A Review: Quorum Sensing Phenomenon: Regulation of biofilm in *Pseudomonas aurogenosa*

Rawa Abdul Redha Aziz[1], SuraAlaa Saud[2], Elaf Sameer Mohameed[2]

[1]Doctor Lecturer; [2] Lecturer Asisstant; Al-Karkh University of Science,College of Science, Department of Microbiology, Iraq

Abstract

It is been mentioned that *Pseudomonas aeruginosa,* a ubiquitous, gram negative, can infect patients with Cystic fibrosis. Treatment of infections can often be difficult due to its ability to produce biofilm. It has been revealed that biofilms are enclosing architecture that would consist of planktonic cells to survive in harsh environments. Biofilm is usually comprised of Pel, Psl, and Alginate, and many others to make a scaffold that bacteria can attach and start making microcolonies. The formation of the biofilm and Alginate layer is shown to be controlled by intricate cell-to-cell communication known as quorum sensing (QS). Finally, in this report, we have taken the basic principles of what is *P.aerogenosa*, and how it is related to the cystic fibrosis disease. Also, we have illustrated the formation of biofilms and the regulation of biofilms to the quorum sensing LasR/LasI and RhlR/RhlI systems.

Keywords: *Cystic Fibrosis, Type IV pili (TFP), Alginate, Quorum sensing*

Introduction

It is a fact that *Pseudomonas aeruginosa* is a ubiquitous, gram negative opportunistic pathogen. It is often found in hospitals and medical equipment and cuase many chest infection (i.e.nosocomial). Treatment of infections can often be difficult due to its ability to produce biofilm [(1;2)].

It is known that biofilm is a community of microorganisms that can be aggregated within extracellular polymeric substances produced by the same microorganisms [(3)]. This mode of production would be as one of the protective mechanisms to survive in any harsh environments and some cells then would disperse to start producing biofilms under favorite conditions. It is studied that biofilm could be found in hospitals, natural, and industrial places [(4;5)].

Biofilms can develop antibiotic resistance up to a thousandfold higher than planktonic cells. The formation of biofilm is shown to be controlled by intricate cell-to-cell communication known as quorum sensing (QS) [(6)].

Corresponding author:
Rawa Abdul Redha Aziz
dr.rawaaziz@kus.edu.iq

Psudomonas aeruginosa and cystic fibrosis disease

When admitting CF patients to hospitals, it is found that they suffer from aggravation of the illness. They show symptoms of fevers, loss of appetite, night sweats, weight loss, and they are treated with antibiotic therapy for a long time [(7;9)].

To define the multisystem disorder Cystic fibrosis (CF), it could be said that it is caused by mutations in the gene encoding the CF transmembrane conductance regulator that known as cyclic AMP. It is usually regulate chloride ion channel. Thus when mutated, it will be mislocalized, or it will loss its action [(8;10)].

Biofilm formation as a virulence factor

It is reported that bacteria can adhere to each other to any desirable surface when exposed to antibiotic treatments and limited nutrients to survive [(11)]. It is mentioned earlier that pathogens could resist antibiotics and any environmental conditions when enclosing

within biofilm architecture [12] . Many researchers show preference to study the bacterial metabolism and signals that are essential in the transition from planktonic cell to biofilm growth [13].

Exopolysaccharides of biofilm plays a significant role in pathogens protection and could make a scaffold barrier for pathogens to attach [14]. Three polysaccharides in *P. aeruginosa* are responsible for antibiotic resistance and structure maintenance, such as Psl, Pel, and alginate [15]. Before talking about the alginate layer in detail, we have to present some of the functions of all EPS involved in the biofilm production of *P. aeruginosa*[16].

Biofilm development

In order to understand the physiology change of *Pseudomonas aeruginosa* during biofilm, it should be aware of biofilm stages in terms of motility mechanisms and regulation of pathogens near the scaffold surface (figure1), alginate production, and quorum sensing [17].

Attachment of bacterial cells on a surface

It is researched that during this step, exopolysaccharides protein could play a significant role in bacterial attachment on the surface where this protein causes significant changes of substratum salvation patterns [18] .There is a significant difference between aqueous and solid environments in terms of the adsorption of organic and inorganic substances on surfaces wherein the aqueous environment is extremely rapid and is estimated to be less than 1s [19].

The Reversible attachment of bacterial cells

This step depends firstly on the interaction of bacteria and substratum that often described to be weak according to vdW electrostatic forces and hydrophobic interactions [DLVO theory]. [17]. It is researched that bacteria could easily be removed in this step by mild shear forces because they still show Brownian motion [20].

Irreversible attachment of bacterial cells

To make monolayers of bacterial cells, they use flagella to bind to the scaffold surface; making the initial step of microcolonies that form mature biofilm by twitching motility via TFP and single flagellum [21;22].

Biofilm Maturation step

In terms of definition, glycocalyx layers are made of organic and inorganic molecules with bacteria embedded in aqueous environments [23;17]. These layers work professionally as glue material to attach the colonized bacteria to the surface and can trap nutrients from the environment such as exogenous DNA, minerals, proteins, and other desirable materials. The glycocalyx is usually composed of Pel, Psl, and Alginate exopolysaccharides [24;17].

Detachment of bacterial cells from the biofilm

Colonized cells at this step could disperse from biofilm architecture and start colonizing on a new scaffold because of the death and lysis of the sub-population of bacterial cells within the mature biofilm [25].

Figure 1, a) Schematic representation of a mature biofilm [22].

Figure 1, b) Biofilm formation [22].

Alginate layer formation

Clinical *P. aeruginosa* isolated from CF patients can produce alginate layer as exopolysaccharide and the overproduction of these layers would turn bacterial cells from non- to mucoid phenotype [26]. There are several excellent reviews have been revealed that alginate precursor guanosine diphosphate mannuronic acid can be synthesized by algD, algA, and algC genes located on AlgT/U operon (figure 2) [28; 29]

Polymerization of polymannuronate

When labeling GDP mannuronic acid with 14C for in vitro experiment of alginate polymerization, alginate chain extents only in cell envelop with the presence of Alg8 protein, suggesting that polymerization requires an association between outer and cytoplasmic membranes. Also, overexpression of Alg8 protein up to 20 fold will increase alginate production and would alter the acetylation and epimerization profile. Another essential protein required for alginate production is Alg44 that has PilZ domains (cytoplasmic N-terminal), which when mutated by point mutation will lose the c-di-

GMP binding thereby stop alginate production. The C-terminal periplasmic domain of Alg44 on other hand resemplesMexA [MexAB-OprM multidrug efflux pump] [16]. These resembling properties could be beneficial to gene therapy [16; 30].

Regulations of biofilm formation

A Flurry of research from a molecular biology perspective showed that gene regulation of any gene expression of pathogens would be beneficial as gene therapy instead of antibiotics regimes [31,3]. In biofilm production as mentioned above, array of genes will be participated in the production, and understanding how they are work and regulates by c-di GMP, 4-2- Gac A/ Gas S two- Component Systems, Alg C- dependent enzymatic regulation, and 4-4- Quorum Sensing, it can be controlled [28; 33].

Regulation of alginate biosynthesis

As mentioned above how to produce alginate by a collection of genes, they need to be positively and/ or negatively regulated [34,35, and 36]. Genes involved in this process are usally found on operon algD- algA PA3540-PA3551 that is controlled by algD promoter. To triger inducing and enhancing transcription of this promoter, AlgT (AlgU or σ^{22}) is used [37; 38], and to deregulate AlgT and convert the strains to mucoid type, mucA and mucB genes produce inhibitors that inactivate algT action. Alg R plays a crucial role in regulating algC as it binds in three positions downstream and [- 479 ; - 457] bp upstream of the transcription starting point [39, 41]. This property resembles enhancers in eukaryotic cells [37, 39 and 40].

Many case report studies revealed that more than eighty percent of CF patients having *P. auroginosa* infections have strains with mutant mucA, stating that this gene is a key point for mutagenesis and cause mucoid patterns. Furthermore, algD transcription requires RpoN (s54) that is observed only in muc23. Besides, response regulators AlgR, AlgB, AlgZ, IHF, AlgP, and AlgQ show transcription process of algD controlling [39].

Quorum sensing regulation phenomenon

Quorum sensing is known to be the bacterial intracellular language that can regulate many virulence factors like bioluminescence, virulence factor expression, antibiotics,, sporulation, and biofilm production depending on cell density and environmental conditions by the production of autoinducers (AIs) (figure 3) [43; 44]. This phenomenon allows bacteria to communicate with each other in any hard circumstances to modulate behaviors of the bacterial population and density [43; 45].

These signaling systems are The LasR/LasI and RhlR/RhlI systems can regulate the activation of more than 300 genes by producing acyl-homoserine lactone (AHL) (figure 4) [49]. The LasI can synthesis PAI1 [N-(3-oxo-dodecanoyl)-L-homoserine lactone (3-oxo-C12-HSL)], which is a signaling molecule, and LasR plays as a transcriptional regulator [42, 43].

The PAI1 molecule can activate LuxR-type transcription factor (LasR), and then LasR-PAI1 would induce the production of LasA protease, LasB elastase, exotoxin A, alkaline protease, and the LasRI[50]. On other hand, PAI2 [N-butanoyl-L-homoserine lactone] along with RhlR regulator enhances the production of many molecules such as hydrogencyanide, LasB elastase, rhamnolipid, pyocyanin, and cytotoxic lectins [50].

Positive regulation by the Lux R family member

It is demonstrated that the LuxR family regulator regulates quorum sensing positively by encoding an autoinducer-responsive transcriptional activator. While LuxI encodes a protein required for autoinducersynthesis[47,48] .

Conclusion

It is shown in this report the basic principles of what is *P.aerogenosa*, and how it is related to the cystic fibrosis disease. Also, advances in biofilm formation of *P.aerogenosa*and regulations with a focus on the biosynthesis of alginate. Besides, the role of LasR/LasI and RhlR/RhlI is demonstrated.

Conflict of Interest – **Nil**

Source of Funding- **Self**

Ethical Clearance – **Not required**

References

1. Bacalso M., Xu T., Yeung K. Biofilm Formation of *Pseudomonas aeruginosa* PA14 Required lasI and was Stimulated by the Pseudomonas Quinolone Signal although Salicylic Acid Inhibition is Independent of the pqs Pathway Mary. Journal of Experimental Microbiology and Immunology (JEMI). 2011; 15: 84 – 89.
2. Wei Q. and Z. Ma . Biofilm Matrix and It's Regulation in Pseudomonas aeruginosa. International Journal of Molecular Sciences. 2011; 14: 20983-21005.
3. Louise F. Roddam, Latham R., David W. Reid. Pseudomonas aeruginosa Uses Multiple Pathways To Acquire Iron during Chronic Infection in Cystic Fibrosis Lungs. Infection and Immunity. 2013; 81 (8): 2697–2704.
4. Chang R., Das T., Manos J., Kutter E. Bacteriophage PEV20 and Ciprofloxacin Combination Treatment Enhances Removal of Pseudomonas aeruginosa Biofilm Isolated from Cystic Fibrosis and Wound Patients. The AAPS Journal. 2019; 21(49).
5. Filkins LM, O'Toole GA. Cystic fibrosis lung infections: polymicrobial, complex, and hard to treat. PLoSPathog. 2015;11(12):e1005258–e.
6. Gonzalez MR, Fleuchot B, Lauciello L, Jafari P. Effect of human burn wound exudate on Pseudomonas aeroginosa. virulence.msphere. 2016; 1(2).
7. Yoon SS, Hennigan RF, Hilliard GM. Pseudomonas aeruginosa anaerobic respiration in biofilms: relationships to cystic fibrosis pathogenesis. Dev. Cell. 2002; 3: 593-603.
8. Mulcahy LR, Burns JL. Emergence of Pseudomonas aeruginosa strains producing high levels of persister cells in patients with cystic fibrosis. J. Bacteriol. 2010; 192: 6191-6199.
9. Xu Z., Fang X., Wood T.K. A Systems-Level Approach for Investigating Pseudomonas aeruginosa Biofilm Formation. PLoSONE . 2013; 8(2): e57050.
10. Ramsey D.M, Wozniak D.J. Understanding the control of Pseudomonas aeruginosa alginate synthesis and the prospects for management of chronic infections in cystic fibrosis. Molecular Microbiology . 2005; 56(2): 309-322.
11. Mauch R., ØstrupJensen P., Moser C. Mechanisms of humoral immune response against Pseudomonas aeruginosa biofilm infection in cystic fibrosis. Journal of Cystic Fibrosis. 2018; 17(2): 143-152.
12. Lee K. and Yoon S. Pseudomonas aeruginosa Biofilm, a Programmed Bacterial Life for Fitness. J. Microbiol. Biotechnol. 2017; 27(6): 1053-1064.
13. Sadovskaya I, Vinogradov E, Li J. High-level antibiotic resistance in Pseudomonas aeruginosa biofilm: the ndvB gene is involved in the production of highly glycerol-phosphorylated beta-(1->3)-glucans, which bind aminoglycosides. Glycobiology. 2010; 20: 895-904
14. Hoiby N, Bjarnsholt T, Givskov M. Antibiotic resistance of bacterial biofilms. Int. J. Antimicrob. Agents . 2010; 35: 322-332
15. Wang S, Liu X, Liu H, Zhang L. The exopolysaccharide Psl-eDNA interaction enables the formation of a biofilm skeleton in Pseudomonas aeruginosa. Environ. Microbiol. Rep. 2015; 7: 330-340.
16. Franklin M.J., Nivens D.E. Biosynthesis of the Pseudomonas aeruginosa extracellular polysaccharides, alginate, Pel, and Psl. Frontiers in Microbiology | Cellular and Infection Microbiology . 2011; 2(176): 1-16.
17. Dagana W. Understanding adhesion of Pseudomonas fluorescens on household surfacesSchool of Chemical Engineering. The University of Birmingham. 2012.
18. Harmsen M, Yang L, Pamp SJ. An update on Pseudomonas aeruginosa biofilm formation, tolerance, and dispersal. FEMS Immunol. Med. Microbiol. 2010; 59: 253-268.
19. Vasseur P, Vallet-Gely I, Soscia C. The pel genes of the Pseudomonas aeruginosa PAK strain are involved at early and late stages of biofilm formation. Microbiology . 2005; 151: 985-997.
20. Baker P, Hill PJ, Snarr BD. Exopolysaccharide biosynthetic glycoside hydrolases can be utilized to disrupt and prevent Pseudomonas aeruginosa biofilms. Sci. Adv. 2016; 2: e1501632.
21. Pezzulo A. A, Tang X., Hoegger M.J., AbouAlaiwa M. H. Reduced Airway Surface pH Impairs Bacterial Killing in the Porcine Cystic Fibrosis Lung. Science. 2016; 351(6272).
22. LembreP., LorentzC., and Di MartinoP. Exopolysaccharides of the Biofilm Matrix: A

Complex Biophysical World. book edited by Desiree NedraKarunaratne, ISBN 978-953-51-0819-1, Published under CC BY 3.0 license. 2012.

23. Yang L, Hengzhuang W, Wu H. Polysaccharides serve as scaffold of biofilms formed by mucoid Pseudomonas aeruginosa. FEMS Immunol. Med. Microbiol. 2012; 65: 366-376. 55.
24. Ryder C, Byrd M, Wozniak DJ. Role of polysaccharides in Pseudomonas aeruginosa biofilm development. Curr. Opin. Microbiol. 2007; 10: 644-648
25. Kim SK, Lee JH. Biofilm dispersion in Pseudomonas aeruginosa. J. Microbiol. 2016; 54: 71-85
26. Tolker-Nielsen T. Pseudomonas aeruginosa biofilm infections: from molecular biofilm biology to new treatment possibilities. APMIS Suppl. 2014; 122: 1-51.
27. O'Toole G, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annu. Rev. Microbiol. 2000; 54: 49-79
28. Borlee BR, Goldman AD, Murakami K. Pseudomonas aeruginosa uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix. Mol. Microbiol. 2010; 75: 827-842.
29. Wozniak DJ, Wyckoff TJ, Starkey M. Alginate is not a significant component of the extracellular polysaccharide matrix of PA14 and PAO1 Pseudomonas aeruginosa biofilms. Proc. Natl. Acad. Sci. USA. 2003; 100: 7907-7912.
30. Colvin KM, Gordon VD, Murakami K, Borlee BR. The Pel polysaccharide can serve a structural and protective role in the biofilm matrix of Pseudomonas aeruginosa. PLoSPathog. 2011; 7: e1001264.
31. Petrova OE, Sauer K. A novel signaling network essential for regulating Pseudomonas aeruginosa biofilm development. PLoSPathog. 2009; 5: e1000668.
32. Jennings LK, Storek KM, Ledvina HE, Coulon C. Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the Pseudomonas aeruginosa biofilm matrix. Proc. Natl. Acad. Sci. USA. 2015; 112: 11353-11358.
33. Wei Q, Ma LZ. 2013. Biofilm matrix and its regulation in Pseudomonas aeruginosa. Int. J. Mol. Sci. 2013; 14: 20983-21005.
34. Friedman L, Kolter R. Two genetic loci produce distinct carbohydrate-rich structural components of the Pseudomonas aeruginosa biofilm matrix. J. Bacteriol. 200; 186: 4457-4465. 53.
35. Yang L, Hu Y, Liu Y, Zhang J. Distinct roles of extracellular polymeric substances in Pseudomonas aeruginosa biofilm development. Environ. Microbiol. 2011; 13: 1705-1717.
36. Calfee MW, Coleman JP, Pesci EC. Interference with Pseudomonas quinolone signal synthesis inhibits virulence factor expression by Pseudomonas aeruginosa.Proc. Natl. Acad. Sci. USA. 2001; 98: 11633-11637.
37. Jackson KD, Starkey M, Kremer S, Parsek MR. Identification of psl, a locus encoding a potential exopolysaccharide that is essential for Pseudomonas aeruginosa PAO1 biofilm formation. J. Bacteriol. 2004; 186: 4466-4475.
38. Franklin M.J., Nivens D. Biosynthesis of the Pseudomonas aeruginosa Extracellular Polysaccharides, Alginate, Pel, and Psl. Frontiers in Microbiology. 2011; 2(167):1-16.
39. Limoli H., Whitfield G., Kitao T., Ivey M. Pseudomonas aeruginosa Alginate Overproduction Promotes Coexistence with Staphylococcus aureus in a Model of Cystic Fibrosis Respiratory Infection Dominique.AmericanSocitey for Microbiology. 2017; 8 (2) e00186-17.
40. Moradali M.F., Rehm B.H.A. The Regulation of Alginate Biosynthesis via Cyclic di-GMP Signaling. In: Chou SH., Guiliani N., Lee V., Römling U. (eds) Microbial Cyclic Di-Nucleotide Signaling. Springer, Cham. 2020.
41. Al Ahmar, Roy, "Understanding Pseudomonas aeruginosa Alginate Regulation and its Link to Chronic Lung Infections in Cystic Fibrosis Patients". Theses, Dissertations and Capstones. 2019; 1248.
42. Ishida T., Ikeda T., Takiguchi N. Inhibition of Quorum Sensing in Pseudomonas aeruginosa by N-Acyl Cyclopentylamides. Applied and Environmental Microbiology . 2007; 73(10), p. 3183–3188.
43. Juhas, M., L. Eberl, 2005. Quorum sensing: the power of cooperation in the world of Pseudomonas. Environmental Microbiology. 2005; 7: 459-471.
44. VittorioV. Regulation of quorum sensing in Pseudomonas. FEMS MicrobiolRev30. 2005; 274–291.
45. Jimenez P. N., KochG., Thompson J. A. The Multiple Signaling Systems Regulating Virulence

in Pseudomonas aeruginosa. Microbiology and Molecular Biology Reviews; 2012; 12, 46–65.

46. Swift, S., et al. "Quorum sensing: A population-density component in the determination of bacterial phenotype." Trends in Biochemical Sciences. 1996; 21:214-219.
47. Wang W., Morohoshi T., Ikeda T. Inhibition of Lux quorum-sensing system by synthetic N-acyl-L-homoserine lactone analogous. ActaBiochimBiophysSin . 2008; 40(12): 1023-1028.
48. Ching- T. H. Microbial metabolism: Quorum Sensing and biofilms. Appl. Microbial Biotechnology. 2010; 86(5): 1267-79.
49. Yang L, Nilsson M. Pyoverdine and PQS mediated subpopulation interactions involved in Pseudomonas aeruginosa biofilm formation. Mol. Microbiol. 2009; 74: 1380-1392.
50. Pearson JP, Pesci EC. Roles of Pseudomonas aeruginosa las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. J. Bacteriol. 1997; 179: 5756-5767.