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Pseudomonas aeruginosa biofilms: role of the alginate exopolysaccharide

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Pseudomonas aeruginosa **synthesizes an exopolysaccharide called alginate in response to environmental conditions. Alginate serves to protect the bacteria from adversity in its surroundings and also enhances adhesion to solid surfaces. Transcription of the alginate biosynthetic genes is induced upon attachment to the substratum and this leads to increased alginate production. As a result, biofilms develop which are advantageous to the survival and growth of the bacteria. In certain circimstances, *P. aeruginosa* produces an alginate lyase enzyme which cleaves the polymer into short oligosaccharides. This negates the anchoring properties of the alginate and results in increased detachment of the bacteria away from the surface, allowing them to spread and colonize new sites. Thus, both alginate biosynthetic and degradative enzymes are important for the development, maintenance and spread of *P. aeruginosa* biofilms.**

Keywords: biofilm anchoring; alginate gene activation; biofilm detachment; alginate lyase

Alginate and *Pseudomonas aeruginosa*

Alginate is a linear (1→4)-linked exopolysaccharide (EPS) of β-D-mannuronic acid and the C-5 epimer α-L-guluronic acid (Figure 1) [31]. This polymer is produced by brown seaweeds (*Phaeophyceae*) and by some prokaryotes, for example *Pseudomonas* and *Azotobacter* species. In bacteria, the mannuronic acid residues can be acetylated at the 2 and/or 3 positions [21]. *P. aeruginosa, P. fluorescens* and *P. syringae* are a few of the *Pseudomonas* species that are capable of synthesizing alginate [31–33,40]. *P. syringae* is a plant pathogen and epiphyte of a wide range of temperate zone crops and trees. *P. fluorescens* is often found as the predominant organism of biofilms contaminating water cooling systems. *P. aeruginosa* is a widespread organism that can be found in a variety of habitats including soil and freshwater streams. In these environments the microbes are found predominantly attached to solid surfaces. Not all *P. aeruginosa* strains produce alginate and some may be capable of synthesizing alternate exopolysaccharides. This bacterium is of particular interest as a human pathogen. It is primarily an opportunistic pathogen, infecting patients with

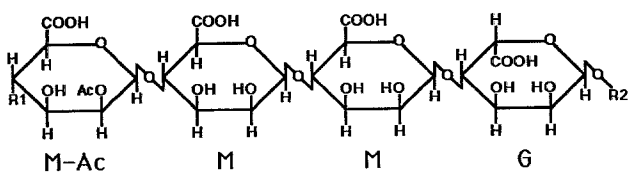

Figure 1 Structure of the alginate exopolysaccharide produced by *P. aeruginosa*. M, mannuronic acid; G, guluronic acid; M-Ac, acetylated mannuronic acid

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predisposing conditions, such as burns, open wounds, immunosuppression or indwelling medical devices. *P. aeruginosa* can also infect the lungs of cystic fibrosis (CF) patients. This disease is characterized by disturbances in electrolyte transport and secretions of the exocrine glands and the secretory epithelium [55]. This results in, amongst other things, secretion of elevated amounts of abnormally viscous mucus by the lung epithelial cells. This impairs lung function and is an open invitation to infection. The CF lung becomes infected with a progression of different bacterial species, including *P. aeruginosa.*

Biofilms and alginate

EPS has been proposed to play an important role in initial adhesion, as well as secure anchoring, of bacteria to solid surfaces [52,53,63]. However, there are also reports which propose that for some bacteria EPS production does not enhance attachment and may even be a hindrance [6,66,80]. Alginate has been shown to improve attachment and anchoring of *P. aeruginosa* to solid surfaces (see below). The interactions of the EPS with the surface and its conditioning film may influence the initial reversible association of bacteria with the substratum and may help overcome the electrostatic repulsion that exists between the cell and the surface, leading to increased irreversible adherence and thus anchoring the cell to the substratum [1,16,54,56]. It is often not possible to distinguish between the effect of alginate in the initial adhesion process and its role in subsequent colonization and cell anchoring. Alginate is important for the development of *P. aeruginosa* monospecies biofilms as it is the main constituent of the glycocalyx. Non-EPS-producing bacterial strains, including those of *P. aeruginosa*, can attach to solid surfaces but are unable to form mature biofilms [6,59]. Mucoid *P. aeruginosa* strains (ie those that produce alginate) attach at higher frequencies to hamster tracheal epithelial cells than nonmucoid (non-alginate

producing) strains [53] and exogenously added alginate can enhance *P. aeruginosa* attachment [63]. Mucoid strains also attach better to inorganic substrates, such as dacron fibers, when compared to nonmucoid strains. This increase can be mitigated by the use of monoclonal antibodies to the alginate or by the addition of a *Bacillus circulans* alginate-degrading enzyme [52]. These experiments implicate alginate as being important for attachment of *P. aeruginosa* to solid surfaces. However, it should be noted that the nature of the surface and of the alginate are both critical factors as to whether or not alginate does have a positive effect on adhesion. Alginate isolated from *P. aeruginosa* strain 492c bound to human buccal and tracheal epithelial cells, whereas alginate from strain 492a bound neither [29]. Also, a mucoid *P. aeruginosa* strain which produced nonacetylated alginate did not attach to a germanium surface, while the parent mucoid strain with acetylated alginate did, forming mature biofilms [59].

P. aeruginosa in the CF lung and in the environment is present predominantly as microcolonies enclosed in a glycocalyx of alginate, forming a biofilm [48]. *P. aeruginosa* is intrinsically recalcitrant to antibiotic and biocide treatment and causes persistent infections, never being completely eradicated [58]. There are several advantages that alginate confers on *P. aeruginosa*. Alginate enhances resistance to antimicrobial agents by hindering the passage of these compounds to the bacteria, by binding the compounds and/or by inactivating them [8,9]. The ability of the host's defense systems to attack the infection are inhibited by the presence of alginate surrounding the cells [8,9]. Exopolysaccharide can protect bacteria from dehydration as it can bind several times its own volume of water and only slowly becomes desiccated [60,65]. The presence of acetylated uronic acids in bacterial alginate increases its hydration capacity. Thus, the production of alginate is a general protection mechanism for the bacteria.

Growth as a biofilm also serves to protect the bacteria (Table 1). The host defense response to *P. aeruginosa* grown in biofilms is greatly reduced compared to free cells [43]. The biofilm mode of growth protects the sessile bacteria from concentrations of antibiotics and biocides which would swiftly kill planktonic cells [41]. These resistances caused by alginate production and biofilm formation are probably responsible for the difficulty involved in the clearing of mucoid *P. aeruginosa* from sites of contamination and infection, including the lungs of CF patients [8]. Plasmid stability is enhanced, as is genetic exchange due to the close proximity of the bacteria to one another. Microenvironments can develop since growth as a biofilm allows modification of the bacterial surroundings. Biofilm cells would also be expected to be influenced greatly by cell-to-cell communication, for instance autoinducer molecules. These molecules are produced when bacteria are present in high concentrations, close together as in a biofilm. Nonmucoid *P. aeruginosa* strains synthesize an autoinducer molecule that is responsible for promoting production of the elastase protein [61]. The ability of a subpopulation of cells that is not actively producing alginate to selectively stimulate elastase activity while in high concentration at a surface which could be a potential substrate for the elastase (eg the CF lung and blood vessels), would be beneficial to the bacteria. Growth in a biofilm may lead to improved viability as it allows accessibility to nutrients that are within the surface or have absorbed to it and to the metabolic products of other microorganisms. Biofilm exopolysaccharides can retain and capture nutrients, ions and other compounds and may be responsible for concentrating foci of extracellular factors of *P. aeruginosa* [19].

The differences between sessile and planktonic bacteria seem to result from a combination of factors: the inaccessibility of underlying cells to the external environment, the surrounding glycocalyx, and the intrinsic physiological properties of biofilm cells compared to planktonic cells [14,19,50]. The glycocalyx and the overlying cells cause a decrease in diffusion throughout the biofilm, by blocking, binding, utilizing or inactivating compounds, leading to gradients of nutrients, pH, metabolic waste products, etc. When an established biofilm of *P. fluorescens* was treated with the antimicrobial agent fleroxacin the underlying cells were less affected than the uppermost ones [47]. Despite the presence of channels within the biofilm, these lower cells were protected by the overlying cells and the glycocalyx. Microenvironments can form and due to the differing conditions within the biofilm the cells become physiologically diverse [50]. It has been shown that the cellular metabolic activity, as measured by the adenylate energy charge, decreases with depth into a biofilm of *P. aeruginosa* [45]. It was proposed that the underlying cells were diffusion limited for nutrients and oxygen, so leading to the low energy status of these bacteria. Biofilm cells generally have slower growth rates than planktonic cells. A decrease in growth rate increases the resistance of bacteria to antibacterial compounds, as well as having a range of other effects [30]. Biofilm cells produce a spectrum of proteins which is distinct from that produced by planktonic cells, and differences in the expression of outer membrane proteins of *P. aeruginosa* in biofilms compared to free cells have been reported [57]. Such changes in the composition of the cell envelope could profoundly affect microbial physiology. Older biofilms are more resistant to antimicrobial action than newly formed biofilms, suggesting that it may take some time for the particular properties of the biofilm to develop [7]. The cells of new biofilms are still metabolically active, few layers have formed and polysaccharide synthesis may not yet be maximal. A combination of all,

Table 1 Advantages of biofilms for *P. aeruginosa* growth and persistence

Protection from environment
Increased resistance to antibiotics and other biocides
Protection from desiccation
Decreased susceptibility to killing by host defense mechanisms
Retention and concentration of nutrients
Retention and binding of cations
Concentration of extracellular virulence factors
Utilization of nutrients at surface
Utilization of metabolic products of other bacteria
Increased plasmid stability and genetic exchange
Enhanced cell-to-cell communication
Microenvironment establishment and maintenance

or some, of these factors is responsible for biofilms conferring on *P. aeruginosa* advantages for survival and growth.

Alginate regulation

The ability of the CF lung environment to trigger the synthesis of alginate by *P. aeruginosa* may provide clues to regulation of production of this EPS in the CF lung, as well as in other situations. *P. aeruginosa* initially infects the CF patient with a nonmucoid phenotype in the upper region of the respiratory tract. As time proceeds the bacteria progress down to the lower respiratory regions and become alginate-producing. The bacteria can adhere to human tracheal epithelial cells and alginate enhances this attachment [53,63]. Mucoid *P. aeruginosa* are characteristic of CF infections. Other clinical infections by *P. aeruginosa* are not normally alginate-producing. The CF lung environment is unique in a number of ways that may be stimulatory for alginate production by *P. aeruginosa*. The CF sputum is of lower water content as compared to mucus secreted by a non-diseased lung. The concentrations of calcium, chloride and sodium ions are elevated and there are increased amounts of free cellular products—DNA, LPS, protein, etc—as well as reactive oxygen species, due to lysis of host and bacterial cells that have been damaged, either by the products of the infecting bacteria or by the host's own defense systems. In addition, CF patients undergo longterm antibiotic treatment in an effort to minimalize bacterial infections. All of these factors contribute to a stressful environment for the bacteria.

A number of environmental signals increase alginate production by mucoid strains and/or turn on alginate synthesis in *P. aeruginosa*, ie they convert nonmucoid strains to mucoidy. These include nutrient starvation, antibiotic treatment, slow growth rate, ethanol dehydration, high osmotic pressure and high ionic strength [27,31,39,77]. In general it seems that it is environmental stress, such as oligotrophic (ie nutrient-poor) conditions, that is the signal for alginate production. A decrease in available nutrients has been observed to lead to a starvation strategy—the cells dwarf and the energy expenditure rises temporarily [46]. Bacteria in such starved conditions attached to solid surfaces coated with lipids which allowed enhanced growth of the bacteria [46]. In non-clinical situations, the oligotrophic nature of the surroundings would induce alginate synthesis allowing increased protection and attachment. Infection by *P. aeruginosa* of the CF lung and subsequent conversion to mucoidy seems to be an unfortunate coincidental adaptation. *P. aeruginosa* infection of the CF lung is a relatively recent development, as it was only as the age of these patients has lengthened due to improved medical treatments that *P. aeruginosa* infection has become a problem. This seems too short a time in which a bacterial species could develop a totally new virulence factor in order to improve viability, but plenty of time in which to put to use a previously-existing adaptative mechanism. The ability of non CF-infecting *Pseudomonas* species to produce alginate supports this idea [33,40]. To a certain extent enzymes for alginate biosynthesis are recruited from other metabolic processes, such as phosphomannomutase-phosphoglucomutase (AlgC) from lipopolysaccharide production [38]. There is phenotypic selection for mucoidy by the CF lung environment stimulating alginate production and also genotypic selection as it is those cells which have undergone changes in the EPS gene expression to produce copious amounts of alginate that will survive (see below).

P. aeruginosa attached to a solid surface produced both a high and a low molecular weight EPS, whereas in liquid culture there was only high molecular weight polymer [3]. Whether the two forms of EPS have differing structures or whether the smaller is a degradation product of the larger one was not reported. Growth rate greatly affected EPS production by the adherent bacteria, with more polymer being produced at slower growth rates. It has also been shown that EPS production by adherent bacteria was greatest at the stage of irreversible attachment and at the microcolony-forming stage, while the bacteria were still metabolically active [5]. When mucoid strains of *P. aeruginosa* attached to a solid surface of either silicone rubber or Teflon fibers there was an increase in the amount of alginate detected [22,42]. These results could have been due to selection of a sub-population of mucoid cells that produced more alginate. It was reported by Vandevivere and Kirchman in 1993 that exopolysaccharide production increased with attachment of bacteria to a solid surface and that this increase was not due to preferential attachment of a genotypic subpopulation with increased exopolysaccharide production, as reinoculation of the biofilm bacteria into liquid medium resulted in reduction of exopolysaccharide production to the level previously found in planktonic cells [78]. Concomitant with the increase in alginate, both of the studies with *P. aeruginosa* [22,42] measured an increase in the activation of promoters controlling production of enzymes essential for alginate synthesis (see below).

Alginate genes

The biosynthetic pathway of alginate in *P. aeruginosa* has been mostly elucidated (Figure 2a) [72]. The sugar precursor that is utilized is fructose-6-phosphate (F6P) from the Entner–Doudoroff pathway. F6P is converted to mannose-6-phosphate (M6P) by the action of phosphomannose isomerase (PMI) which is one of the functions of the bifunctional AlgA protein [73]. Phosphomannomutase (PMM), encoded by the *algC* gene, converts M6P to mannose-1-phosphate (M1P) [82]. M1P is the substrate for GDP-mannose pyrophosphorylase (GMP), the other enzymatic activity of AlgA, and with GTP forms GDP-mannose (GDP-Man). GDP-Man is oxidized by GDP-mannose dehydrogenase (GMD, AlgD) to produce GDP-mannuronic acid (GDP-ManA) [67]. This is the unit for polymerization and export through the inner membrane, processes in which the *alg8* and *alg44* gene products, both probably membrane-bound proteins, may be involved [51]. The enzymes responsible for epimerization of mannuronic acid to guluronic acid (AlgG), for acetylation of mannuronic residues (AlgF) and for cleavage of the alginate polymer (AlgL) are all located within the periplasmic space [11,34,35,68,74]. Alg60 is a protein required for alginate synthesis, which may also be involved in the process of alginate acetylation as a domain of its sequence shows similarity to acyl carrier proteins (S Mukhopadhayay and A Boyd, unpublished

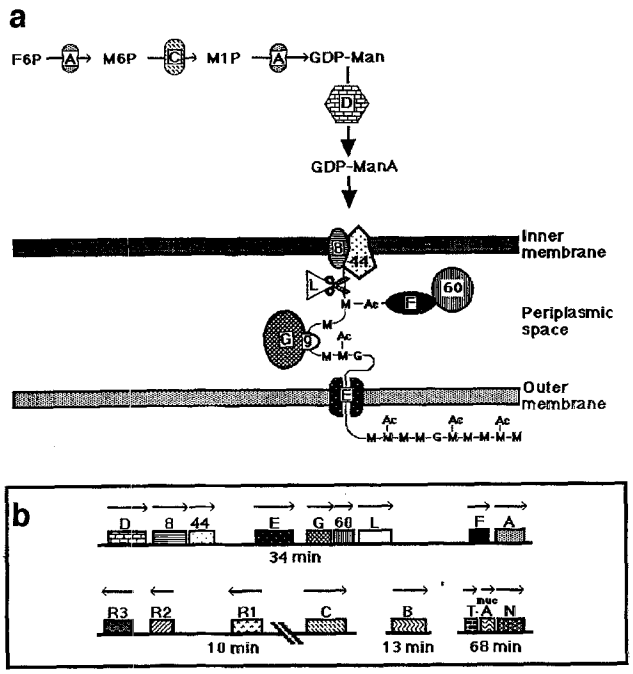

Figure 2 (a) The pathway of alginate exopolysaccharide synthesis by *P. aeruginosa*. For details see text. (b) The genes involved in alginate biosynthesis. The minutes refer to the position of these operons on the *P. aeruginosa* chromosome. Arrows indicate direction of transcription. See text for further details

data). AlgE is an outer membrane protein which acts as an ionic channel and could export the alginate polymer out of the cell [64]. All but one of the identified biosynthetic genes are located in an operonic-like cluster at 34 min on the chromosome (Figure 2b) [17]. The exception is *algC* which is located at the 10 min region of the chromosome [75].

A number of proteins have been identified as playing a role in the regulation of alginate production. Most of the known regulators act at the transcriptional level. There is translational control of the *algC* and *algD* transcripts, but the *trans*-acting factors involved, if any, are as yet unidentified [36]. Two response regulator transcription factors, AlgB and AlgR1 (also known as AlgR), are involved in controlling alginate biosynthesis [24,79]. Response regulators operate with a sensor protein in a two-component regulatory system, but no sensor protein is known for alginate regulation. AlgT (AlgU) is a putative sigma factor that globally regulates the genes involved in alginate production [25]. The activity of AlgT is negatively regulated by MucA and AlgN (MucB), the genes of which are all present in the same operon (Figure 2b) [25]. Spontaneous mutations in *algT* lead to conversion to nonmucoidy, whereas mutations in *mucA* lead to mucoidy [28,71]. Recently, the AlgT protein has been purified and its role as an alternate sigma factor (σ^E) has been demonstrated (CD Hershberger *et al*, Proc Natl Acad Sci USA, in press). AlgR3 is a histone-like protein that is necessary for optimal transcription of the alginate structural genes [44]. *P. aeruginosa* strains carrying mutations in *algR2 (algQ)* are nonmucoid and show a decrease in the activities of nucleoside diphosphate kinase (Ndk), which regulates the pool of nucleotides in the cell, and succinyl coenzyme A synthetase (Scs), which is involved in the TCA cycle, leading to a decrease in the energy status of the cell [69,70]. It has been proposed that carbon flux, ATP synthesis and EPS production are all integrated processes in the cell [49]. For efficient alginate production there must be a balance between carbon flow, energy generation (such as TCA cycle operation) and growth of the organism. Any significant alteration in these processes affects EPS production.

Regulation of alginate genes during biofilm development

The activation of transcription of the *algD* promoter has been studied extensively [10,25–27,42]. The *algD* promoter appears to control the transcription of all the known alginate biosynthetic structural genes apart from *algC* and as such regulation of this promoter is a major control point in alginate synthesis [17]. The activity of this promoter is influenced by external factors similarly to how environmental conditions affect alginate production, as may be expected. For example, high osmolarity, nutritional deprivation and the presence of ethanol all increase *algD* transcriptional activity [10,26,27]. The transcriptional activity has been measured by fusing the DNA containing the *algD* promoter region to a reporter gene such as *lacZ* or *xylE*. Both of these genes code for proteins whose activities are easily and reliably quantified *in vitro*. Increased activation of the *algD* promoter leads to increased transcription of the reporter gene and so to increased reporter enzyme expression and activity.

A mucoid strain of *P. aeruginosa* that carried a plasmid with an *algD-lacZ* transcriptional fusion was used by Hoyle *et al* to investigate the effect of attachment to a solid surface of silicone rubber on the transcription of genes controlled by the *algD* promoter [42]. Attached cells had higher *algD* promoter activity than free swimming cells. In addition, there was an increased amount of carbohydrate associated with the biofilm cells as compared to the cells in the aqueous seeding medium. Davies *et al* performed similar experiments with an *algC-lacZ* transcriptional fusion [22]. *P. aeruginosa* attached to Teflon fibers had higher transcription of the *algC* gene than unattached cells. Davies and Geesey showed further that the *algC* promoter is specifically activated subsequent to attachment of the bacteria [23]. Individual cells were observed over time by fluorescence microscopy to detect *lacZ* activity. A cell having no fluorescence prior to adhesion would become fluorescent a few minutes after it had attached to the surface. This experiment provides direct evidence for activation of transcription of an alginate biosynthetic gene following attachment to a solid surface. Those cells that initially adhered to the surface and did not activate *algC* transcription, quickly dissociated from the substratum, while those cells that did activate *algC* remained attached. This indicates that transcription of alginate biosynthetic genes and concomitant alginate production are required to anchor *P. aeruginosa* securely to the substratum.

These experiments show that attachment to a solid surface activates alginate biosynthesis in *P. aeruginosa*, and that this is brought about by increased transcription of the alginate structural genes. Specific activation of a subset of

Table 2 Effect of alginate and alginate degradation on cell detachment of *P. aeruginosa*

P. aeruginosa strains	Phenotype	Alginate lyase activity[a]	Extensive alginate degradation	Cell detachment (fold increase)
8821	mucoid	+	no	1
8830	mucoid	±	no	1
8822	nonmucoid	±	N/A[d]	57
8830(pMMB22)[b]	mucoid	±	no	1
8830(pSK700)[c]	mucoid	+++	yes	20

[a]±, barely detectable lyase activity; +, low lyase activity; +++, high lyase activity
[b]Plasmid pMMB22 acts as the vector control
[c]Plasmid pSK700 allows enhanced production of the alginate lyase protein. Even though this leads to alginate degradation, the 8830 strain remains mucoid
[d]N/A, not applicable, since strain 8822 is a non-alginate producer

genes in *Pseudomonas* species S9 occurs upon growth in the presence of a solid surface but not in liquid broth or on agar plates, although the identity of these genes has not been determined [20]. A number of suggestions have been put forward as to how attachment to the substratum enhances alginate synthesis. Outer membrane proteins have been implicated in the regulation of EPS synthesis in other bacterial species [18]. These could be membrane sensors that are activated upon attachment ('touch receptors'). If such a sensing protein(s) is involved in alginate biosynthesis by *P. aeruginosa*, it should be possible to isolate mutants that show no activation of *algC* or *algD* transcription upon attachment. Physiological studies of such mutants would provide interesting information on how alginate genes are regulated in biofilms. Another possibility is that integral changes in the cellular membrane occur when it comes in contact with another surface, which could lead to signaling for EPS production. Alternatively, it is possible that the differences in the physiological and energy status of biofilm cells as compared to planktonic cells are responsible for increasing alginate synthesis, rather than attachment *per se*.

Influence of alginate on biofilm detachment

Biofilm detachment refers to the dissociation of biofilm particles away from the biofilm into the surrounding environment. This process balances microbial growth and thereby determines the steady state accumulation of the biofilm and overall biofilm activity. The detached bacteria provide an inoculum for growth of a non-attached population and for colonization at new sites. Five categories of detachment have been identified—erosion, sloughing, abrasion, human intervention and predator grazing [15]. Erosion is the detachment of single cells and small clusters of cells, while sloughing refers to the removal of large areas of biofilm. Abrasion is caused by collisions of solid particles with the biofilm. Physical or chemical elimination by human intervention and predation grazing by organisms such as protozoa and snails also lead to a decrease in biofilm mass.

Turbulence in the surrounding environment may cause bacteria to detach from a solid surface. However, in many situations the shear stress would not be sufficient to influence the biofilm. It has been reported that a decrease in metabolic activity brought about by starvation leads to increased detachment of bacteria from surfaces [13]. Starvation also caused cells of *Pseudomonas* species S9 to detach from a hydrophobic surface of siliconized glass, due to production and release of a neutral exopolysaccharide [80]. It has been shown that detachment and growth are directly related, as factors which limit the growth rate of a biofilm will also limit the detachment rate [62,76]. This seems to be due to cell-cycle-mediated events. Detachment of both *Escherichia coli* and *P. aeruginosa* are influenced by these events [2,4]. Analysis of newly detached cells showed that compared to cells in the biofilm they had a low hydrophobicity which steadily increased with time following detachment. The surface hydrophobicity of cells is lower at and just after division, and so the greatest dislodgment of bacteria occurs at this stage in the cell cycle [37]. Thus, in addition to external conditions, the status of the cells within the biofilm also influences detachment rates.

Alginate production is associated with an increase in adhesion of *P. aeruginosa* to solid surfaces [52,53,63]. It has been shown that a nonmucoid strain of *P. aeruginosa* detached with a much higher frequency from an agar surface than did mucoid strains (Table 2) [12]. Alginate anchored the bacteria onto the substratum with greater tenacity, decreasing detachment of the cells, whereas the bacteria that did not produce copious amounts of alginate exopolysaccharide could freely disperse away from the substratum. *P. aeruginosa* has a multifactorial system for the activation of alginate synthesis. It would be beneficial also to have a way to remove the alginate when it is no longer useful, or maybe even detrimental, to the bacteria. *P. aeruginosa* possesses an alginate lyase enzyme which is capable of cleaving the bond between two uronic acids by an eliminase mechanism to yield two shorter alginate polymers [11,68]. Further action of the lyase results in continued depolymerization of the alginate to oligosaccharides.

The gene encoding the alginate lyase (*algL*) has been cloned under the control of the *tac* promoter. Induction of the alginate lyase in a mucoid *P. aeruginosa* strain carrying this construct led to a decrease in the length of the alginate polymer. Associated with this decrease in size were elevated levels of bacterial detachment (Table 2) [12]. The action of the alginate lyase affects the ability of the alginate to anchor the cells to the substratum. In an analogous man-

ner the bacterium *Methanosarcina mazei* produces a capsular depolymerase under conditions of stress that causes cell aggregates to dissociate [81]. It could be that under certain circumstances in *P. aeruginosa*, the alginate lyase activity may be preferentially enhanced resulting in increased detachment of the bacteria into the surrounding environment and dispersal of the microbes to new locations. This seeding of the aqueous medium can be problematic in both environmental and industrial situations. *P. aeruginosa* progresses from the upper to the lower respiratory tract during the course of infection of the CF patient and spread between sufferers of this disease is a problem in CF clinics. Detachment would allow the bacteria to disperse to colonize new surfaces when local conditions became unsuitable for survival and growth. Such stressful conditions that could be found in a biofilm include starvation, anaerobiosis or toxicity. Any of these could be signals for enhanced alginate lyase activity.

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