **188:** 7335–7343.

Morici LA, Carterson AJ, Wagner VE, Frisk A, Schurr JR, Honer zu Bentrup K, Hassett DJ, Iglewski BH, Sauer K, Schurr MJ. 2007. *Pseudomonas aeruginosa* AlgR represses the Rhl quorum-sensing system in a biofilm-specific manner. *J Bacteriol* **189:** 7752–7764.

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ. 2005. The bactericidal effect of silver nanoparticles. *Nanotechnology* **16:** 2346–2353.

Mulvey MA, Lopez-Boado YS, Wilson CL, Roth R, Parks WC, Heuser J, Hultgren SJ. 1998. Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. *Science* **282:** 1494–1497.

Nakayama J, Tanaka E, Kariyama R, Nagata K, Nishiguchi K, Mitsuhata R, Uemura Y, Tanokura M, Kumon H, Sonomoto K. 2007. Siamycin attenuates fsr quorum sensing mediated by a gelatinase biosynthesis-activating

pheromone in *Enterococcus faecalis*. *J Bacteriol* **189:** 1358–1365.

Nallapareddy SR, Singh KV, Sillanpaa J, Garsin DA, Hook M, Erlandsen SL, Murray BE. 2006. Endocarditis and biofilm-associated pili of *Enterococcus faecalis*. *J Clin Invest* **116:** 2799–2807.

Nilsson LM, Yakovenko O, Tchesnokova V, Thomas WE, Schembri MA, Vogel V, Klemm P, Sokurenko EV. 2007. The cysteine bond in the *Escherichia coli* FimH adhesin is critical for adhesion under flow conditions. *Mol Microbiol* **65:** 1158–1169.

Njoroge J, Sperandio V. 2009. Jamming bacterial communication: New approaches for the treatment of infectious diseases. *EMBO Mol Med* **1:** 201–210.

Okada A, Gotoh Y, Watanabe T, Furuta E, Yamamoto K, Utsumi R. 2007. Targeting two-component signal transduction: A novel drug discovery system. *Methods Enzymol* **422:** 386–395.

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

O'Toole G, Kaplan HB, Kolter R. 2000. Biofilm formation as microbial development. *Annu Rev Microbiol* **54:** 49–79.

Otto K, Silhavy TJ. 2002. Surface sensing and adhesion of *Escherichia coli* controlled by the Cpx-signaling pathway. *Proc Natl Acad Sci* **99:** 2287–2292.

Palchevskiy V, Finkel SE. 2006. *Escherichia coli* competence gene homologs are essential for competitive fitness and the use of DNA as a nutrient. *J Bacteriol* **188:** 3902–3910.

Parsek MR, Singh PK. 2003. Bacterial biofilms: An emerging link to disease pathogenesis. *Annu Rev Microbiol* **57:** 677–701.

Patel RB, Welling PG. 1980. Clinical pharmacokinetics of co-trimoxazole (trimethoprim-sulphamethoxazole). *Clin Pharmacokinet* **5:** 405–423.

Patrauchan MA, Sarkisova S, Sauer K, Franklin MJ. 2005. Calcium influences cellular and extracellular product formation during biofilm-associated growth of a marine *Pseudoalteromonas sp. Microbiology* **151:** 2885–2897.

Percival SL, Kite P, Eastwood K, Murga R, Carr J, Arduino MJ, Donlan RM. 2005. Tetrasodium EDTA as a novel central venous catheter lock solution against biofilm. *Infect Control Hosp Epidemiol* **26:** 515–519.

Pihl M, Davies JR, Chavez de Paz LE, Svensater G. 2010. Differential effects of *Pseudomonas aeruginosa* on biofilm formation by different strains of *Staphylococcus epidermidis*. *FEMS Immunol Med Microbiol* **59:** 439–446.

Pinkner JS, Remaut H, Buelens F, Miller E, Aberg V, Pemberton N, Hedenstrom M, Larsson A, Seed P, Waksman G, et al. 2006. Rationally designed small compounds inhibit pilus biogenesis in uropathogenic bacteria. *Proc Natl Acad Sci* **103:** 17897–17902.

Pompilio A, Scocchi M, Pomponio S, Guida F, Di Primio A, Fiscarelli E, Gennaro R, Di Bonaventura G. 2011. Antibacterial and anti-biofilm effects of cathelicidin peptides against pathogens isolated from cystic fibrosis patients. *Peptides* **32:** 1807–1814.

Pratt LA, Kolter R. 1998. Genetic analysis of *Escherichia coli* biofilm formation: Roles of flagella, motility, chemotaxis and type I pili. *Mol Microbiol* **30:** 285–293.

Prigent-Combaret C, Lejeune P. 1999. Monitoring gene expression in biofilms. *Methods Enzymol* **310:** 56–79.

Prigent-Combaret C, Brombacher E, Vidal O, Ambert A, Lejeune P, Landini P, Dorel C. 2001. Complex regulatory network controls initial adhesion and biofilm formation in *Escherichia coli* via regulation of the csgD gene. *J Bacteriol* **183:** 7213–7223.

Pruss BM, Besemann C, Denton A, Wolfe AJ. 2006. A complex transcription network controls the early stages of biofilm development by *Escherichia coli*. *J Bacteriol* **188:** 3731–3739.

Purevdorj-Gage B, Costerton WJ, Stoodley P. 2005. Phenotypic differentiation and seeding dispersal in non-mucoid and mucoid *Pseudomonas aeruginosa* biofilms. *Microbiology* **151:** 1569–1576.

Qin X, Singh KV, Weinstock GM, Murray BE. 2000. Effects of *Enterococcus faecalis fsr* genes on production of gelatinase and a serine protease and virulence. *Infect Immun* **68:** 2579–2586.

Qin Z, Ou Y, Yang L, Zhu Y, Tolker-Nielsen T, Molin S, Qu D. 2007. Role of autolysin-mediated DNA release in biofilm formation of *Staphylococcus epidermidis*. *Microbiology* **153:** 2083–2092.

Qin Z, Yang L, Qu D, Molin S, Tolker-Nielsen T. 2009. *Pseudomonas aeruginosa* extracellular products inhibit staphylococcal growth, and disrupt established biofilms produced by *Staphylococcus epidermidis*. *Microbiology* **155:** 2148–2156.

Raad I, Buzaid A, Rhyne J, Hachem R, Darouiche R, Safar H, Albitar M, Sherertz RJ. 1997. Minocycline and ethylenediaminetetraacetate for the prevention of recurrent vascular catheter infections. *Clin Infect Dis* **25:** 149–151.

Raad I, Chatzinikolaou I, Chaiban G, Hanna H, Hachem R, Dvorak T, Cook G, Costerton W. 2003. In vitro and ex vivo activities of minocycline and EDTA against microorganisms embedded in biofilm on catheter surfaces. *Antimicrob Agents Chemother* **47:** 3580–3585.

Raad II, Fang X, Keutgen XM, Jiang Y, Sherertz R, Hachem R. 2008. The role of chelators in preventing biofilm formation and catheter-related bloodstream infections. *Curr Opin Infect Dis* **21:** 385–392.

Rasko DA, Moreira CG, Li de R, Reading NC, Ritchie JM, Waldor MK, Williams N, Taussig R, Wei S, Roth M, et al. 2008. Targeting QseC signaling and virulence for antibiotic development. *Science* **321:** 1078–1080.

Reigstad CS, Hultgren SJ, Gordon JI. 2007. Functional genomic studies of uropathogenic *Escherichia coli* and host urothelial cells when intracellular bacterial communities are assembled. *J Biol Chem* **282:** 21259–21267.

Rendueles O, Kaplan JB, Ghigo JM. 2012. Antibiofilm polysaccharides. *Environ Microbiol* doi: 10.1111/j.1462-2920.2012.02810.x.

Romling U. 2002. Molecular biology of cellulose production in bacteria. *Res Microbiol* **153:** 205–212.

Rosen DA, Hooton TM, Stamm WE, Humphrey PA, Hultgren SJ. 2007. Detection of intracellular bacterial communities in human urinary tract infection. *PLoS Med* **4:** e329.

Rosen DA, Pinkner JS, Jones JM, Walker JN, Clegg S, Hultgren SJ. 2008a. Utilization of an intracellular bacterial community pathway in *Klebsiella pneumoniae*

urinary tract infection and the effects of FimK on type 1 pilus expression. *Infect Immun* **76:** 3337–3345.

Rosen DA, Pinkner JS, Walker JN, Elam JS, Jones JM, Hultgren SJ. 2008b. Molecular variations in *Klebsiella pneumoniae* and *Escherichia coli* FimH affect function and pathogenesis in the urinary tract. *Infect Immun* **76:** 3346–3356.

Rowe MC, Withers HL, Swift S. 2010. Uropathogenic *Escherichia coli* forms biofilm aggregates under iron restriction that disperse upon the supply of iron. *FEMS Microbiol Lett* **307:** 102–109.

Rupp ME, Lisco SJ, Lipsett PA, Perl TM, Keating K, Civetta JM, Mermel LA, Lee D, Dellinger EP, Donahoe M, et al. 2005. Effect of a second-generation venous catheter impregnated with chlorhexidine and silver sulfadiazine on central catheter-related infections: A randomized, controlled trial. *Ann Intern Med* **143:** 570–580.

Sauer K, Cullen MC, Rickard AH, Zeef LA, Davies DG, Gilbert P. 2004. Characterization of nutrient-induced dispersion in *Pseudomonas aeruginosa* PAO1 biofilm. *J Bacteriol* **186:** 7312–7326.

Schaeffer AJ, Amundsen SK, Jones JM. 1980. Effect of carbohydrates on adherence of *Escherichica coli* to human urinary tract epithelial cells. *Infect Immun* **30:** 531–537.

Schembri MA, Kjaergaard K, Klemm P. 2003. Global gene expression in *Escherichia coli* biofilms. *Mol Microbiol* **48:** 253–267.

Schmidt J, Musken M, Becker T, Magnowska Z, Bertinetti D, Moller S, Zimmermann B, Herberg FW, Jansch L, Haussler S. 2011. The *Pseudomonas aeruginosa* chemotaxis methyltransferase CheR1 impacts on bacterial surface sampling. *PLoS ONE* **6:** e18184.

Schwartz DJ, Chen SL, Hultgren SJ, Seed PC. 2011. Population dynamics and niche distribution of uropathogenic *Escherichia coli* during acute and chronic urinary tract infection. *Infect Immun* **79:** 4250–4259.

Secinti KD, Ozalp H, Attar A, Sargon MF. 2011. Nanoparticle silver ion coatings inhibit biofilm formation on titanium implants. *J Clin Neurosci* **18:** 391–395.

Shak S, Capon DJ, Hellmiss R, Marsters SA, Baker CL. 1990. Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum. *Proc Natl Acad Sci* **87:** 9188–9192.

Shanks RM, Sargent JL, Martinez RM, Graber ML, O'Toole GA. 2006. Catheter lock solutions influence staphylococcal biofilm formation on abiotic surfaces. *Nephrol Dial Transplant* **21:** 2247–2255.

Snyder JA, Lloyd AL, Lockatell CV, Johnson DE, Mobley HL. 2006. Role of phase variation of type 1 fimbriae in a uropathogenic *Escherichia coli* cystitis isolate during urinary tract infection. *Infect Immun* **74:** 1387–1393.

Sokurenko EV, Courtney HS, Ohman DE, Klemm P, Hasty DL. 1994. FimH family of type 1 fimbrial adhesins: Functional heterogeneity due to minor sequence variations among fimH genes. *J Bacteriol* **176:** 748–755.

Sokurenko EV, Courtney HS, Maslow J, Siitonen A, Hasty DL. 1995. Quantitative differences in adhesiveness of type 1 fimbriated *Escherichia coli* due to structural differences in fimH genes. *J Bacteriol* **177:** 3680–3686.

Sokurenko EV, Chesnokova V, Dykhuizen DE, Ofek I, Wu XR, Krogfelt KA, Struve C, Schembri MA, Hasty DL. 1998. Pathogenic adaptation of *Escherichia coli* by natural variation of the FimH adhesin. *Proc Natl Acad Sci* **95:** 8922–8926.

Sokurenko EV, Feldgarden M, Trintchina E, Weissman SJ, Avagyan S, Chattopadhyay S, Johnson JR, Dykhuizen DE. 2004. Selection footprint in the FimH adhesin shows pathoadaptive niche differentiation in *Escherichia coli*. *Mol Biol Evol* **21:** 1373–1383.

Sperling O, Fuchs A, Lindhorst TK. 2006. Evaluation of the carbohydrate recognition domain of the bacterial adhesin FimH: Design, synthesis and binding properties of mannoside ligands. *Org Biomol Chem* **4:** 3913–3922.

Spurbeck RR, Stapleton AE, Johnson JR, Walk ST, Hooton TM, Mobley HL. 2011. Fimbrial profiles predict virulence of uropathogenic *E. coli* strains: Contribution of Ygi and Yad fimbriae. *Infect Immun* **79:** 4753–4763.

Stanley NR, Britton RA, Grossman AD, Lazazzera BA. 2003. Identification of catabolite repression as a physiological regulator of biofilm formation by *Bacillus subtilis* by use of DNA microarrays. *J Bacteriol* **185:** 1951–1957.

Starkey M, Hickman JH, Ma L, Zhang N, De Long S, Hinz A, Palacios S, Manoil C, Kirisits MJ, Starner TD, et al. 2009. *Pseudomonas aeruginosa* rugose small-colony variants have adaptations that likely promote persistence in the cystic fibrosis lung. *J Bacteriol* **191:** 3492–3503.

Sutherland IW, Hughes KA, Skillman LC, Tait K. 2004. The interaction of phage and biofilms. *FEMS Microbiol Lett* **232:** 1–6.

Thankavel K, Madison B, Ikeda T, Malaviya R, Shah AH, Arumugam PM, Abraham SN. 1997. Localization of a domain in the FimH adhesin of *Escherichia coli* type 1 fimbriae capable of receptor recognition and use of a domain-specific antibody to confer protection against experimental urinary tract infection. *J Clin Invest* **100:** 1123–1136.

Thomas VC, Thurlow LR, Boyle D, Hancock LE. 2008. Regulation of autolysis-dependent extracellular DNA release by *Enterococcus faecalis* extracellular proteases influences biofilm development. *J Bacteriol* **190:** 5690–5698.

Thomas VC, Hiromasa Y, Harms N, Thurlow L, Tomich J, Hancock LE. 2009. A fratricidal mechanism is responsible for eDNA release and contributes to biofilm development of *Enterococcus faecalis*. *Mol Microbiol* **72:** 1022–1036.

Thumbikat P, Berry RE, Zhou G, Billips BK, Yaggie RE, Zaichuk T, Sun TT, Schaeffer AJ, Klumpp DJ. 2009. Bacteria-induced uroplakin signaling mediates bladder response to infection. *PLoS Pathog* **5:** e1000415.

Tielker D, Hacker S, Loris R, Strathmann M, Wingender J, Wilhelm S, Rosenau F, Jaeger KE. 2005. *Pseudomonas aeruginosa* lectin LecB is located in the outer membrane and is involved in biofilm formation. *Microbiology* **151:** 1313–1323.

Toledo-Arana A, Valle J, Solano C, Arrizubieta MJ, Cucarella C, Lamata M, Amorena B, Leiva J, Penades JR, Lasa I. 2001. The enterococcal surface protein, Esp, is involved in *Enterococcus faecalis* biofilm formation. *Appl Environ Microbiol* **67:** 4538–4545.

Ton-That H, Schneewind O. 2003. Assembly of pili on the surface of *Corynebacterium diphtheriae*. *Mol Microbiol* **50:** 1429–1438.

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Ton-That H, Marraffini LA, Schneewind O. 2004. Sortases and pilin elements involved in pilus assembly of *Corynebacterium diphtheriae*. *Mol Microbiol* **53:** 251–261.

Toutain CM, Caizza NC, Zegans ME, O'Toole GA. 2007. Roles for flagellar stators in biofilm formation by *Pseudomonas aeruginosa*. *Res Microbiol* **158:** 471–477.

Ubeda C, Taur Y, Jenq RR, Equinda MJ, Son T, Samstein M, Viale A, Socci ND, van den Brink MR, Kamboj M, et al. 2010. Vancomycin-resistant *Enterococcus* domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *J Clin Invest* **120:** 4332–4341.

Uhlich GA, Cooke PH, Solomon EB. 2006. Analyses of the red-dry-rough phenotype of an *Escherichia coli* O157:H7 strain and its role in biofilm formation and resistance to antibacterial agents. *Appl Environ Microbiol* **72:** 2564–2572.

Ulett GC, Valle J, Beloin C, Sherlock O, Ghigo JM, Schembri MA. 2007. Functional analysis of antigen 43 in uropathogenic *Escherichia coli* reveals a role in long-term persistence in the urinary tract. *Infect Immun* **75:** 3233–3244.

Vallet I, Olson JW, Lory S, Lazdunski A, Filloux A. 2001. The chaperone/usher pathways of *Pseudomonas aeruginosa*: Identification of fimbrial gene clusters (cup) and their involvement in biofilm formation. *Proc Natl Acad Sci* **98:** 6911–6916.

Vasseur P, Vallet-Gely I, Soscia C, Genin S, Filloux A. 2005. The pel genes of the *Pseudomonas aeruginosa* PAK strain are involved at early and late stages of biofilm formation. *Microbiology* **151:** 985–997.

Venditti M, Biavasco F, Varaldo PE, Macchiarelli A, De Biase L, Marino B, Serra P. 1993. Catheter-related endocarditis due to glycopeptide-resistant *Enterococcus faecalis* in a transplanted heart. *Clin Infect Dis* **17:** 524–525.

Vidal O, Longin R, Prigent-Combaret C, Dorel C, Hooreman M, Lejeune P. 1998. Isolation of an *Escherichia coli* K-12 mutant strain able to form biofilms on inert surfaces: Involvement of a new ompR allele that increases curli expression. *J Bacteriol* **180:** 2442–2449.

Vilain S, Pretorius JM, Theron J, Brozel VS. 2009. DNA as an adhesin: *Bacillus cereus* requires extracellular DNA to form biofilms. *Appl Environ Microbiol* **75:** 2861–2868.

Vuong C, Voyich JM, Fischer ER, Braughton KR, Whitney AR, DeLeo FR, Otto M. 2004. Polysaccharide intercellular adhesin (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system. *Cell Microbiol* **6:** 269–275.

Waksman G, Hultgren SJ. 2009. Structural biology of the chaperone-usher pathway of pilus biogenesis. *Nat Rev Microbiol* **7:** 765–774.

Walters MC 3rd, Roe F, Bugnicourt A, Franklin MJ, Stewart PS. 2003. Contributions of antibiotic penetration, oxygen limitation, and low metabolic activity to tolerance of *Pseudomonas aeruginosa* biofilms to ciprofloxacin and tobramycin. *Antimicrob Agents Chemother* **47:** 317–323.

Wang X, Preston JF 3rd, Romeo T. 2004. The pgaABCD locus of *Escherichia coli* promotes the synthesis of a polysaccharide adhesin required for biofilm formation. *J Bacteriol* **186:** 2724–2734.

Wang X, Dubey AK, Suzuki K, Baker CS, Babitzke P, Romeo T. 2005. CsrA post-transcriptionally represses *pgaABCD*, responsible for synthesis of a biofilm polysaccharide adhesin of *Escherichia coli*. *Mol Microbiol* **56:** 1648–1663.

Wang X, Wang Q, Yang M, Xiao J, Liu Q, Wu H, Zhang Y. 2011. QseBC controls flagellar motility, fimbrial hemagglutination and intracellular virulence in fish pathogen *Edwardsiella tarda*. *Fish Shellfish Immunol* **30:** 944–953.

Watanabe T, Okada A, Gotoh Y, Utsumi R. 2008. Inhibitors targeting two-component signal transduction. *Adv Exp Med Biol* **631:** 229–236.

Watnick PI, Kolter R. 1999. Steps in the development of a *Vibrio cholerae* El Tor biofilm. *Mol Microbiol* **34:** 586–595.

Webb JS, Thompson LS, James S, Charlton T, Tolker-Nielsen T, Koch B, Givskov M, Kjelleberg S. 2003. Cell death in *Pseudomonas aeruginosa* biofilm development. *J Bacteriol* **185:** 4585–4592.

Weissman SJ, Beskhlebnaya V, Chesnokova V, Chattopadhyay S, Stamm WE, Hooton TM, Sokurenko EV. 2007. Differential stability and trade-off effects of pathoadaptive mutations in the *Escherichia coli* FimH adhesin. *Infect Immun* **75:** 3548–3555.

Welch RA, Burland V, Plunkett G 3rd, Redford P, Roesch P, Rasko D, Buckles EL, Liou SR, Boutin A, Hackett J, et al. 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *Proc Natl Acad Sci* **99:** 17020–17024.

Wellens A, Garofalo C, Nguyen H, Van Gerven N, Slattegard R, Hernalsteens JP, Wyns L, Oscarson S, De Greve H, Hultgren S, et al. 2008. Intervening with urinary tract infections using anti-adhesives based on the crystal structure of the FimH-oligomannose-3 complex. *PLoS ONE* **3:** e2040.

Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS. 2002. Extracellular DNA required for bacterial biofilm formation. *Science* **295:** 1487.

Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, Lory S, Greenberg EP. 2001. Gene expression in *Pseudomonas aeruginosa* biofilms. *Nature* **413:** 860–864.

Wood TK, Hong SH, Ma Q. 2010. Engineering biofilm formation and dispersal. *Trends Biotechnol* **29:** 87–94.

Wright KJ, Seed PC, Hultgren SJ. 2007. Development of intracellular bacterial communities of uropathogenic *Escherichia coli* depends on type 1 pili. *Cell Microbiol* **9:** 2230–2241.

Wu Y, Outten FW. 2009. IscR controls iron-dependent biofilm formation in *Escherichia coli* by regulating type I fimbria expression. *J Bacteriol* **191:** 1248–1257.

Yamanaka M, Hara K, Kudo J. 2005. Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. *Appl Environ Microbiol* **71:** 7589–7593.

Yang D, Biragyn A, Kwak LW, Oppenheim JJ. 2002. Mammalian defensins in immunity: More than just microbicidal. *Trends Immunol* **23:** 291–296.

Yang L, Barken KB, Skindersoe ME, Christensen AB, Givskov M, Tolker-Nielsen T. 2007. Effects of iron on

DNA release and biofilm development by *Pseudomonas aeruginosa*. *Microbiology* **153:** 1318–1328.

Zhang L, Mah TF. 2008. Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. *J Bacteriol* **190:** 4447–4452.

Zhou G, Mo WJ, Sebbel P, Min G, Neubert TA, Glockshuber R, Wu XR, Sun TT, Kong XP. 2001. Uroplakin Ia is the urothelial receptor for uropathogenic *Escherichia coli*: Evidence from in vitro FimH binding. *J Cell Sci* **114:** 4095–4103.

Zhu Y, Weiss EC, Otto M, Fey PD, Smeltzer MS, Somerville GA. 2007. *Staphylococcus aureus* biofilm metabolism and the influence of arginine on polysaccharide intercellular adhesin synthesis, biofilm formation, and pathogenesis. *Infect Immun* **75:** 4219–4226.

Zogaj X, Nimtz M, Rohde M, Bokranz W, Romling U. 2001. The multicellular morphotypes of *Salmonella typhimurium* and *Escherichia coli* produce cellulose as the second component of the extracellular matrix. *Mol Microbiol* **39:** 1452–1463.

Zogaj X, Bokranz W, Nimtz M, Romling U. 2003. Production of cellulose and curli fimbriae by members of the family Enterobacteriaceae isolated from the human gastrointestinal tract. *Infect Immun* **71:** 4151–4158.

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