CRITICAL REVIEWS IN ORAL BIOLOGY & MEDICINE

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Biofilm Dispersal: Mechanisms, Clinical Implications, and Potential Therapeutic Uses

ABSTRACT

Like all sessile organisms, surface-attached communities of bacteria known as biofilms must release and disperse cells into the environment to colonize new sites. For many pathogenic bacteria, biofilm dispersal plays an important role in the transmission of bacteria from environmental reservoirs to human hosts, in horizontal and vertical cross-host transmission, and in the exacerbation and spread of infection within a host. The molecular mechanisms of bacterial biofilm dispersal are only beginning to be elucidated. Biofilm dispersal is a promising area of research that may lead to the development of novel agents that inhibit biofilm formation or promote biofilm cell detachment. Such agents may be useful for the prevention and treatment of biofilms in a variety of industrial and clinical settings. This review describes the current status of research on biofilm dispersal, with an emphasis on studies aimed to characterize dispersal mechanisms, and to identify environmental cues and inter- and intracellular signals that regulate the dispersal process. The clinical implications of biofilm dispersal and the potential therapeutic applications of some of the most recent findings will also be discussed.

KEY WORDS: biofilm, detachment, dispersal, dispersion, erosion, matrix, plaque, seeding, sloughing, transmission.

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INTRODUCTION

Surface-associated communities of bacteria known as biofilms play a role in the pathogenesis of many chronic infections. In the oral cavity, biofilms that form on teeth produce acids that cause dental caries, and biofilms that grow in the gingival sulcus contribute to the pathogenesis of periodontitis (Marsh, 2006). Biofilms that form in other organs of the body cause numerous, often life-threatening, infections such as cystic fibrosis pneumonia and catheter-related endocarditis (Costerton *et al*., 1999). All biofilms, regardless of their location, share several common features. These include the synthesis of an extracellular polymeric matrix that holds the bacterial cells together, and an increase in resistance to killing by host defenses and antimicrobial agents compared with the resistance exhibited by free-living or 'planktonic' cells (Mah and O'Toole, 2001). The inherent protective nature of the biofilm colony makes most biofilm-associated infections difficult or impossible to eradicate.

Biofilm development can be divided into three distinct stages: attachment of cells to a surface, growth of the cells into a sessile biofilm colony, and detachment of cells from the colony into the surrounding medium. The initial, reversible interaction between a bacterial cell and a surface is mediated by non-specific Lifshitz-van der Waals, Lewis acid-base, and electrostatic forces. This transient attachment is reinforced by host- and tissue-specific adhesins that are located on the bacterial cell surface or on cellular appendages such as pili and fimbriae (Rosan and Lamont, 2000). This results in the irreversible attachment of the bacterial cell to the surface. In the case of dental plaque, which can be comprised of hundreds of bacterial species, colonization of tooth surfaces follows an ordered progression, with initial adhesion of early 'pioneer' species to the enamel surface followed by attachment of later-colonizing species to the already-attached early colonizers (Marsh, 2004).

The second stage of biofilm development involves the multiplication of bacteria on the surface and the concomitant synthesis of an extracellular polymeric matrix. The matrix holds the bacterial cells together in a mass and firmly attaches the bacterial mass to the underlying surface. Some examples of polymeric biofilm matrix components produced by oral bacteria include the well-studied glucan polysaccharides of *Streptococcus mutans* (Banas and Vickerman, 2003), proteinaceous fimbriae produced by *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* (Kachlany *et al*., 2001; Lamont *et al*., 2002), and extracellular, double-stranded DNA in biofilms produced by *A. actinomycetemcomitans*, *S. mutans,* and *S. intermedius* (Inoue *et al*., 2003; Petersen *et al*., 2004, 2005). In addition to providing a structural 'scaffold' for the biofilm colony, the matrix also contributes to biofilm-mediated antimicrobial resistance, either by acting as a diffusion barrier, or by binding directly to antimicrobial agents and preventing their access to the biofilm cells (Mah and O'Toole, 2001).

Continued growth of bacterial cells on a surface leads to the development of mature biofilm colonies containing millions of tightly packed cells gathered into

pillar- and mushroom-shaped masses that project outward into the surrounding medium for hundreds of microns (Hall-Stoodley *et al*., 2004). These structures are interspersed with fluid-filled channels which act as a primitive circulatory system, allowing for the exchange of nutrients and waste products with the bulk fluid phase. In addition, masses of biofilm cells often contain demarcated internal spaces that are devoid of cells. Thus, mature biofilm colonies are complex, highly differentiated structures. Numerous microenvironments that differ with respect to pH, oxygen concentration, nutrient availability, and cell density exist within the biofilm colony. This results in a great deal of heterogeneity in metabolic and reproductive activity among cells located in different parts of the colony. Metabolically inactive cells located in the interior of the colony may be resistant to the actions of antimicrobial agents that target actively growing cells (Fux *et al*., 2004).

The final stage of biofilm development is the detachment of cells from the biofilm colony and their dispersal into the environment. This is an essential stage of the biofilm life cycle that contributes to biological dispersal, bacterial survival, and disease transmission. Like other stages of biofilm development, dispersal can be a complex process that involves numerous environmental signals, signal transduction pathways, and effectors (Karatan and Watnick, 2009). No single mechanism of biofilm dispersal is utilized by all bacteria.

This article reviews the current literature on biofilm dispersal. Although dispersal is the least-understood stage of the biofilm life cycle, increasing numbers of studies on this process are being published. An important rationale for these studies is that understanding the mechanisms of biofilm dispersal is expected to lead to the development of clinically useful agents that inhibit biofilm formation or promote biofilm detachment. This review is divided into two main sections. The first section describes the known mechanisms of biofilm dispersal, and the second section describes the known chemical signals that regulate the dispersal process. The clinical implications of biofilm dispersal will also be discussed, as will the potential therapeutic applications of some of the most recent findings.

MECHANISMS OF BIOFILM DISPERSAL

Bacterial biofilm dispersal can be divided into three distinct phases: (i) detachment of cells from the biofilm colony; (ii) translocation of the cells to a new location; and (iii) attachment of the cells to a substrate in the new location. Thus, *S. mutans* cells that detach from dental plaque can be transported to the saliva of an infant by direct contact or by means of a vector such as a shared spoon, and then attach to the tooth surface and initiate colonization of the new host. Similarly, cells that detach from a *Legionella* biofilm growing in a cooling tower can be transported by means of air-borne water droplets to the lungs of a susceptible host, where they can attach to alveolar macrophages and initiate infection. In the literature and in this review, the terms 'detachment', 'dispersal', and 'dispersion' are used interchangeably to refer to the cell-detachment phase of the dispersal process. Studies on the movement of detached cells to a new location fall mostly under the discipline of disease transmission.

In general, mechanisms of biofilm dispersal can be divided into two broad categories: active and passive. Active dispersal refers to mechanisms that are initiated by the bacteria themselves, whereas passive dispersal refers to biofilm cell detachment that is mediated by external forces such as fluid shear, abrasion (collision of solid particles with the biofilm), predator grazing, and human intervention (Lawrence *et al*., 2002; Choi and Morgenroth, 2003; Ymele-Leki and Ross, 2007). In a complex community such as dental plaque, close relationships between species based on competition, mutualism, predation, or parasitism are likely to have resulted in the evolution of various other passive dispersal mechanisms. These may include interspecific antimicrobial compounds, quorum-sensing signals, or matrix-degrading enzymes. Phagocytosis, a form of predator grazing, may also contribute to the passive dispersal of oral biofilms (Erard *et al*., 1989).

At least three distinct modes of biofilm dispersal have been identified: erosion, sloughing, and seeding. Erosion refers to the continuous release of single cells or small clusters of cells from a biofilm at low levels over the course of biofilm formation. Sloughing refers to the sudden detachment of large portions of the biofilm, usually during the later stages of biofilm formation (Marshall, 1988; Lappin-Scott and Bass, 2001; Stoodley *et al*., 2001; Wilson *et al*., 2004). Seeding dispersal, also known as central hollowing, refers to the rapid release of a large number of single cells or small clusters of cells from hollow cavities that form inside the biofilm colony (Boles *et al*., 2005; Ma *et al*., 2009). Erosion and sloughing can be either active or passive processes, whereas seeding dispersal is always an active process. The following sections describe some of the mechanisms of active biofilm dispersal that have been described to date.

Enzymatic Degradation of the Biofilm Matrix

A basic mechanism of biofilm dispersal that is utilized by phylogenetically diverse bacteria is the production of extracellular enzymes that degrade adhesive components in the biofilm matrix. Since the biofilm matrix encases the bacterial cells within the biofilm colony, degradation of the matrix results in the detachment of cells from the colony and their release into the environment. Matrix-degrading enzymes implicated in active biofilm dispersal include glycosidases, proteases, and deoxyribonucleases (Table).

One well-studied biofilm-matrix-degrading enzyme is dispersin B, a glycoside hydrolase produced by the periodontopathogen *A. actinomycetemcomitans* (Kaplan *et al*., 2003b). Dispersin B degrades poly-*N*-acetylglucosamine (PNAG), a biofilm matrix polysaccharide that mediates attachment of *A. actinomycetemcomitans* cells to abiotic surfaces, intercellular adhesion (autoaggregation), and resistance to killing by detergents and human phagocytic cells (Kaplan *et al*., 2003b, 2004b; Izano *et al*., 2007, 2008b; Venketaraman *et al*., 2008). Evidence that dispersin B is involved in biofilm dispersal comes from studies utilizing mutant strains that do not produce the enzyme (Kaplan *et al*., 2003b). When cultured in broth, these mutant strains produce biofilm colonies that are similar in morphology to wild-type colonies, but the mutant colonies fail to release cells into the medium and disperse (Fig. 1).

Table. Bacterial Enzymes Implicated in Active Biofilm Dispersal

Enzyme	Molecular Weight (kDa)	Substrate	Bacterium	Reference
Alginate lyase	43	Alginate (polymer of mannuronic and guluronic acids)	*Pseudomonas aeruginosa*	Boyd and Chakrabarty, 1994
Aureolysin	33	Unknown	*Staphylococcus aureus*	Boles and Horswill, 2008
Chitinase	57	Chitin	*Pseudoaltermonas* sp. S91	Baty *et al.*, 2000
Disaggregatase	180	Polymer of *N*-acetylgalactosamine and galacturonic and glucuronic acids	*Methanosarcina mazei*	Xun *et al.*, 1990
Dispersin B	42	Poly-β(1,6)-*N*-acetyl-D-glucosamine (PNAG)	*Aggregatibacter actinomycetemcomitans*	Kaplan *et al.*, 2003b
Endo-β-1,4-mannanase	33	Unknown	*Xanthomonas campestris*	Dow *et al.*, 2003
Exopolysaccharide lyase	Unknown	Unknown	*Pseudomonas fluorescens*	Allison *et al.*, 1998
Hemagglutinin protease (HAP)	66	Bacterial receptors on human intestinal cells	*Vibrio cholerae*	Finkelstein *et al.*, 1992
Hyaluronidase	117	Hyaluronan	*Streptococcus intermedius*	Pecharki *et al.*, 2008
LapG protease	24	LapA exopolysaccharide-binding protein	*Pseudomonas putida*	Gjermansen *et al.*, 2009
Spl protease	23	Unknown	*Staphylococcus aureus*	Boles and Horswill, 2008
Surface-protein-releasing enzyme (SPRE)	Unknown	Antigen P1	*Streptococcus mutans*	Lee *et al.*, 1996
Thermonuclease	32	Extracellular DNA	*Staphylococcus aureus*	Mann *et al.*, 2009

Further evidence that dispersin B is involved in biofilm dispersal comes from studies showing that purified dispersin B enzyme detaches pre-formed biofilm colonies produced by *A. actinomycetemcomitans* and other PNAG-producing bacteria (Itoh *et al*., 2005; Izano *et al*., 2007). In addition, over-expression of dispersin B in wild-type *A. actinomycetemcomitans* biofilms results in a hyper-dispersal phenotype (unpublished data). An orthologous dispersin B enzyme is produced by the porcine respiratory pathogen *Actinobacillus pleuropneumoniae* (Kaplan *et al*., 2004b), although its role in biofilm dispersal has not been investigated. Genes homologous to the *A. actinomycetemcomitans* dispersin B structural gene (*dspB*) are present in the genomes of several other bacteria, including the human oral commensal *Aggregatibacter aphrophilus*, and the bovine ruminal species *Actinobacillus succinogenes* and *Mannheimia succiniciproducens*. There is no evidence that these other species produce a functional dispersin B enzyme. A homologue of *dspB* was identified in the genome of *Staphylococcus lugdunensis*, although its role in biofilm dispersal was not investigated (Frank and Patel, 2007).

The cariogenic bacterium *S. mutans* also produces an enzyme that mediates the release of cells from biofilms (Lee *et al*., 1996). This enzyme, referred to as surface-protein-releasing enzyme, or SPRE, degrades salivary receptor P1 (also known as antigen I/II or PAc), a 185-kDa surface protein that mediates attachment of *S. mutans* cells to the tooth surface (Vats and Lee, 2000). Degradation of P1 by exogenously added SPRE results in the detachment of a *S. mutans* monolayer formed on saliva-coated hydroxyapatite rods (Lee *et al*., 1996). In addition, a Tn*917* SPRE-defective mutant strain was shown to detach from the rods at a significantly lower rate than the parental strain (Vats and Lee, 2000). Interestingly, active detachment of *S. mutans* biofilms occurs more rapidly as the pH of the medium drops (Tam *et al*., 2007). Since the production of SPRE is optimal at pH 5-6, this suggests that increased acidogenicity may trigger *S. mutans* biofilm detachment by the induction of SPRE activity. SPRE is also produced by *S. gordonii*, a pioneer colonizer and important endocarditis pathogen, and by several other non-oral pathogenic streptococci, including *S. pneumoniae*, *S. pyogenes,* and *S. agalactiae* (Vats and Lee, 2000).

Several other non-oral bacteria produce extracellular enzymes that degrade endogenous matrix components and mediate biofilm cell detachment. Mucoid strains of the human opportunistic pathogen *Pseudomonas aeruginosa*, for example, produce both alginate, a biofilm matrix polysaccharide composed of mannuronic and guluronic acids, and alginate lyase, an enzyme that degrades alginate. Increased expression of alginate lyase promotes the detachment of cells from *P. aeruginosa* biofilms cultured on agar surfaces (Boyd and Chakrabarty, 1994), and exogenously added alginate lyase increases the effectiveness of some antibiotics against *P. aeruginosa* biofilms cultured in broth (Alkawash *et al*., 2006; Alipour *et al*., 2009). Polysaccharide lyases that promote biofilm detachment are also produced by *P. fluorescens* and *P. syringae* (Allison *et al*., 1998; Preston *et al*., 2000). Other polysaccharide-degrading enzymes implicated in biofilm cell detachment include endo-β-1,4-mannanase, produced by the plant pathogen *Xanthomonas campestris* (Dow *et al*., 2003), and disaggregatase, produced by the archaeon *Methanosarcina mazei* (Xun *et al*., 1990).

Several extracellular proteases have also been implicated in biofilm cell detachment. In the plant saprophyte *Pseudomonas putida*, it has been shown that LapG protease cleaves a periplasmic protein (LapA) which anchors an unidentified biofilm matrix polysaccharide to the cell (Gjermansen *et al*., 2009). This process results in the release of cells from biofilms cultured in microplate wells or flow cells. In *Staphylococcus aureus*, deletion of the genes encoding the extracellular proteases aureolysin and Spl resulted in a significant increase in biofilm formation in the flow cells, and a concomitant decrease in planktonic cells

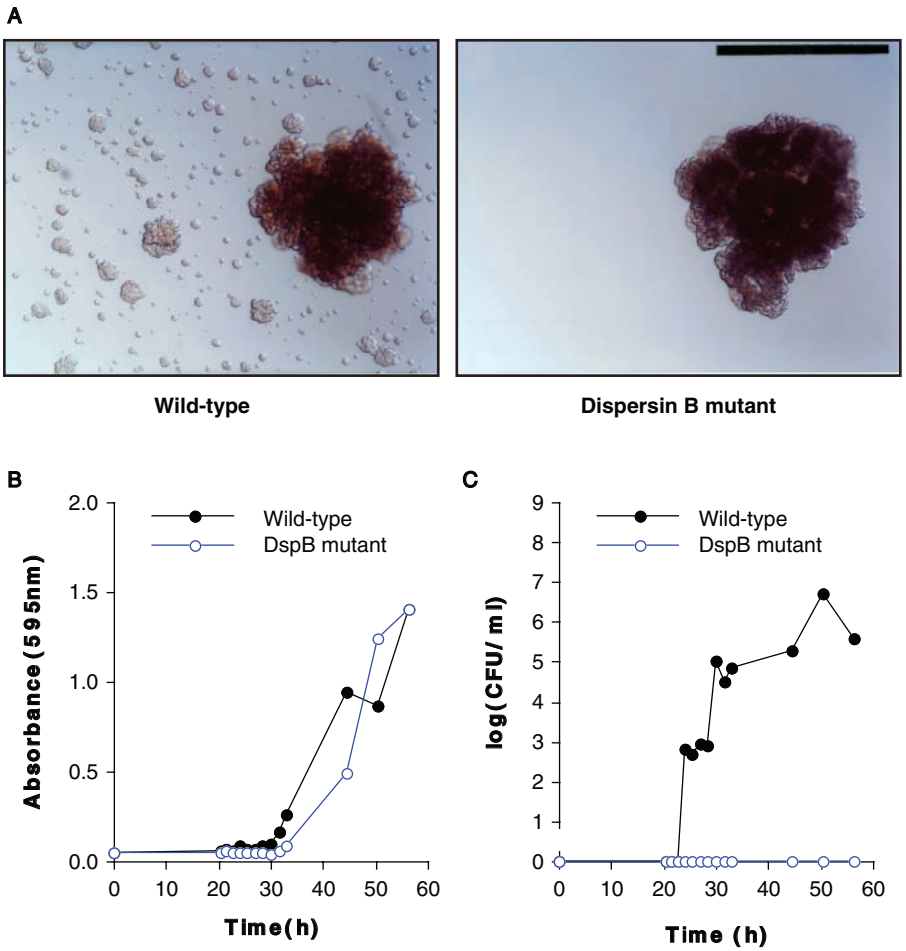

Figure 1. Biofilm dispersal phenotypes of wild-type and dispersin B mutant strains of *Aggregatibacter actinomycetemcomitans*. (**A**) Dispersal of *A. actinomycetemcomitans* strain CU1000 (wild-type) and JK1023 (dispersin B mutant) in broth. (**B**) Biofilm formation by strains CU1000 and JK1023 over time as measured by crystal violet staining. (**C**) Detachment of cells from CU1000 and JK1023 biofilms over time as measured by CFU/mL in the broth.

present in the fluid effluent (Boles and Horswill, 2008). These findings indicate that *S. aureus* biofilm dispersal requires protease activity, although the targets for these proteases are not known.

In addition to the glycosidases and proteases mentioned above, the deoxyribonuclease known as thermonuclease or micrococcal nuclease has been implicated in cell detachment in *S. aureus* biofilms (Mann *et al*., 2009). *S. aureus* biofilms are readily detached from microplate wells by exogenously added deoxyribonucleases, including thermonuclease, indicating that extracellular DNA is a major biofilm matrix adhesin in this species (Izano *et al*., 2008a). It has been shown (Mann *et al*., 2009) that a thermonuclease-deficient mutant strain of *S. aureus* exhibited significantly increased biofilm formation in flow cells compared with the amount of biofilm exhibited by a wild-type strain. These findings suggest that thermonuclease may function as an endogenous mediator of biofilm dispersal in this species.

All of the matrix-degrading enzymes described above mediate biofilm dispersal by enabling bacteria to degrade their own biofilm matrix polymers. An alternative mechanism of enzyme-mediated biofilm dispersal may occur in multi-species biofilms such as dental plaque, where some bacteria may produce enzymes that degrade biofilm matrix polymers produced by other species. Interspecific matrix-degrading enzymes may have evolved to provide bacteria with a source of nutrients, or as a defense mechanism that detaches and displaces competing species. Zhang and Bishop (2003) provided evidence that this mechanism of biofilm dispersal may occur in complex biofilm communities found in wastewater treatment plants. Using a small-scale biofilm reactor that was seeded with wastewater and perfused with synthetic wastewater, they showed that bacteria present in activated sludge can degrade the extracellular matrix of biofilms produced by the wastewater bacteria, and then utilize the degradation products as an additional food source. Such action would likely result in the passive detachment of the wastewater biofilms.

Enzymatic Degradation of the Biofilm Substrate

Another mechanism of enzyme-mediated biofilm dispersal involves the production of extracellular enzymes that degrade the substrate on which the biofilm colony is growing. An interesting example of this type of dispersal may be found in the oral bacterium *Streptococcus intermedius*, which produces hyaluronidase, an enzyme that degrades the glycosaminoglycan hyaluronan (HA) found in the extracellular matrix of connective tissue. By breaking down the connective tissue, hyaluronidase may provide nutrients for the bacteria or allow the spread of bacteria and toxins deeper into the tissue. It was recently shown (Pecharki *et al*., 2008) that hyaluronidase may play a role in *S. intermedius* biofilm dispersal. These authors found that a hyaluronidase mutant strain formed significantly more biofilm in broth supplemented with HA than did a wild-type strain. Also, exogenous hyaluronidase dispersed *S. intermedius* biofilms grown in HA-supplemented medium. This mechanism may have clinical relevance to the dispersal of *S. intermedius* biofilms in the oral cavity.

In the intestinal pathogen *Vibrio cholerae*, a Zn-metalloprotease known as hemagglutinin protease (HAP) may promote detachment of vibrios from human intestinal epithelial cells by digesting epithelial cell receptors that bind to *V. cholerae* adhesins (Finkelstein *et al*., 1992). Evidence supporting this model comes from experiments showing that mutant vibrio strains lacking HAP remained attached to cultured intestinal cells, whereas wild-type strains

readily detached (Finkelstein *et al*., 1992). In addition, pretreatment of the cultured epithelial cells with purified HAP inhibited attachment of the vibrios in a time- and dose-dependent manner. Another example of this dispersal mechanism may be found in the marine bacteria *Pseudoaltermonas* sp. strain S91 and *Vibrio furnissii* (Yu *et al*., 1991; Baty *et al*., 2000). These bacteria form biofilms on solid chitin surfaces in marine environments, and also produce chitinase, which degrades the chitin for use as a food source. Chitin degradation results in detachment of the attached biofilm cells from artificially prepared chitin surfaces and from natural squid chitin surfaces *in vitro* (Baty *et al*., 2000).

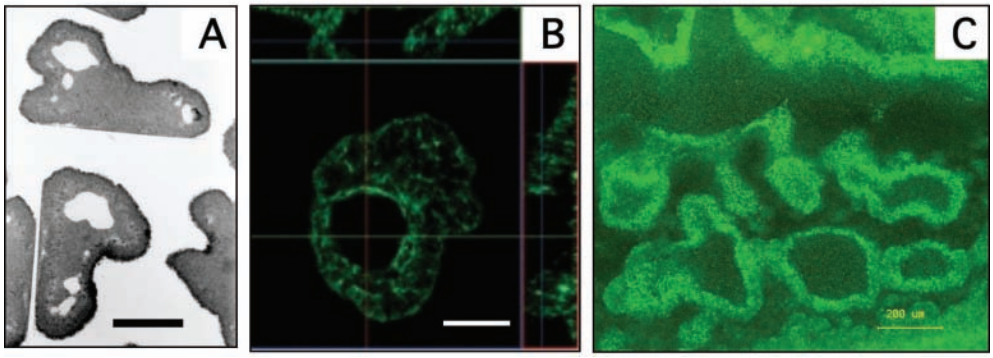

Figure 2. Cross-sections of biofilm colonies produced by (**A**) *A. actinomycetemcomitans*, (**B**) *P. aeruginosa*, and (**C**) *S. marcescens*. Scale bars = 100 μm in (A), 50 μm in (B), and 200 μm in (C). Panels (B) and (C) are from Ma *et al.* (2009) and Koh *et al.* (2007), respectively. Used with permission.

Seeding Dispersal

Microscopic studies have shown that many bacteria produce biofilm colonies that contain hollow, internal cavities (Fig. 2). These cavities have been observed in biofilms produced by the human pathogens *A. actinomycetemcomitans*, *P. aeruginosa*, *Serratia marcescens,* and *S. aureus* (Kaplan *et al*., 2003a; Yarwood *et al*., 2004; Koh *et al*., 2007; Ma *et al*., 2009); by the plant-associated bacteria *P. putida* and *Chromobacterium violacium* (Tolker-Nielsen *et al*., 2000; Mai-Prochnow *et al*., 2008); and by the aquatic bacteria *Caulobacter crescentus*, *Pseudoalteromonas tunicate,* and *Marinomonas mediterranea* (Mai-Prochnow *et al*., 2004, 2008). Increasing evidence suggests that these hollow cavities play a role in biofilm cell detachment through a process termed 'seeding dispersal'. In this process, the hollow cavities become filled with non-aggregated planktonic cells. Seeding dispersal results when a breach in the colony wall releases the planktonic cells from the cavities into the surrounding medium.

Seeding dispersal has been well-characterized in the oral bacterium *A. actinomycetemcomitan*s (Kaplan and Fine, 2002; Kaplan *et al*., 2003a,b). Two lines of evidence support the notion that *A. actinomycetemcomitans* biofilm colonies undergo seeding dispersal. First, microscopic analyses show that mature *A. actinomycetemcomitans* biofilm colonies cultured in broth develop internal, hollow cavities that are surrounded by a layer of non-aggregated cells (Fig. 2A) (Kaplan *et al*., 2003a). Second, when *A. actinomycetemcomitans* biofilms are cultured in broth, the release of cells from the biofilm into the broth over time is sudden rather than gradual, consistent with a seeding dispersal event (Fig. 1C). *A. actinomycetemcomitans* biofilm dispersal can be visualized by the culturing of biofilm colonies in the presence of buoyancy-driven convection currents under conditions of low vibration (Fig. 3) (Kaplan and Fine, 2002). Under these conditions, dispersed cells move along the surface by convection current, reattach to the surface, and then form new biofilm colonies. This results in the appearance of streamers of satellite colonies emanating from the dispersed biofilm colony. Biofilm colonies produced by mutant strains of *A. actinomycetemcomitans* deficient in the production of dispersin B still contain internal voids, but the void spaces are not surrounded by a layer of non-aggregated cells, and the colonies do not release cells into the medium and disperse (Figs. 1A, 1C) (Kaplan *et al*., 2003b). These findings indicate that depolymerization of PNAG by dispersin B is required for the production of the non-aggregated cell layer, but not for the formation of the hollow cavities themselves. Other oral bacteria that exhibit a similar mode of biofilm dispersal include *Neisseria subflava* and *S. mutans* (Fig. 3), and *Aggregatibacter aphrophilus* and *S. mitis* (Kaplan and Fine, 2002). In the case of *N. subflava*, a sudden spike in the number of CFU in the medium during biofilm formation was also observed (Kaplan and Fine, 2002).

Seeding dispersal has been studied extensively in *P. aeruginosa* biofilms (Sauer *et al*., 2002; Hunt *et al*., 2004; Schooling *et al*., 2004; Boles *et al*., 2005; Purevdorj-Gage *et al*., 2005; Kirov *et al*., 2007; Pamp and Tolker-Nielsen, 2007; Ma *et al*., 2009). These studies have shown that the hollow cavities that form inside *P. aeruginosa* biofilm colonies are devoid of biofilm matrix polysaccharide, but contain numerous swimming bacterial cells (Ma *et al*., 2009). In some cases, motile cells in the hollow cavities can be seen swimming through openings in the colony wall and entering the bulk liquid (Sauer *et al*., 2002). Central hollowing and seeding dispersal in *P. aeruginosa* is evidently triggered by an increase in colony size, because a threshold colony diameter of > 80 μm is required for hollow cavity formation to occur (Purevdorj-Gage *et al*., 2005).

The mechanism of central hollowing is not fully understood, but evidence suggests that it involves the death and lysis of a subpopulation of cells located in the center of the colony. In *P. aeruginosa*, for example, strains that are deficient in the production of the Cid/Lrg toxin-antitoxin system produce biofilm colonies that do not undergo central hollowing (Webb *et al*., 2003; Ma *et al*., 2009). The Cid/Lrg proteins are structurally and functionally related to bacteriophage-encoded holins, which regulate host cell lysis during the lytic cycle of infection by modulating the expression of murein hydrolase (Bayles, 2007). An orthologous Cid/Lrg system was shown to be responsible for

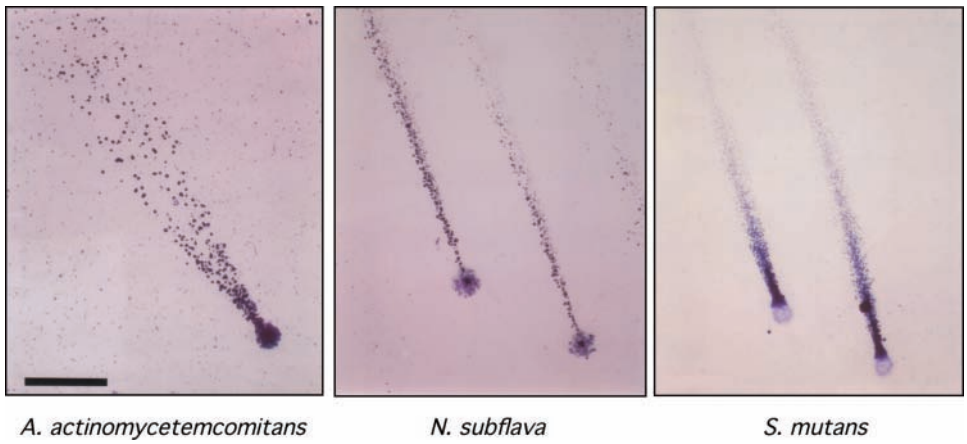

Figure 3. Dispersal of *A. actinomycetemcomitans, N. subflava,* and *S. mutans* biofilms in broth. Biofilms were stained with crystal violet. Scale bar = 1 mm. The panel on the left is from Kaplan and Fine (2002). Used with permission.

cell death and DNA release in *S. aureus* biofilms, although the role of this system in biofilm dispersal has not been investigated (Rice *et al*., 2007; Mann *et al*., 2009). Genes that encode homologues of the Cid/Lrg system are present in many bacterial species, including the oral bacteria *A. actinomycetemcomitans*, *Fusobacterium nucleatum,* and *S. mutans*. Thus, the Cid/Lrg system may mediate cell death and biofilm dispersal in a variety of species (Bayles, 2007).

Several studies have shown that phage-mediated cell lysis can also lead to central hollowing and seeding dispersal in *P. aeruginosa* biofilms. A bacteriophage capable of superinfecting and lysing *P. aeruginosa* (bacteriophage Pf4) was detected in the fluid effluent from *P. aeruginosa* biofilms cultured in flow cells (Webb *et al*., 2003), and the amount of bacteriophage activity in the effluent paralleled the degree of cell death seen in the biofilm (Kirov *et al*., 2007). In addition, deletion of the entire Pf4 prophage genome from the *P. aeruginosa* chromosome resulted in the formation of biofilms that did not exhibit cell lysis, central hollowing, or seeding dispersal (Rice *et al*., 2009).

For motile bacteria, it has been shown that motility is: (i) required for the initial attachment of bacteria to surfaces (O'Toole and Kolter, 1998; Chiang and Burrows, 2003); (ii) repressed in mature biofilms (Sauer and Camper, 2001; Whiteley *et al*., 2001; Sauer *et al*., 2002, 2004); and (iii) induced upon biofilm dispersal (Jackson *et al*., 2002; Sauer *et al*., 2002; Purevdorj-Gage *et al*., 2005). These observations suggest that the induction of motility may itself represent a specific mechanism of biofilm dispersal, or may play a role in the initiation of seeding dispersal. However, studies have shown that seeding dispersal is not influenced by motility in *P. aeruginosa* and *P. putida* biofilms (Gjermansen *et al*., 2005; Morgan *et al*., 2006). In addition, non-motile species such as *A. actinomycetemcomitans* undergo seeding dispersal. Thus, other gene products that reduce the cohesiveness of the biofilm in the center of the colony evidently mediate seeding dispersal.

Seeding dispersal also occurs in biofilms produced by the marine bacterium *Pseudoalteromonas tunicata* (Mai-Prochnow *et al*., 2004). Dispersal involves the formation of central voids within the biofilm colony, extensive cell killing, and detachment of the biofilm from the substratum. Mutant strains deficient in the production of the autolytic protein AlpP did not exhibit cell death and biofilm dispersal. It was subsequently shown that AlpP acts as a lysine oxidase that generates hydrogen peroxide, which is directly responsible for cell death within the biofilm colony (Mai-Prochnow *et al*., 2008). Orthologous AlpP proteins mediate cell death and biofilm dispersal in *C. crescentus*, *C. violaceum,* and *M. mediterranea* (Mai-Prochnow *et al*., 2008).

Production of Rhamnolipids

Rhamnolipids are extracellular surfactants produced by *P. aeruginosa* (Soberón-Chávez *et al*., 2005). These compounds typically contain a dimer of 3-hydroxyfatty acids linked through a β-glycosidic bond to a mono- or di-rhamnose moiety. *P. aeruginosa* produces several rhamnolipids, including L-rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate (mono-rhamnolipid) (Fig. 4A), and L-rhamnosyl-L-rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate (di-rhamnolipid). Due to their amphipathic nature, rhamnolipids exhibit surface-acting properties that decrease the adhesiveness of cell-cell, cell-matrix, and cell-surface interactions (Neu, 1996). It has been shown (Boles *et al*., 2005) that inactivation of the rhamnolipid biosynthetic genes (*rhaAB*) in *P. aeruginosa* biofilms inhibits central hollowing and cell detachment, and that over-expression of *rhaAB* in wild-type biofilms increases cell detachment. Also, exogenous rhamnolipids induce central hollowing and biofilm detachment in wild-type *P. aeruginosa* biofilms (Boles *et al*., 2005; Dong *et al*., 2008). These findings suggest that rhamnolipids can mediate central hollowing in *P. aeruginosa* biofilms during seeding dispersal.

The mechanism by which rhamnoloipids induce central hollowing is not known. These compounds most likely act by disrupting interactions among various cellular and matrix component within the biofilm colony. This model is supported by the fact that exogenously added rhamnolipids can inhibit *P. aeruginosa* biofilm formation on glass surfaces when added to the broth at the time of inoculation (Schooling *et al*., 2004). In addition, exogenously added *P. aeruginosa* rhamnolipids can disperse biofilms produced by other species of bacteria, including *Bordetella bronchiseptica* and *Salmonella enterica* serovar Typhimurium (Mireles *et al*., 2001; Irie *et al*., 2005). Interestingly, another surfactant, sodium dodecyl sulfate, can also induce central hollowing in *P. aeruginosa*

biofilm colonies when exogenously added to the colonies (Boles *et al*., 2005). All of these observations support the notion that rhamnolipids disrupt biofilm cohesiveness in a non-specific manner. However, there is still no explanation for the fact that cells in the center of the biofilm colony are more susceptible to the actions of surfactants. Unknown phenotypic differences in the cells or matrix at the interior of the colony must account for the central hollowing induced by exogenous surfactants (Boles *et al*., 2005).

Modulation of Fimbrial Adherence

Two different pathogenic strains of *E. coli*, enteroaggregative *E. coli* (EAEC) and enteropathogenic *E. coli* (EPEC), have evolved unique mechanisms to achieve detachment from biofilms that form on intestinal epithelial cells. In EAEC, autoaggregation and adherence to the human intestinal mucosa are mediated by aggregative adherence fimbriae (AAFs). The adhesive properties of AAFs are modulated by a small protein named dispersin, which binds non-covalently to lipopolysaccharide (LPS) on the surface of the bacterium (Sheikh *et al*., 2002; Velarde *et al*., 2007). LPS is negatively charged, and AAFs, unlike most enteric pili, are positively charged. When dispersin is bound to LPS, it neutralizes the negative charge and allows the AAFs to extend away from the bacterial cell surface, where they can mediate their adhesive effects. In the absence of dispersin, the AAFs collapse onto the bacterial cell surface and become non-adhesive, due to their electrostatic interaction with the LPS. Thus, biofilm dispersal is achieved by down-regulation of dispersin protein.

A similar mechanism of dispersal is displayed by EPEC, which produces type IV bundle-forming pili (BFP) that mediate microcolony formation on human intestinal mucosa (Cleary *et al*., 2004). BFP can undergo structural alterations that result in the formation of either thin or thick BFP bundle structures (Knutton *et al*., 1999). Thin BFP bundles mediate microcolony formation, whereas thick BFP bundles are associated with a non-aggregative, planktonic phenotype. EPEC can therefore modulate microcolony cohesion and dispersal by modulating the structure of the BFP bundles, possibly thorough a mechanism that involves pilus retraction (Knutton *et al*., 1999).

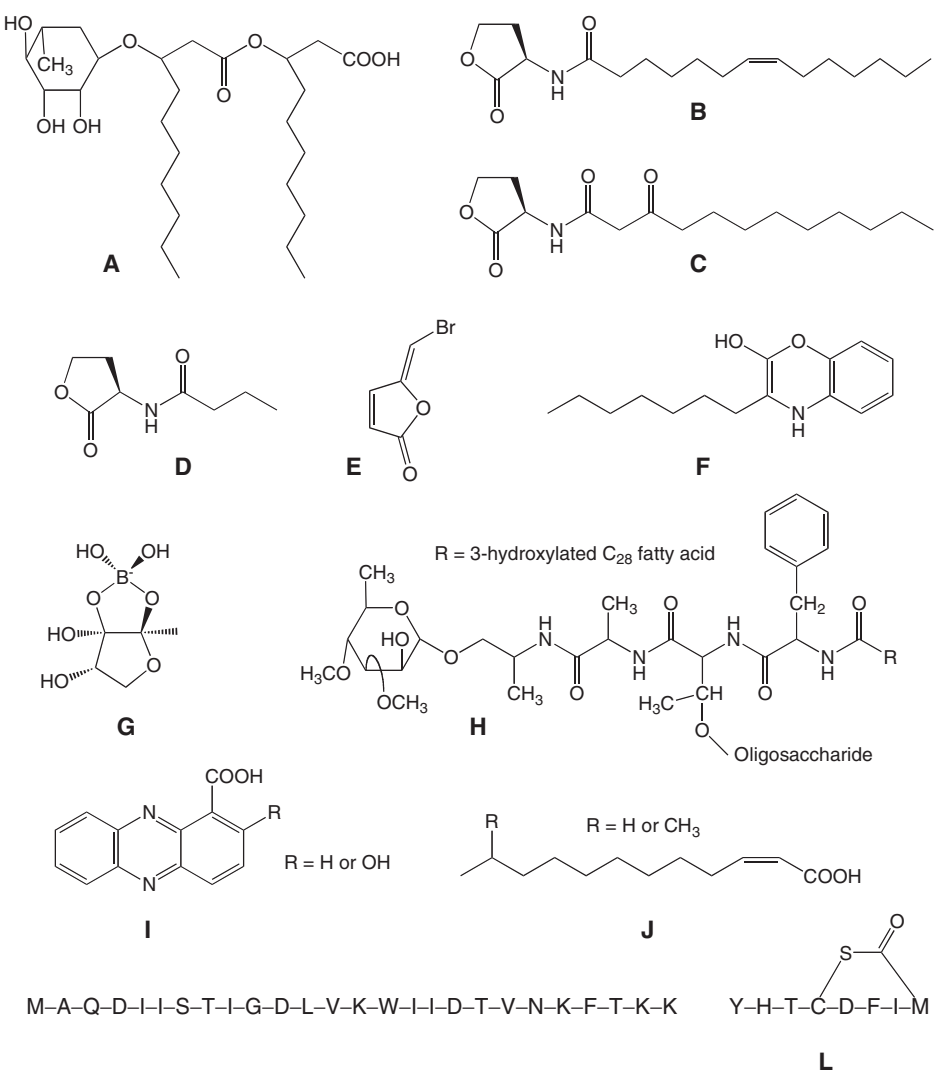

Figure 4. Chemical signals implicated in biofilm dispersal.

Cell-division-mediated Biofilm Dispersal

Another possible mechanism of biofilm dispersal involves cell detachment due to cell division at the outer surface of the biofilm colony (Allison *et al*., 1990). When biofilm cells on the outer surface of the colony divide, one of the progeny cells may be located at a sufficient distance from the colony so that it is not subjected to the attractive forces of the biofilm matrix, and would therefore be liberated into the bulk fluid following cell separation (Gilbert *et al*., 1993). This dispersal mechanism has been exploited to obtain synchronous populations of bacteria from cells attached to a membrane filter and perfused with fresh medium (Helmstetter and Cummings, 1964; Gilbert *et al*., 1989). This mechanism of dispersal may account for biofilm erosion in some natural biofilms.

REGULATION OF BIOFILM DISPERSAL

Many studies on the regulation of biofilm dispersal have focused on the identification of environmental conditions that trigger the

dispersal process. Factors such as nutrient levels, oxygen tension, pH, and temperature have been shown to induce dispersal of biofilms produced by a variety of species (Karatan and Watnick, 2009). The rationale for these studies is the notion that dispersal of biofilm cells is a selective advantage when environmental conditions become unfavorable. However, dispersal is a selective advantage even when conditions are favorable. Biological dispersal has fundamental importance for the expansion, reproduction, and survival of all species. In addition to environmentally induced biofilm dispersal, therefore, it is likely that mechanisms of genetically programmed biofilm dispersal also exist. If this is true, then the life cycle of a biofilm may be analogous to that of a primitive, multicellular organism (Stoodley *et al*., 2002; Klausen *et al*., 2006). One example of programmed dispersal may be seen in *A. actinomycetemcomitans* biofilms, which undergo a reproducible, periodic detachment of biofilm biomass, even under conditions where nutrients are not limited (Fig. 1B). A similar reproducible pattern of biomass detachment is observed in biofilms produced by *P. putida* (Gjermansen *et al*., 2009) and *Serratia marcescens* (Rice *et al*., 2005).

The following sections describe some of the environmental cues and inter- and intracellular signals that have been shown to influence and regulate biofilm dispersal.

Nutrient Cues

Several studies have shown that sudden changes in nutrient availability can induce biofilm dispersal. Most work in this area has been performed with biofilms produced by pseudomonads. In experiments with *P. aeruginosa*, it was found that biofilms cultured in minimal medium in flow cells underwent dispersal in response to both a sudden decrease and a sudden increase in carbon substrate availability (Hunt *et al*., 2004; Sauer *et al*., 2004). Both of these responses may make sense from an ecological point of view. Cells may detach to escape unfavorable conditions when nutrients are scarce, or may choose to invest metabolic energy into reproduction and detachment when nutrients are plentiful. Nutrient starvation has also been shown to induce detachment of biofilms produced by *P. fluorescens* (Delaquis *et al*., 1989), *P. putida* (Gjermansen *et al*., 2009), and *Pseudomonas* sp. S9 (Wrangstadh *et al*., 1989). In *P. fluorescens*, starvation induced an increase in exopolysaccharide lyase production and biofilm dispersal (Allison *et al*., 1998), whereas in *P. aeruginosa*, alginate lyase activity was maximally induced in rapidly growing cells (Ott *et al*., 2001). Again, both of these observations suggest that increased biofilm dispersal rates may occur under both favorable and unfavorable conditions. Among non-pseudomonads, nutrient starvation was shown to increase biofilm dispersal in aquatic *Aeromonas hydrophila* (Sawyer and Hermanowicz, 1998), whereas high-nutrient conditions induced biofilm dispersal in environmental *Acinetobacter* sp. (James *et al*., 1995). In *E. coli*, exogenous glucose blocked biofilm dispersal induced by CsrA, a global regulator of central carbon flux, which further supports the hypothesis that nutrient cues can induce biofilm dispersal (Jackson *et al*., 2002).

Oxygen tension may be another environmental signal that modulates biofilm dispersal. In *P. putida*, oxygen-limited biofilms exhibited significantly lower shear removal rates and significantly greater sloughing dispersal when compared with biofilms cultured under oxygen-rich conditions (Applegate and Bryers, 1991). It was hypothesized that this difference may be due to a higher amount of extracellular polymer present in oxygen-limited biofilms. In contrast, a sudden downshift in molecular oxygen induced a rapid and efficient dispersal of biofilms produced by *Shewanella oneidensis*, an anaerobic, metal-reducing bacterium found in deep sea sediments (Thormann *et al*., 2005). Carbon limitation did not induce *S. oneidensisis* biofilm dispersal.

Acyl Homoserine Lactones

The expression of biofilm-specific genes is often regulated by quorum-sensing, a regulatory mechanism that involves the synthesis, secretion, and sensing of small chemical signals called autoinducers (Irie and Parsek, 2008). As the cell density and autoinducer concentration increase, a threshold concentration of autoinducer triggers an increase in the transcription of biofilm-specific genes by activating transcription factors that bind to sequences upstream from these genes. Autoinducers have been shown to control several stages of biofilm formation, including surface attachment, matrix synthesis, the formation of fluid channels and pillar-like architecture, and dispersal (Hall-Stoodley *et al*., 2004; Stanley and Lazazzera, 2004). *N*-acyl-homoserine lactones (AHLs), produced by Gram-negative bacteria, are one of the best-studied classes of autoinducers (Fuqua and Greenberg, 2002). AHLs implicated in biofilm dispersal include 7,8-*cis*-*N*-(tetradecenoyl)homoserine lactone, produced by *Rhodobacter sphaeroides* (Fig. 4B) (Puskas *et al*., 1997), *N*-3-oxo-dodecanoyl homoserine lactone (3-oxo-C12-HSL), produced by *P. aeruginosa* (Fig. 4C) (Wilson *et al*., 2004; Purevdorj-Gage *et al*., 2005), and *N*-butanoyl-L-homoserine lactone (C4-HSL), produced by *Serratia marcescens* and *P. aeruginosa* (Fig. 4D) (Schooling *et al*., 2004; Rice *et al*., 2005).

In *P. aeruginosa*, biofilms formed by mutant strains deficient in the production of C4-HSL (Δ*lasI*/*rhlI*) do not undergo central hollowing (Purevdorj-Gage *et al*., 2005), and the addition of exogenous C4-HSL to wild-type biofilms induces biofilm dispersal (Schooling *et al*., 2004; Dong *et al*., 2008). C4-HSL may induce dispersal by up-regulation of the rhamnolipid biosynthetic gene *rhaA* (Davey *et al*., 2003). C4-HSL signaling is also required for sloughing in *Serratia marcescens* biofilms (Rice *et al*., 2005). In contrast, treatment of wild-type *P. aeruginosa* biofilms with furanone 56 (Fig. 4E), a synthetic inhibitor of AHL signaling, induces biofilm dispersal (Hentzer *et al*., 2002). These findings suggest that *P. aeruginosa* biofilm dispersal is regulated by multiple AHL signaling networks.

In *R. sphaeroides*, a mutant strain deficient in the production of 7,8-*cis*-*N*-(tetradecenoyl)homoserine lactone formed large aggregates of cells in broth, and clumping was reversed by the addition of the AHL signal (Puskas *et al*., 1997). In *P. aeruginosa*, however, the detachment rates and size distributions of detached cell clumps were the same in wild-type and 3-oxo-C12-HSL-mutant biofilms (Wilson *et al*., 2004). These findings suggest that AHL signaling regulates biofilm dispersal differently in different species.

Pseudomonas Quinolone Signal

Another autoinducer produced by *P. aeruginosa* is 2-heptyl-3-hydroxy-4-quinolone, also known as *Pseudomonas* quinolone signal, or PQS (Fig. 4F) (Pesci *et al*., 1999). It has been shown

(Dong *et al*., 2008) that exogenous PQS induces dispersal of wild-type *P. aeruginosa* biofilms cultured in microplate wells. Previous studies showed that PQS mediates cell death and DNA release in *P. aeruginosa* biofilms (Allesen-Holm *et al*., 2006), which suggests that PQS may induce central hollowing.

Furanosylborate

The cholera bacterium *V. cholerae* produces the autoinducer furanosylborate, also known as AI-2 (Fig. 4G). *V. cholerae* AI-2 mutants form thicker biofilms than wild-type strains on glass coverslips (Hammer and Bassler, 2003), and are also deficient in biofilm detachment (Liu *et al*., 2007). Thus, AI-2 may regulate *V. cholerae* biofilm dispersal.

Glycopeptidolipids

Glycopeptidolipids (GPLs) are monoglycosylated, fatty acylated peptides that are further modified by small variable oligosaccharides (Fig. 4H). GPLs are the dominant immunogenic glycolipids of many mycobacteria (Brennan *et al*., 1981). It has been shown (Freeman *et al*., 2006) that GPLs mediate biofilm dispersal in the opportunistic pathogen *Mycobacterium avium*. Using a recirculating water biofilm reactor meant to simulate the conditions of a drinking water distribution system, these authors found that wild-type and GPL-mutant strains bound equally well to stainless steel coupons in the reactor, but that the mutant cells were present in relatively small numbers in the recirculating water phase compared with the number of wild-type cells. It was hypothesized that the mutants detached inefficiently from the biofilm due to enhanced cell-to-cell interactions.

Phenazines

Phenazines are tricyclic pyrazines that are produced by various bacteria, including members of the genera *Pseudomonas* and *Streptomyces* (Laursen and Nielsen, 2004). Phenazines have been shown to increase the survival of bacteria in natural environments, possibly due to their antimicrobial activity against other microorganisms. *Pseudomonas chloroaphis*, a biological control bacterium, produces two major phenazines, phenazine-1-carboxylic acid (PCA) and 2-hydroxy-PCA (2OH-PCA) (Fig. 4I). It has been shown (Maddula *et al*., 2008) that a *P. chloroaphis* strain deficient in the production of 2OH-PCA and a strain that overproduces 2OH-PCA both exhibited lower biofilm dispersal rates than a wild-type strain when cultured in a flow cell biofilm reactor. The mechanisms by which phenazines regulate biofilm dispersal are not known.

Fatty Acid Signals

Several bacteria secrete unsaturated fatty acids that function as intra- and interspecies cell-to-cell communication signals (Wang *et al*., 2004). In *P. aeruginosa*, the fatty acid *cis*-2-decenoic acid (Fig. 4J) acts as a positive signal that is sensed by bacteria, thereby inducing a cascade of event that results in degradation of the biofilm matrix and biofilm dispersal (Davies and Marques, 2009). Exogenous *cis*-2-decenoic acid has been shown to induce biofilm dispersal in *P. aeruginosa* and other phylogenetically diverse bacteria and some fungi (Davies and Marques, 2009). Another unsaturated fatty acid, *cis*-11-methyl-2-dodecenoic acid, also known as diffusible signal factor or DSF, causes biofilm dispersal in *X. campestris* by up-regulating expression of the biofilm-matrix-degrading enzyme endo-β-1,4-mannanase (Dow *et al*., 2003). Numerous other bacteria secrete fatty acid signals, although their role in biofilm dispersal has not been investigated (Ryan and Dow, 2008).

Peptide Signals

Staphylococci produce and secrete a number of peptide signals that accumulate in the extracellular environment. Evidence suggests that two of these peptides—δ-toxin produced by *S. epidermidis* and *S. aureus* (Fig. 4K), and autoinducing peptide I (AIP-I) produced by *S. aureus* (Fig. 4L)—may play a role in biofilm dispersal.

Mutant strains of *S. epidermidis* that do not produce δ-toxin form significantly more biofilm biomass in microplate wells compared with the amount produced by wild-type strains (Vuong *et al*., 2003). In addition, exogenous δ-toxin decreases biofilm attachment in δ-toxin mutant strains of both *S. epidermidis* and *S. aureus* (Vuong *et al*., 2000, 2003). Microarray analysis has shown that the genes encoding δ-toxin and other related peptide signals are highly down-regulated in *S. epidermidis* biofilms (Yao *et al*., 2005). It is possible that δ-toxin contributes to biofilm dispersal through a physical mechanism that involves its detergent-like properties (Vuong *et al*., 2003).

Autoinducing peptides comprise a family of extracellular cyclic peptide signals produced by different strains of *S. aureus* (Ji *et al*., 1997). Exogenous autoinducing peptide AIP-I induces sloughing of *S. aureus* biofilms cultured in flow cells (Boles and Horswill, 2008; Lauderdale *et al*., 2009). AIP-I may mediate biofilm dispersal by up-regulating expression of the matrix-degrading proteases aureolysin and Spl (Boles and Horswill, 2008).

Nitric Oxide

Nitric oxide (NO), an endogenous product of anaerobic metabolism, has been shown to induce dispersal of *P. aeruginosa* biofilms (Barraud *et al*., 2006). A *P. aeruginosa* strain lacking nitrate reductase, the only enzyme capable of generating metabolic NO through anaerobic respiration, does not disperse, whereas a NO reductase mutant exhibited a hyper-dispersal phenotype. In addition, exogenously added sodium nitroprusside, a NO donor, induces detachment of pre-formed *P. aeruginosa* biofilm colonies. In these experiments, NO was used at sublethal concentrations (25 to 500 nm), suggesting that NO functions as an extracellular signal to mediate the dispersal effect (Romeo, 2006).

Cyclic Diguanyl Monophosphate

Cyclic dimeric GMP (c-di-GMP) is an intracellular signal that regulates the transition from sessile to planktonic growth in a variety of bacteria (Yildiz, 2008). Increased levels of c-di-GMP generally result in an increase in exopolysaccharide and fimbriae production, and a decrease in motility, whereas decreased

levels of c-di-GMP exert the opposite effects and induce biofilm dispersal. c-di-GMP-regulated biofilm dispersal has been observed in many species, including *P. aeruginosa*, *P. fluorescens*, *Salmonella enterica* serovar Typhimurium, *E. coli*, *Shewanella oneidensis*, and various vibrios (Simm *et al*., 2004; Thormann *et al*., 2005, 2006; Morgan *et al*., 2006; Boehm *et al*., 2009; Gjermansen *et al*., 2009; Newell *et al*., 2009; Yildiz and Visick, 2009). It has been shown that c-di-GMP up-regulates the biofilm-matrix-degrading protease LapG in *P. fluorescens* and *P. putida* (Gjermansen *et al*., 2009; Newell *et al*., 2009), up-regulates biofilm matrix polysaccharide production in *E. coli* (Boehm *et al*., 2009), and down-regulates motility in *P. putida* (Gjermansen *et al*., 2006).

CLINICAL RELEVANCE OF BIOFILM DISPERSAL

Biofilm formation is the primary mode of growth for bacteria in most natural and clinical environments. For many pathogenic bacteria, therefore, biofilm dispersal plays a critical role in the transmission of bacteria from environmental reservoirs to human hosts, in the transmission of bacteria between hosts, and in the exacerbation and spread of infection within a single host.

Many pathogens are transmitted to human hosts from environmental reservoirs. The opportunistic pathogen *P. aeruginosa*, for example, lives in soil, water, vegetation, sinks, faucets, respiratory therapy equipment, and on the hands of healthcare workers (Foca *et al*., 2000). Other important pathogens that colonize environmental reservoirs include *Legionella*, *Vibrio*, *Mycobacterium,* and *Listeria*. Biofilm formation plays a key role in the ability of these bacteria to colonize most environmental niches, and biofilm dispersal is their primary means of escaping the biofilm to be translocated to their human hosts. Sloughing dispersal may be an important factor in the transmission of some environmental pathogens, because sloughing can result in the detachment of a sufficient number of cells for an infective dose that is not typically found in bulk fluid (Hall-Stoodley and Stoodley, 2005).

Biofilm dispersal also plays a key role in the communicable transmission of many pathogens. For example, *S. mutans* can detach from dental biofilms in a mother's mouth and be transmitted to an infant by direct or indirect contact (Berkowitz and Jones, 1985). Similarly, *A. actinomycetemcomitans* can be transmitted from person to person by means of a shared toothbrush (Stabholz *et al*., 1998). This type of dispersal may contribute to the host-to-host transmission of many respiratory pathogens (Morris, 2007), and of *V. cholerae* during cholera epidemics (Nielsen *et al*., 2008).

The intra-host spread and persistence of bacteria are also mediated by biofilm dispersal. For example, detached *S. mutans* cells can be translocated to adjacent or opposing teeth by means of salivary flow (Svanberg and Loesche, 1978), and transient bacteremias are frequently detected following dental procedures (Kinane *et al*., 2005). Other examples of intra-host spread include: hospital-acquired pneumonia, caused by bacteria detached from biofilms in a patient's endotracheal tube (Adair *et al*., 1999); infectious kidney stones, caused by bacteria detached from a biofilm in a patient's bladder (Mathoera *et al*., 2000); and embolic events in endocarditis (Parsek and Singh, 2003). Twitching motility, a mechanism of surface translocation and a potential mode of biofilm dispersal, may contribute to persistence of *P. aeruginosa* in the lungs (Chiang and Burrows, 2003).

Although biofilm dispersal clearly plays a major role in disease transmission, few studies have examined the role of biofilm dispersal in pathogenesis. It has been shown (Bieber *et al*., 1998) that biofilm dispersal is required for full virulence of enteropathogenic *E. coli* in human volunteers. Using a model that measures diarrhea following oral inoculation, these authors found that a mutant strain deficient in the production of type IV bundle-forming pili, which are required for biofilm dispersal, was 200-fold less virulent than a wild-type strain. In the plant pathogen *X. campestr*is, biofilm dispersal mediated by production of the matrix-degrading enzyme endo-β-1,4-mannanase was required for full virulence of the bacterium in plants (Dow *et al*., 2003).

POTENTIAL THERAPEUTIC USES

An anticipated offshoot of research on biofilm dispersal is the development of novel therapeutic and prophylactic approaches for the treatment of biofilm infections. Some classes of agents that may have clinical utility are biofilm-matrix-degrading enzymes, quorum-sensing signals, surfactants, and small molecule inhibitors of bacterial diguanylate cyclases.

Among the biofilm-matrix-degrading enzymes, dispersin B of *A. actinomycetemcomitans* has received the most attention. *In vitro*, exogenously added dispersin B has been shown to inhibit biofilm formation, detach pre-formed biofilms, and sensitize pre-formed biofilms to killing by antimicrobial agents, bacteriophages, and host defenses in phylogenetically diverse bacteria (Kaplan *et al*., 2004a; Itoh *et al*., 2005; Lu and Collins, 2007; Izano *et al*., 2008a,b; Venketaraman *et al*., 2008). *In vivo*, dispersin B completely eliminated *S. aureus* port-related bloodstream infections in catheterized sheep when used in combination with teicoplanin as a catheter lock solution (Jose del Pozo, personal communication), and reduced the rate of *S. aureus* catheter colonization from 97% to 3% when used in combination with triclosan as a catheter coating in a rabbit model of subcutaneous implant infections (Darouiche *et al*., 2009). One of the main advantages of dispersin B and other matrix-degrading enzymes is that they do not kill bacteria or inhibit their growth. This reduces the chances for the evolution of resistance to these agents.

Alginate lyase is another matrix-degrading enzyme that has therapeutic potential against biofilm-related pulmonary infections caused by *P. aeruginosa*. *In vitro*, alginate lyase reduces the viscoelasticity of purulent sputum from individuals with cystic fibrosis (Mrsny *et al*., 1994) and enhances the antibiotic killing of mucoid *P. aeruginosa* in biofilms (Alkawash *et al*., 2006; Alipour *et al*., 2009). *In vivo*, alginate lyase increases the effectiveness of amikacin against mucoid strains of *P. aeruginosa* in a rabbit model of infective endocarditis (Bayer *et al*., 1992). Alginate lyase, when used in combination with deoxyribonuclease, may be useful for the treatment of alginate polysaccharide build-up in the lungs of individuals with cystic fibrosis (Wong *et al*., 2000; VanDevanter and Van Dalfsen, 2005).

Many small molecules have been shown to induce biofilm cell detachment *in vitro* (Fig. 4), and some of these may have clinical applications. The NO donor sodium nitroprusside, for example, induces detachment of pre-formed *P. aeruginosa* biofilm colonies *in vitro* and greatly enhances the efficacy of antibiotics in the

removal of the biofilms (Barraud *et al*., 2006). However, one study showed that sodium nitroprusside increased biofilm formation by *P. aeruginosa* and *Burkholderia cenocepacia in vitro* (Zaitseva *et al*., 2009). Quorum-sensing autoinducers, analogues, and antagonists are another class of promising anti-biofilm agents, but their utility in the clinic has still not been demonstrated. This may be because quorum-sensing circuitry is extremely complex, and the biological activity of these compounds is difficult to predict from *in vitro* studies. Unsaturated fatty acids are another example of signaling molecules that exhibit broad-spectrum biofilm-detaching activity *in vitro* (Davies and Marques, 2009), but their effectiveness *in vivo* has not been evaluated.

The c-di-GMP signaling pathways have received considerable attention over the past few years. Diguanylate cyclase enzymes are a very attractive target for antimicrobial therapy, because they are found only in bacteria and not in eukaryotic cells. Since decreased levels of intracellular c-di-GMP induce biofilm dispersal, inhibitors of these enzymes should inhibit biofilm formation or promote biofilm dispersal. However, diguanylate cyclases comprise a large super-family of enzymes, with many bacteria having dozens of homologues. The biological effect of diguanylate cyclase inhibition, therefore, is not easy to predict (Yildiz, 2008). The recent reporting of the three-dimensional structures of two guanylate cyclases, PleD from *Caulobacter crescentus* and FimX from *P. aeruginosa* (Wassmann *et al*., 2007; Navarro *et al*., 2009), should facilitate the discovery of small-molecule diguanylate cyclase inhibitors by rational drug design.

CONCLUSIONS

Research on biofilm dispersal is in its infancy. Virtually all dispersal studies have been performed *in vitro* under the controlled conditions of a laboratory, and most of these were performed with monospecies biofilms. It is extremely difficult to extrapolate these results to any environmental biofilm, especially a complex biofilm community such as dental plaque. Although numerous potential dispersal-inducing agents have been identified, it remains to be seen whether any of these agents will have clinical significance.

Sessile organisms such as plants and fungi have evolved a multitude of ingenious strategies to disperse seeds and spores into the environment to colonize new sites. In fact, burrs, helicopter seeds, and spore-shooting fungi represent some of the most remarkable evolutionary adaptations in nature. It is likely that biofilm bacteria have evolved similarly diverse dispersal mechanisms that are waiting to be discovered. There is a good chance that continued advances in biofilm dispersal research will soon lead to the development of novel therapies based on these findings.

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