Pseudomonas Aeruginosa Biofilm Formation In Cystic Fibrosis

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Abstract- *The persistence of chronic Pseudomonas aeruginosa lung infections in cystic fibrosis (CF) patients is due to biofilm-growing mucoid (alginate-producing) strains. A biofilm is a structured consortium of bacteria, embedded in a self-produced polymer matrix consisting of polysaccharide, protein and DNA. In CF lungs, the polysaccharide alginate is the major part of the P. aeruginosa biofilm matrix. Bacterial biofilms cause chronic infections because they show increased tolerance to antibiotics and resist phagocytosis, as well as other components of the innate and the adaptive immune system. As a consequence, a pronounced antibody response develops, leading to immune complex-mediated chronic inflammation, dominated by polymorphonuclear leukocytes. The chronic inflammation is the major cause of the lung tissue damage in CF. Biofilms can be prevented and reduced by early aggressive antibiotic prophylaxis or therapy, and they can be treated by chronic suppressive therapy.*

Keywords- CFTR, PMNs, QS

I. INTRODUCTION

A biofilm is any group of microorganisms in which cells stick to each other and often these cells adhere to a surface. Surface-associated communities of bacteria are known as biofilms bacteria and they play very important role in the pathogenesis of many chronic infections. According to researchers, a biofilm bacterium is 1000 times immune to lone bacteria against antibiotics.

Biofilms can contain many different types of microorganism.
E.g. bacteria, archaea, protozoa, fungi and algae.

This paper would be mainly being focusing on *Pseudomonas aeruginosa.* It is a multidrug resistant pathogen recognized for its ubiquity, its intrinsically advanced antibiotic resistance mechanisms. Biofilms of *P. aeruginosa* can cause chronic opportunistic infections, which are a serious problem for medical care in industrialized societies. They often cannot be treated effectively with traditional antibiotic therapy. Biofilms seem to protect these bacteria from adverse environmental factors. *P. aeruginosa* can cause nosocomial infections and is considered a model organism for the study of antibiotic-resistant bacteria. Cystic fibrosis is one disease where *P. aeruginosa* plays a very big role to make this disease incurable. Microscopic examinations of sputum samples and lung tissue sections have revealed the presence of micro colonies and large biofilm of *P. aeruginosa.* [1]

II. PSEUDOMONAS AERUGINOSA AND CYSTIC FIBROSIS

P. aeruginosa is a common Gram-negative, rod-shaped bacterium that can cause disease in plants and animals, including humans. The organism is considered opportunistic insofar as serious infection often occurs during existing diseases or conditions – most notably cystic fibrosis and traumatic burns. It is found in soil, water, skin flora, and most man-made environments throughout the world. It thrives not only in normal atmospheres, but also in low-oxygen atmospheres, thus has colonized many natural and artificial environments. Such colonization's occur in critical body organs, such as the lungs, the urinary tract, and kidneys, and the results can be fatal.
Genome of *P. aeruginosa* is 5.5–6.8 Mb and they often cannot be treated effectively with traditional antibiotic therapy.

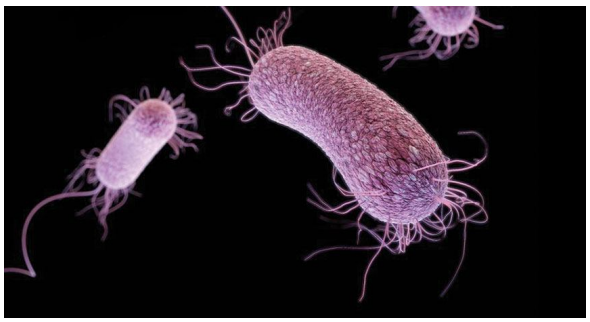

Figure 1- *Pseudomonas aeruginosa*

Now Cystic fibrosis is an inherited disease characterized by the buildup of thick, sticky mucus that can damage many of the body's organs. In people with cystic fibrosis, the body produces mucus that is abnormally thick and sticky. This abnormal mucus can clog the airways, leading to severe problems with breathing and bacterial infections in the lungs. These infections cause chronic coughing, wheezing,

and inflammation. Over time, mucus buildup and infections result in permanent lung damage, including the formation of scar tissue (fibrosis) and cysts in the lungs.

Cystic fibrosis is a common genetic disease within the white population in the United States. The disease occurs in 1 in 2,500 to 3,500 white newborns. Cystic fibrosis is less common in other ethnic groups, affecting about 1 in 17,000 African Americans and 1 in31,000 Asian Americans. [4]

Mutations in the *CFTR* gene cause cystic fibrosis.

It provides instructions for making a channel that transports negatively charged particles called chloride ions into and out of cells. Chloride is a component of sodium chloride, a common salt found in sweat. Chloride also has important functions in cells; for example, the flow of chloride ions helps control the movement of water in tissues, which is necessary for the production of thin, freely flowing mucus.

Mutations in the *CFTR* gene disrupt the function of the chloride channels, preventing them from regulating the flow of chloride ions and water across cell membranes. As a result, cells that line the passageways of the lungs, pancreas, and other organs produce mucus that is unusually thick and sticky. This mucus clogs the airways and various ducts, causing the characteristic signs and symptoms of cystic fibrosis. [3]

III. BIOFILM FORMATION

Numerous lines of evidence have confirmed that P. aeruginosa persists in CF lungs as biofilms. Biofilms can grow to larger than 100 μM in diameter, and the bacteria within these biofilms have been identified as P. aeruginosa by fluorescent in situ hybridization (FISH)Biofilms are enclosed within an exopolymer matrix that can restrict the diffusion of substances and bind antimicrobials. This will provide effective resistance for biofilm cells against large molecules such as antimicrobial proteins lysozyme and complement. The diffusion barrier is also probably effective against smaller antimicrobial peptides, numerous defensins. The negatively charged exopolysaccharide is very effective in protecting cells from positively charged aminoglycosididic antibiotics by restricting their permeation, possibly through binding. Hence the disease may assume the chronic forms.

There are 3 stages of biofilm development:

1. Irreversible attachment
2. Maturation
3. Dispersion

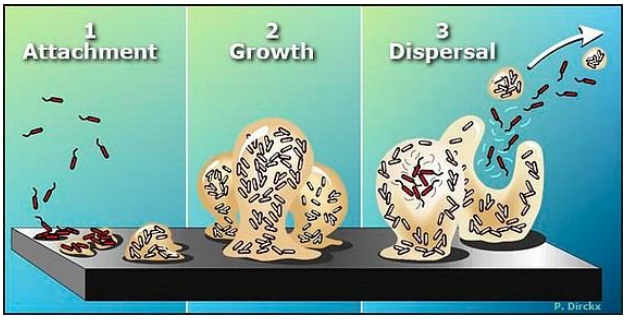

Figure 2- Biofilm dispersal

Also bacterial communication via quorum sensing has been reported to be important in the production of virulence factors and antibiotic resistance. It has therefore been suggested that quorum sensing may contribute to the ability of *P. aeruginosa* to initiate infection and to persist in a host as a biofilm. Data from many models of both acute infection and chronic infection have supported the hypothesis that quorum sensing is important in *P. aeruginosa* pathogenesis and biofilm formation.

One protein known to play a key role in biofilm formation is RpoS, the σ^S subunit of RNA polymerase. It governs the expression of many genes induced during the stationary phase of growth and is considered to be the master regulator of the general stress response in *E. coli*. It was noted that 46% of the genes that were found to be differentially expressed during biofilm growth were under the control of RpoS, and deletion of *rpoS* rendered *E. coli* incapable of establishing sessile communities. In *P. aeruginosa*, the role of a homolog of *E. coli* RpoS seems to be the opposite of the role of RpoS in *E. coli:* The *P. aeruginosa rpoS* gene was found to be repressed in biofilms, and *rpoS*-deficient mutants not only formed better biofilms than wild-type cells but were more resistant to antimicrobial treatment. There are several differences between the roles of RpoS in *E. coli* and *P. aeruginosa*, and it is therefore not surprising that RpoS plays different roles in biofilm development in *E. coli* and *P. aeruginosa.* [5]

In the CF lung, this process is not fully understood; however, many in vitro studies have outlined key factors responsible for the methodical formation of P. aeruginosa biofilms. First, the initial phase of attachment to a surface seems to be motility-mediated, in that P. aeruginosa overcomes the surface repulsion forces for bacteria and comes into contact with a surface most likely when propelled by its own method of movement. The presence of type-IV pili also allows the further development of the thin monolayer.

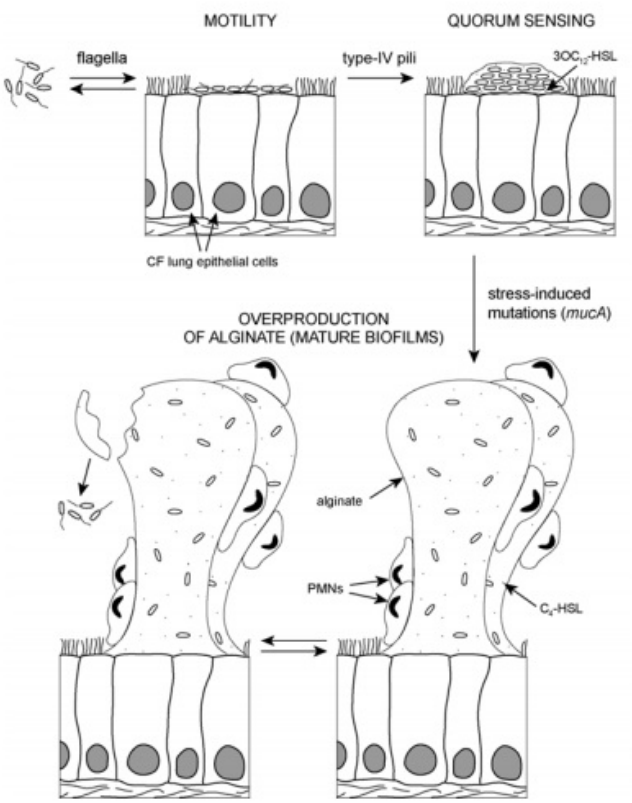

Figure3-Biofilm Formation

Other surface-associated proteins are thought to be involved in early biofilm formation including curly as well as a new chaperone/usher pathway involved in novel adhesion assembly.

After the formation of the monolayer, further biofilm development also requires a cell-density dependent communication ability known as quorum sensing. A hypothetical process of P. aeruginosa colonization leading to biofilm formation in CF. Flagella-mediated motility is involved in the initial attachment. Type-IV pili and a quorum sensing signal participate in micro colony differentiation. Overproduction of alginate, which results in the formation of mature biofilms, can be caused by mucA mutations induced through stresses such as oxidants released by PMNs.

Biofilms may be different from each other physiologically because of differences in motility, quorum sensing- and overproduction of alginate-mediated activity. 12 two systems, las and rhl. These two systems act together to coordinate requires a cell-density dependent communication ability known as quorum sensing, consisting of two systems, las and rhl. These two systems act together to coordinate P. aeruginosa gene expression relevant to survival in the biofilm phenotype.

N-(3- oxododecanoyl)-L-homoserine lactone (3OC12-HSL) and N-butyryl-L-HSL (C4-HSL) are known to be involved in cell-to-cell communication. 3OC12-HSL has been shown to be involved in micro colony differentiation.

A recent study reported levels of C4-HSL were higher than 3OC12-HSL when analyzed in CF sputum carrying P. aeruginosa. This suggests that C4-HSL may be important in later stages of biofilm formation. Alginate does not seem to be required for initial biofilm formation.

Later due to the depth of bacteria within a biofilm, immune cells find it difficult to effectively remove the large mass of microorganisms. Even coupled with the response of phagocytic macrophages, the mere size of a grouping of bacteria surrounded 13 by alginate proves difficult to ingest, leading to a phenomenon known as “frustrated phagocytosis.” Ultimately, this continued immunologic assault on the colonized CF lung leads to further lung deterioration and subsequent respiratory failure and death.

The formation of P. aeruginosa biofilms promotes chronic infection in the CF airways. Several unique properties of biofilms contribute to this bacterial persistence, including antibiotic resistance, resistance to the activities of the immune system, and the high-frequency generation of bacterial mutants. [6]

IV. MATRICES OF BIOFILMS

ALGINATE PRODUCTION

P. aeruginosa isolates from the CF lungs are very distinctive from those that cause other types of infections, as well as those found throughout the environment. This unique in vivo environment is the only situation where P. aeruginosa is converted to the alginate overproduction phenotype (mucoid). Alginate is a polysaccharide, consisting of a polyuronic acid, which is exported outside of the cell in the form of a capsule. This phenotype manifests in genetic mutations to the bacterium while residing within the CF lung.
The overproduction of alginate is an essential process by P. aeruginosa regarding its survival in the CF lung. Alginate is produced by the proteins encoded by the alginate biosynthetic operon, consisting of 12 genes, beginning with algD. These genes are responsible for the creation of the sugars, placement in the correct orientation, assembly of the polysaccharide, as well as the machinery used to export the polysaccharide outside the bacterium. One of the main factors involved in up-regulation of algD expression, AlgU/AlgT, is an alternative sigma factor and is the key regulator of alginate production. AlgU is referenced to as an “alternative” sigma factor as an

alternative to primary housekeeping sigma factors. AlgU interacts with the core polymerase to form the holoenzyme and drive algD synthesis. Mutations in algU are the most common cause of mucoid conversion of P. aeruginosa in CF patients. AlgU auto regulates its expression from two promoters and is negatively regulated by the immediate downstream cluster mucABCD, where MucA is the cognate anti-sigma factor for AlgU. MucA is an inner membrane protein with its C-terminus proposed to interact with MucB, and its N-terminus interacting with AlgU. Inactivation of MucA, MucB and MucD causes conversion to mucoidy. MucD, a DegP (HtrA) homolog, is a periplasmic serine protease that degrades improperly folded or damaged proteins.

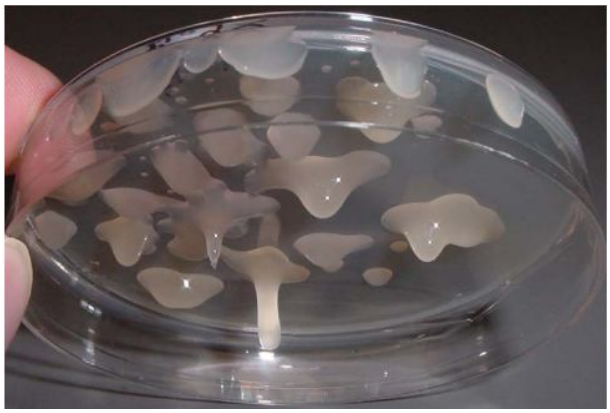

Figure 4-Alginate Biofilms

Psl POLYSACCHARIDE

The Psl cluster consists of 15 co-transcribed genes (*pslA* to *pslO*, PA2231-2245) encoding proteins to synthesize Psl, which is important to initiate and maintain biofilm structure by providing cell-cell and cell-surface interactions.

The roles of Psl in biofilm formation have been thoroughly investigated due to its major contribution to the biofilm formation in *P. aeruginosa.* Firstly, overproduction of the Psl polysaccharide led to enhanced cell-surface and intercellular adhesion of *P. aeruginosa*, suggesting its importance in adhesion, which is critical for initiation and maintenance of the biofilm structure. It was found that Psl polysaccharide anchors on bacterial cell surface in a helical shape, which promotes strong bacterial cell-cell interactions. This results in the assembly of a biofilm and its matrix at the early stage of biofilm development. Later on, Psl polysaccharide accumulated on the periphery of three dimension-structured macro colonies during biofilm maturation. This localization pattern provided the structure support and allowed for later biofilm dispersion. In addition, Psl staining demonstrated that Psl can form a fiber-like matrix that enmeshes bacteria within biofilms. In addition, Psl was found to have roles in pathogenesis and protection against the immune system. [4]

V. REGULATION OF BIOFILM MATRIX

Gene regulation is important for our understanding of biofilm formation.

Biofilm formation is a multicellular process involving environmental signals and a concerted regulation combining both environmental signals and regulatory networks. Due to the major roles of EPS matrix in biofilm formation, its regulation is discussed.

A. c-di-GMP

Bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP), a ubiquitous intracellular second messenger widely distributed in bacteria, was discovered in 1987 as an allosteric activator of the cellulose synthase complex in *Gluconacetobacter xylinus.* In general, c-di-GMP stimulates the biosynthesis of adhesions and exopolysaccharide mediated biofilm formation and inhibits bacterial motilities, which controls the switch between the motile planktonic and sessile biofilm-associated lifestyle of bacteria . Moreover, c-di-GMP controls the virulence of animal and plant pathogens, progression through the cell cycle, antibiotic production and other cellular functions.

C-di-GMP is synthesized from two molecules of GTP by diguanylate cyclases (DGC) containing GGDEF domains and is broken down into 5'-phosphoguanylyl-(3'-5')-guanosine (pGpG) by specific phosphodiesterases (PDE) containing EAL or HD-GYP domains; pGpG is subsequently split into two GMP molecules.

In *P. aeruginosa*, there are 41 of such proteins, including 3 HD-GYP, 17 GGDEF, and 5 EAL domain proteins, as well as 16 proteins with both GGDEF and EAL domains. Most proteins that contain these domains show a multi-modular arrangement. In addition to GGDEF, EAL, or HD-GYP domains, there are a variety of sensory domains such as signal receiver or transmission domains and response regulator domains that are likely to receive signals from the environment. These signals are proposed to be translated as an alteration of the enzymatic activity that would result in local or global fluctuations in c-di-GMP levels, which in turn would lead to behavioral adjustments.

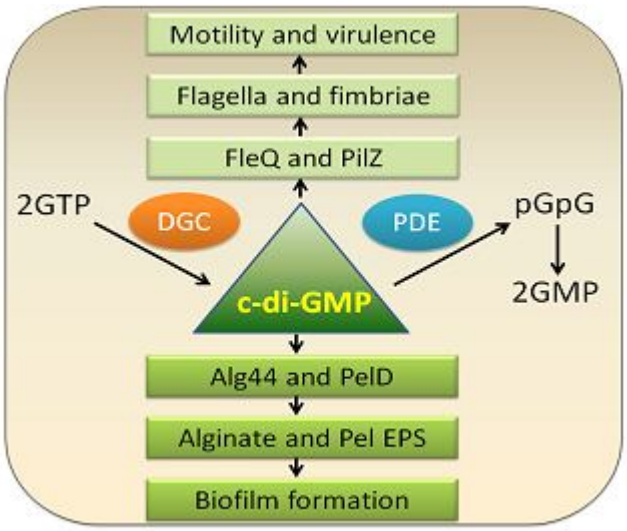

Figure5- c-di-GMP regulatory cycle

In *P. aeruginosa*, at least four c-di-GMP effectors are present including FleQ, PelD, Alg44, and PilZ. FleQ is a c-di-GMP-binding transcription factor, which generally functions as an activator in flagella biosynthesis.

PelD is part of *pel* operon of *P. aeruginosa* activated by direct binding c-di-GMP through a site that resembles the I site motif in GGDEF domain proteins.

c-di-GMP signaling has been shown to be involved in mediating the formation of small colony variants (SCV) in *P. aeruginosa*. It, has been identified to control the wild type-like, antibiotics susceptible revertants. [3]

B. QUORUM SENSING

Quorum sensing (QS), known as bacterial cell-cell communication system, represents another multicellular activity which involves the production, secretion, and detection of molecules called autoinducers (AIs) to modulate behaviors of the bacterial population. QS provides a strategy for bacteria to detect each other's presence and to regulate gene expression in response to changes of bacterial population density. Up to now, many biological processes have been found to be controlled by QS, such as bioluminescence, biofilm formation, virulence factor expression, antibiotics production, sporulation, and competence for DNA uptake *P. aeruginosa* employs three quorum sensing signaling systems (LasR/LasI, RhlR/RhlI and PQS) to control cellular processes involved in the production of extracellular virulence factors and to control biofilm formation. A large number of genes, including virulence factor genes and genes involved in biofilm development, are activated by two typical, interconnected and homologous acyl-homoserine lactone (AHL) quorum sensing systems, namely the LasR/LasI and RhlR/RhlI systems.

It has been shown that signal molecule 3-oxo-C12-HSL (synthesized by LasI) is necessary for the establishment of a differentiated *P. aeruginosa* biofilm since a *lasI* mutant forms flat, undifferentiated biofilms unlike wild-type biofilms and *lasI* is expressed in a large number of cells during the initial stage of biofilm formation. In contrast, the RhlR/RhlI system was found to be activated during the maturation stage of *P. aeruginosa* biofilm development, and might be important for the survival of bacterial cells growing in anaerobic conditions in biofilms. [3]

C. GacA/GacS TWO COMPONENT SYSTEM

Expression of the *pel and psl* genes for exopolysaccharide production in *P. aeruginosa* can be regulated by GacA/GacS two-component system. One of the mechanisms involves two histidine kinases, RetS and LadS that act in opposing ways on the GacA/GacS two-component system.

The GacA/GacS system subsequently controls the transcription of two small regulatory RNAs (sRNAs), *rsmY* and *rsmZ*, leading to the decrease or increase in the translation of the *pel* or *psl* operon. Transcriptomic analysis showed that GacS directly controls the transcription of *rsmY* and *rsmZ*, thereby antagonizing the activities of RNA-binding translational regulator, RsmA, to control the expression of over 500 genes. It was further proved that upon binding of RsmA with the promoter of the *psl* operon, the region spanning the ribosome binding site of *psl* mRNA forms a secondary stem-loop structure that prevents ribosome access and the subsequent translation, without affecting transcription. This translational control of Psl expression constitutes a novel example of translational repression by RsmA. Furthermore, analyses of the mRNA levels using microarray analysis have shown that RetS is required for the expression of genes involved in virulence such as the type III secretion system (T3SS), yet acts as a repressor for the type VI secretion system (T6SS) and genes involved in exopolysaccharide synthesis, leading to the inhibition of biofilm formation. This defines RetS as a pleiotropic regulator of multiple virulence phenotypes that mediates the activation of genes involved in acute infections and the repression of genes associated with chronic persistence. A recent report showed that RetS could directly interact with GacS to modulate its phosphorylation state. During the acute infection phase, RetS interacts with GacS to form heterodimers, blocking GacS auto phosphorylation and leading to reduction in *rsmZ* expression. Finally, RsmA lacking RsmZ will promote the translation of genes required for acute virulence factors. While sensing unknown environmental signals, GacS and RetS each form homodimers, allowing GacS autophosphorylation and

subsequent phosphorylation of GacA, finally resulting in the expression of genes involved in chronic infections. Very recently, a novel RetS interacting protein, PA1611 was identified and characterized as able to both promote biofilm formation and repress T3SS and swarming motility, adding complexity to the classical GacS/GacA regulatory cascade. On the other hand, LadS was found to antagonize the effect of RetS, contributing to the repression of T3SS and the activation of genes necessary for exopolysaccharide production promoting biofilm formation.

However, there is a paucity of information detailing whether LadS affects the GacS or RetS. Interestingly, one *P. aeruginosa* reference strain PA14 was found to have a natural *ladS* mutation, explaining why PA14 exhibits increased virulence and displays attenuated biofilm formation as compared to PAO1. In addition, the histidine phosphotransfer (Hpt) protein HptB signaling pathway was found to control biofilm formation and T3SS, and fine-tunes *P. aeruginosa* pathogenesis. Typically, Hpt protein acts as a phosphorylation relay that transits the activation signal from a sensor kinase to the cognate response regulator. Bordi and co-workers found that in contrast to the double control of *rsmYZ* expression by RetS, HptB exclusively regulates *rsmY* expression. Importantly, in this study, they demonstrated a redundant effect of the two sRNAs on T3SS gene expression, while the influence on *pel* gene expression is additive, further underpinning the novel mechanism of fine-tuned regulation of gene expression. [3]

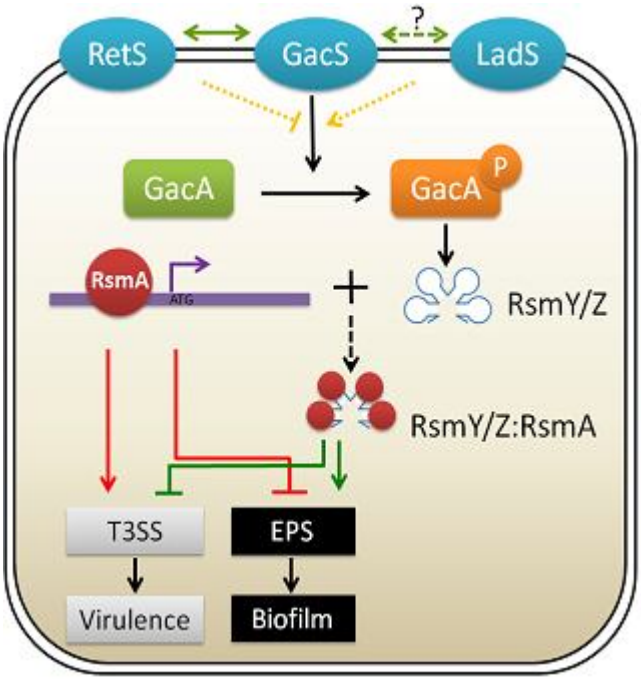

Figure 6- GacA/GacS Two component system

D. OTHER TYPES OF REGULATIONS

In addition to the typical regulation of biofilm development, the biofilm matrix is also under control by other types of regulation. One example is the metabolic regulation mediated by AlgC, a checkpoint enzyme that coordinates the total amount of exopolysaccharides in *P. aeruginosa* by control of sugar precursors pool for exopolysaccharides synthesis. It was demonstrated that overexpression of one exoplysaccharide could reduce the production of the other. For example, overproduction of Psl led to reduced level of alginate, Pel overexpression resulted in less Psl production, and overproduction of alginate and Psl caused decreased levels of B-band LPS. The enzymatic regulation of exopolysaccharide provided us a very interesting clue about the survival strategy used by *P. aeruginosa* in diverse conditions. It is easily speculated that *P. aeruginosa* produces one major type of exopolysaccharide in certain phases whereas it generates another major type of exopolysaccharide upon changing environments. As a key polysaccharide for biofilm formation, Psl expression is regulated at multiple levels. In addition to the aforementioned mechanisms, Psl was found to be regulated by RpoS transcriptionally, and post-transcriptionally by RsmA, an RNA binding protein. The transcriptional regulator AmrZ, previously shown to positively regulate twitching motility and alginate synthesis, was also shown to directly bind to the promoter region of the *psl* operon to repress its transcription. The AmrZ-mediated switch from Psl production to alginate production provides another example of acute-to-chronic transition strategy used by *P. aeruginosa*. [11]

VI. TREATMENTS

Treatment of *P. aeruginosa* lung infections remains challenging. The best course of action might be prevention of infection through aggressive infection control procedures. These procedures are meant to prevent person-to-person transmission as well as transmission from contaminated surfaces. It has been found that sputum-encased *P. aeruginosa* can survive on inanimate surfaces for up to 8 days. Thus, thorough cleaning and sterilization of clinical rooms, apparatuses, and home respirators is recommended. Furthermore, healthcare workers should practice good hand and respiratory hygiene. Isolation and separation of individuals infected with particular pathogens, such as *P. aeruginosa* and multi-drug resistant bacteria, has also been suggested to reduce patient-to-patient spread.

As discussed below, there are a number of antimicrobial therapies implemented to control lung infection with *P. aeruginosa*. [5]

A. ANTIBIOTIC TREATMENTS

Numerous antibiotics have been used to treat CF lung infection with *P. aeruginosa*, although the aminoglycoside antibiotic tobramycin has most often been used and has been studied the most. In order to achieve high concentration in the airways, tobramycin and other antibiotics are often inhaled in a nebulized form. Studies have investigated the efficacy of a number of inhaled antibiotics, including tobramycin, colistin, gentamicin, ceftazidime, cephaloridine, aztreonam lysine, taurolidine, and a gentamicin/carbenicillin combination. The use of inhaled antibiotics, can lead to increased lung function and decreased exacerbation frequency over placebo. During stable periods, inhaled antibiotics such as tobramycin or colistin can be given as chronic suppressive therapies to maintain low bacterial levels within the airways. An economics study estimated that increased usage of inhaled tobramycin would lead to increased cost for medication but decreased physician and hospital visits. This would have a net decrease in healthcare costs. It has also been suggested that this maintenance therapy be supplemented with 2-week courses of intravenous (IV) antibiotic combinations every 3 months for added anti-Pseudomonal pressure. In addition to antimicrobial therapies, other medications such as DNase and hypertonic saline are widely used to increase airway clearance. A recent study has found that twice daily treatments of tobramycin and ceftazidime are just as effective as thrice daily infusions, and this reduced treatment regimen can be safer and more convenient than a three times a day schedule. Studies have demonstrated that the bacterial response to antibiotic treatment is completed within 14 days, although in some cases, patients respond better to shorter or longer treatments. Home-based IV antibiotic therapy of exacerbation has also been explored as an alternative to inpatient treatment. Athome therapy, while requiring specialized training for family members and friends, can reduce costs to families and hospitals, reduce incidence of hospital-acquired infections, improve disease manifestations, and can be more convenient for the affected individual.

B. EARLY COLONIZATION IRRADICATION

The period of intermittent infection, before the establishment of chronic *P. aeruginosa* biofilms, presents a unique opportunity for therapeutic intervention. Many studies have shown the efficacy of early aggressive antibiotic therapy to eradicate *P. aeruginosa* during this early colonization period. In the Copenhagen Model, which has been in place for over 20 years in the Copenhagen CF center, infected CF patients are given inhaled colistin and IV ciprofloxacin for 3 months. 80% of patients treated with this regimen were free of chronic *P. aeruginosa* infection for up to 15 years, and the bacterial isolates recovered exhibited little resistance to colistin and ciprofloxacin. In a different study of other European CF centers, treatment with inhaled colistin and IV ciprofloxacin for 3 months was found to be 81% effective. In this study, treated patients were completely free from *P. aeruginosa* infection for an average of 18 months, and 73% of subsequent *P. aeruginosa* infections were found to involve a genotypically distinct strain, suggesting that the original isolate had been eradicated. This treatment was also associated with reduced overall treatment costs and little development of antibiotic resistance. There are numerous variations on eradication therapies, and many studies evaluating the efficacy of these treatments. In an effort to develop standardized treatment guidelines for early eradication therapies, there have been 2 large, multicenter studies: The Early Inhaled Tobramycin for Eradication (ELITE) study in Europe, and the Early *Pseudomonas* Infection Control (EPIC) program in the United States. The EPIC program, the results of which have yet to be published, is comparing standard culture based therapy with twice daily inhaled tobramycin (300 mg) for 28 days every yearly quarter. The tobramycin-treatment group is further split into groups that additionally receive 14 days of either oral ciprofloxacin or oral placebo. The ELITE study treated participants for 28 days with twice daily inhaled tobramycin (300mg/5mL), and found that 93% of those treated were *P. aeruginosa*-free after 1 month. 66% of participants were free of *P. aeruginosa* infection for 2 years. Similar results were obtained with individuals treated for 56 days. The early promise of eradication therapy studies demonstrates that these treatments will likely enhance overall patient health and reduce healthcare costs related to *P. aeruginosa* infection. Indeed, such early eradication protocols have dramatically increased the age at which chronic *P. aeruginosa* infection is established. Furthermore, eradication can be achieved regardless of the age of the patient, provided there has been no evidence of prior *P. aeruginosa* infection. [10]

VII. NOVEL THERAPIES AND METHODS

A. ANTI BIOFILM FORMATION

Because biofilm formation plays an integral role in the persistence and antibiotic resistance of *P. aeruginosa* in the CF lung, many researchers have begun searching out ways to specifically destroy biofilms. The most obvious place to start in the development of anti-biofilm therapies is testing the efficacy of our current antibiotics against *P. aeruginosa* biofilms in one retrospective analysis, the reported planktonic antibiotic susceptibilities of *P. aeruginosa* CF isolates were compared to the antibiotic susceptibilities of these strains grown as biofilms. Those patients that were treated with antibiotics that could kill biofilm state bacteria experienced lower treatment failure, decreased exacerbation risk, and decreased hospital stays. Other studies have also shown that

treatment tailored to biofilm susceptibility patterns can be effective. The recent development of the Minimum Biofilm Eradication Concentration (MBEC) Assay (also known as the Calgary Biofilm Device), has permitted high throughput analysis of biofilm formation and biofilm susceptibilities of *P. aeruginosa* and other CF pathogens. Ant biofilm therapies developed using the MBEC or other biofilm assays can thus be of great clinical benefit. Another promising avenue of anti-biofilm research is the identification of molecules that interrupt QS signaling. By interfering with inter-bacterial communication and gene regulation, these compounds can lead to the dispersion of biofilm bacteria as well as alter virulence factor production. Generally, QS inhibitors fall into one of three categories:

1) Those that block production of the QS signaling molecule,
2) Those that degrade the QS molecule, and
3) Those that prevent bacterial recognition of the QS signal

Many large screens of natural compounds have been completed or are taking place to identify novel QS inhibitors, and several active compounds have emerged from these studies. For instance, halogenated furanones from the alga *Delisea pulchra* and synthetic furanones have been shown to block *P. aeruginosa* QS and biofilm formation, and they lead to increased *P. aeruginosa* killing when used in combination with traditional antibiotics. Likewise, garlic extract, patulin and penicillic acid from *Penicillium* species, *cis*-2-decanoic acid from *P. aeruginosa*, salicylic acid, 4-nitro-Pyridine-N-oxide (4-NPO), and solenopsin A form fire ant venom has all demonstrated inhibition of *P. aeruginosa* QS, and some have shown direct biofilm disruption activity. Some of these QS inhibitors, such as garlic extract, patulin, 4-NPO, and furanones, have also displayed a therapeutic effect in models of *P. aeruginosa* infection.

Importantly, it is thought that resistance to QS inhibitors will not develop because these compounds do not directly affect bacterial growth, and thus exert little selective pressure.

B. NEWER ANTIMICROBIAL STRATERGIES

On the other hand, the development of inhaled versions of existing antibiotics has been shown to improve delivery times and concentrate the antibiotic at the site of infection. Nebulized tobramycin has been used for years as an effective anti-Pseudomonal therapy, and many other inhaled antibiotic formulations have been studied. Recently, Aztreonam Lysine for Inhalation has been approved in many countries for treatment of chronic CF lung infections, and studies have shown that use of this drug can improve quality of life and pulmonary function of CF patients, while decreasing *P. aeruginosa* burden and lower exacerbation severity. Work continues on other inhaled antibiotics, including aerosolized levofloxacin, fosfomycin/tobramycin, and inhalable dry powers of tobramycin and ciprofloxacin. Inhalation of liposomally-encased antibiotics shows great promise for therapy of biofilm infections, as liposome delivery is thought to increase the penetration of biofilms. Patients treated with liposomally-encased amikacin showed improved lung function and reduction in sputum *P. aeruginosa* levels. [5]

C. VACCINATIONS

Vaccines against *P. aeruginosa* have also been proposed as a potential therapy for preventing chronic CF infections. Researchers have explored vaccines against *P. aeruginosa* LPS, alginate, flagella, outer membrane proteins, pili, T3SS components, DNA, and killed whole bacteria. Many of these vaccines have been tested in clinical trials, with moderate efficacy. It is thought that clearance and prevention of *P. aeruginosa* infection by aggressive early eradication programs masks the true effectiveness of these vaccines, and none of them have reached the market. Passive immunotherapy with monoclonal antibodies or pooled immune serum has also been investigated for anti-Pseudomonal therapy. [5]

VIII. CONCLUSION

There have been a number of great advances in recent years in anti-Pseudomonal therapy of CF lung infections. In particular, early eradication treatments appear to show much promise in delaying the onset of chronic *P. aeruginosa* biofilm formation. The increasing arsenal against *P. aeruginosa*, including inhaled aztreonam and liposomal amikacin, will likely prove a benefit for *P. aeruginosa* treatment. Eradication of chronic *P. aeruginosa* may be possible, but it will take creative thinking. It is clear that new anti-biofilm treatments need to be discovered and implemented. The development of clinically-relevant models will further aid this process by providing appropriate systems for testing novel molecules. With renewed focus on the biofilm nature of the infection, much pro

IX. ACKNOWLEDGEMENT

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