Advances in Biotechnology

Chapter 4

Biofilm Formation and its Role in Antibiotic Resistance

Sree Samanvitha K[1]; Antony V Samrot[1*]; Senthil Kumar P[1]; Raji P[1]

[1]Department of Biotechnology, School of Bio and Chemical Engineering, Sathyabama Institute of Science and Technology, Jeppiar Nagar, Rajiv Gandhi Salai, Chennai, Tamil Nadu, India–600 119.

***[*]Correspondence to: Antony V Samrot,** Department of Biotechnology, School of Bio and Chemical Engineering, Sathyabama Institute of Science and Technology, Jeppiar Nagar, Rajiv Gandhi Salai, Chennai, Tamil Nadu, India–600 119.*

Email: antonysamrot@gmail.com

1. Introduction

Most of the life forms in the world can develop skills for their continued existence against a constantly changing and challenging environment. Amongst all the organisms, bacteria show a tremendous adaptation, by natural selection through transformation crafting genetic variants [1] and show survival instincts in many ways. They can form surface attachments, three dimensional edifices that are sustained by self-synthesised extracellular polymeric matrix. This consortium of cell-cell interaction can be described as biofilms [2], which represents the defence and communication system of a bacterial community. Naturally, biofilms are constructed by a diverse group of microorganisms like *Pseudomonas aeruginosa*, *Escherichia coli*, *Mycobacterium tuberculosis, Streptococcus mutans* which co-exists as a community challenging the hostile environment created by the host defense mechanism followed by the resulting antibiotic exploitation in order to eradicate the formed biofilm [3]. The transmission of a microbial invasion to a chronic pathological condition in not less than 65%, percentage is associated with biofilm formation especially in lung infection in cystic fibrosis, peridontitis of the teeth, middle ear infections, osteomyelitis, wound infections and nosocomial infections in prosthetics of joints, intravenous catheters, urinary catheters and stents [4,5].

Microbes restrain from its planktonic form to sessile mode and pin down to a location to grow into a microcolony like assembly concealed in a polymeric matrix organically synthesised. This dynamic environment evolves the siocio-microbial association a characteristic

physiological and behavioural modification conferring antibiotic resistance as a survival strategy. This alarms the WHO which recognised the antibiotic resistance is a serious problem not only for the human population but for the other organisms the domestic and wildlife. Indeed, it is difficult to restrain antibiotic resistance to one ecological niche but tends to spread universally through horizontal gene transfer [6,7]. Antimicrobial agents are the only existing therapy for treating microbial infections, infections; nevertheless, they could not completely eradicate biofilms conferring persistent infections in living organisms. The biofilm architecture comprising high cell densities protected in an exopolysaccharride matrix requires higher concentration of antibiotics approximately 10-1000 times than that of their planktonic counterparts. Administration of antibiotics in such heavy doses is in itself impossible due to the complications associated with the cellular damages in course of the metabolism and elimination process [8].

2. Stages of Biofilm Formation

The formation of biofilm is a gradual process and independent of the phenotype of the host microorganism [9]. Adhesion, growth, motility, and extracellular matrix production are the steps involved in the development of biofilm which is divided into several stages that are cyclic in nature. Stage 1 is a phase of reversible adhesion of the microbial cell to a surface which is mainly driven by motion, gravitational forces and hydrodynamic forces [10]. It has been studied recently that rough and hydrophobic surfaces such as bone, cartilage and heart valves as well as foreign body implants like catheters and Orthopaedic devices are mostly preferred for surface adhesion. They are highly influenced by pH, temperature, nutrients and their concentration, oxygen concentration osmolality and iron levels [11]. Stage 2 involves production of signals for communication between cells which helps in their growth. Stage 3 is a primary maturation phase where the production of an extracellular polysaccharide matrix is enhanced and motility is gradually decreased. Stage 4 is a phase of cell dispersion in which some bacteria leave the biofilm due to planktonic phenotype development. This results in release of free floating cells capable of reforming biofilm in a different place [12].The consortium of microorganisms within a hydrated environment possibly Exhibits a survival strategy against predation **(Figure 1)**., defence (protection from toxins in the host), colonisation (sequestration in a nutrient rich media), community (utilization of public benefits in a multispecies environment), d efault mode of growth (bacteria normally grow as biofilms only).

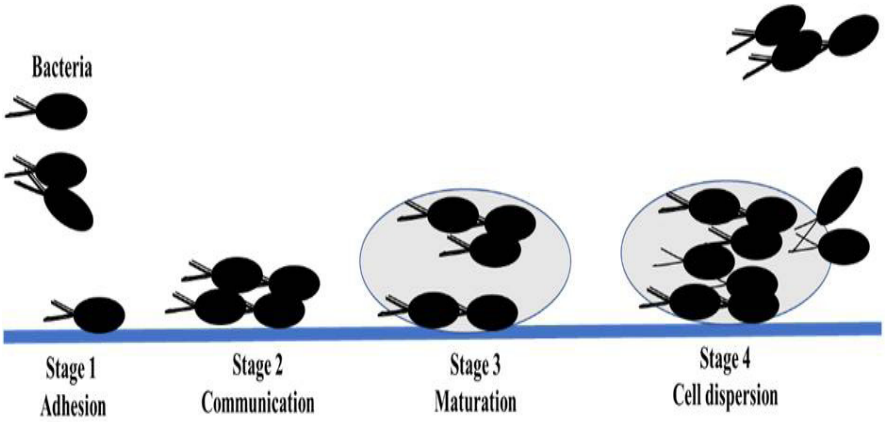

Figure 1: Stages of Biofilm formation

3. Role of EPS in Biofilm Formation

The polysaccharide component, also known as exopolysaccharide (EPS), provides the biofilm with benefits including attachment or adhesion to biotic or abiotic factors, architecture and protection from environment especially from dehydration [13,14,15]. The environmental stress on the biofilm, the maturation period of biofilm and the type of microorganisms are responsible for the constituents and mass of EPS [16]. EPS contributes 50-90% of the entire organic matter found in the biofilm [10]. The attachment of biofilm to the *invitro* and *invivo* substrate like prosthetics and endothelial valves of tissues respectively? is enhanced through the divalent cations present in the outer membrane of a bacterium; the divalent cations like Ca2+, Na+ and Mg2+ aid in maintaining the stability of the structures in the outer membrane [17]. In gram negative bacteria the polysaccharides that constitute the EPS are either of neutral or of negative charge which associates with the divalent ions strengthening the biofilm organisation while the gram positive bacteria, has a positive charge and hence doesn't involve ions presenting a compositional variance of the EPS [10]. The surface to which the biofilm attaches itself and the degree of adhesion of biofilm are directly related to each other; an uneven surface which is hydrophobic in nature is advantageous since the unevenness allows the biofilm to be protected by providing confined spaces [10].

3.1. Chemical Composition of EPS

EPS is made up of a variety of constituents ranging from carbohydrates [18], proteins [19] nucleic acids [20], humic substances [21], organic bases (hydroxyl groups) and organic acids (carboxylic groups) [22]. Presence of polysaccharides, proteins and nucleic acids in the EPS was well evaluated by NMR and FTIR analysis [23] Pal and Paul (2008) confirmed the presence of carbohydrates, proteins, nucleic acids and small amounts of uronic acid in EPS collected from a waste water treatment plant [24]. Sand and Gehrke [25] reported the presence of neutral sugars and lipids in *Acidithiobacillus ferrooxidans*. The EPS constituents like polysaccharides (dextran and kefiran) from lactic acid bacteria, *Weissela, Fructobacillus, Lactococcus* and *Streptococcus* are commercially promoted [26]. Guo-Ping Sheng [27]

concluded that extraction methods are vital in determining the amount of EPS. The total biofilm enzyme activity elucidation could be overlooked because of the disrupted matrix suspensions of the older biofilms of more than 30 days old and intact biofilms of young cells. Hence forth appropriate extraction methods are needed in the assessment of biofilm studies. The composition of EPS depends on the expression of the genes, environment and also the available or attached substrate [28,29]. *Staphylococcus epidermidis* was reported to produce polysaccharides responsible for binding to the medical devices, where the similar kind of polysaccharide i.e. poly-N-acetylglucosamine is produced by *Staphylococcus aureus* [30,31]. There are reports for production of exopolysaccharide like β-1-6 linked 2-amino-2-deoxy-d-glucopyranosyl residues by *S. aureus* [31,32]. Bacterial colonisation studies could also reveal the enzyme activity which deciphers thein metabolically active state of cells. The secretion of enzymes and molecules into the polymer matrix reveals the 'altruistic' behaviour where as the liberated molecules are not only used by the producer but also by every member of the microcolony. Role of these molecules in the biofilm could leave us a clue in spotting a better biofilm target [(32)].

3.2. Applications of EPS

The EPS secreted by microorganisms is employed in various fields such as food, industrial, mining & metallurgy [33] pharmaceutical, biomedical and the diverse structure of EPS has allowed it to be useful in the fields of bioremediation and bioleaching [26] rather than the preceding physical and chemical methods. EPS is responsible for the removal of toxic components from the environment by flocculation [24] or by metal chelation [21] and EPS showed an effect on termination of sulphates [25] as well as organic matter dissolved within aquatic systems [34]. Bioremediation through biofilms is more efficient than planktonic bacteria as biofilms are capable of adapting to the critical environmental conditions [10]. EPS has also been reported to remove remazol (dye) from effluent efficiently, due to its tremendous biosorption ability [35].

4. Role of Biofilm in Antibiotic Resistance in Bacteria

Antibiotic resistance is a phenomenon where Pathogenic bacteria cannot be inhibited by any one or more antibiotics. In such cases the bacteria become resistant to the antibiotics and continue to persist even in the presence of antibiotics. The resistance may be due to biochemical or evolutionary routes that confer resistance to the antibiotic used [6]. The evolutionary factors may influence antibiotic resistance through the formation of a biofilm. Bacteria within a biofilm correspond to a fundamental survival mechanism in which the organisms are protected through various biochemical pathways [37]. Multi drug resistant organisms have a major impact on public health as they exhibit resistance against a wide range of antibacterial agents [38]. Biofilms are responsible for almost 60% of nosocomial diseases related to contact lenses,

pacemakers, prosthetic joints, mechanical heart valves, central venous catheters, urinary catheters, prosthetic devices and orthopaedic devices [39]. These devices act as substrates for biofilm that causes infections and thus demands regular removal and replacement of these devices [40]. Cells from a disrupted biofilm become susceptible to antibiotics when grown in a planktonic state [41,42].

4.1. Slow Permeability of Antibiotics

It is regarded that exopolysaccharide secretion prevents the inlet of antibiotics into cells [42]. Various strains of *Pseudomonas aeruginosa* produce alginate, a negatively charged polysaccharide which helps in maintaining the integrity of the biofilm and further more prevent the entry of positively charged antibiotics such as amikacin and gentamicin [43]. *Staphylococcus aureus* involves in formation of PIA (polysaccharide intercellular adhesion) which helps in the gathering of nutrients during biofilm formation and plays a significant role in the development of biofilm related infections therefore escalating its resistance to antibiotics [44]. The cell membrane of *Staphylococcus epidermis* is surrounded by a glycoprotein polysaccharide called glycocalyx which effectively reduces the susceptibility to various antibiotics [45,46]. The slime secreted by *S. aureus* and *S. epididermis* decreases the susceptibility of the organism towards the activity of glycopeptides and pefloxacin [30,46,47]. De Beer et al [48] confirmed that vancomycin sufficiently penetrated *Staphylococcus epidermidis* biofilm but eradication of biofilm was not favoured. *Invitro* studies are also reporting that biofilms surrounded with polysaccharides possess additional resistance towards any harsh environment [49]. Even the host mechanism does not impact the defence gained by the biofilm towards antibiotics.

4.2. Alteration of Antibiotics

4.2.1. Alteration of efflux pumps

Alteration in pumps lead to infiltration of various antibiotics into the biofilm, which is caused by mutation of genes or enzyme mediated drug modification [37,50]. Singh et al [51] speculated that bacteria enter a phenotypic differentiation that confers resistance either by modification of drug binding sites or through expression of efflux pumps. Bacteria can also obtain supplementary resistance from different organisms through mobile genetic elements [52]. Mutation in genes coding for porins leads to resistance against β-lactam antibiotics [53,54,55]. Mutation in five major classes of efflux pumps leads to drug resistance and they are: ATP Binding Cassette (ABC) superfamily, the Major Facilitator Superfamily (MFS), the Multidrug and Toxic-compound Extrusion (MATE) family, the Small Multidrug Resistance (SMR) family and the Resistance Nodulation Division (RND) family [56]. Multidrug efflux pump expels chemical agents and also the antibiotics from the cells. Up regulation of mar operon in *E. coli* is associated with the multidrug efflux pump AcrAB [50]. MexAB–OprM and MexCD-OprJ pumps found in *Pseudomonas aeruginosa* confer fluoroquinolone resistance [43,50] and

it also expels few antibiotics such as β-lactams macrolides, trimethoprim, chloramphenicol, novobiocin and tetracycline [57]. Few efflux pumps belonging to the resistance nodulation division family such as AcrAB–TolC, MexAB–OprM, CmeABC and MtrCDE enhance cohesion and colonisation of biofilms on the host surface [56].

4.2.2. Alteration of Antibiotic Binding Site

Alteration of the binding site or the target sites where antibiotics bind is commonly exhibited by bacteria. Mutation at enzymes like RNA polymerase and DNA gyrase leads to resistance against enzyme inhibiting antibiotics [58]. Mutation is the major cause for this alteration. One example is the Mutation in the rifampin binding site i.e. RNA polymerase which leads to resistance against rifampin [59], which is observed in *Mycobaterium tuberculosis* [60].

4.2.3. Inactivation of Antibiotics

Enzymes produced by the microorganisms are responsible for the inactivation of antibiotics. From past century there are more examples, even penicillin is cleaved by β-lactamase enzymes. As the microorganisms have evolved there are more mechanism which conferantibiotic resistance including integrons (gene expression cassettes) [61]. These enzymes convert the antibiotics by either doing one or more modification as follows – a) adenylation b) phosphorylation and c) acetylation. Multiple aminoglycoside modifying enzymes are reported to possess transferase activity against aminoglycoside and leads to resistance against aminoglycoside [62].

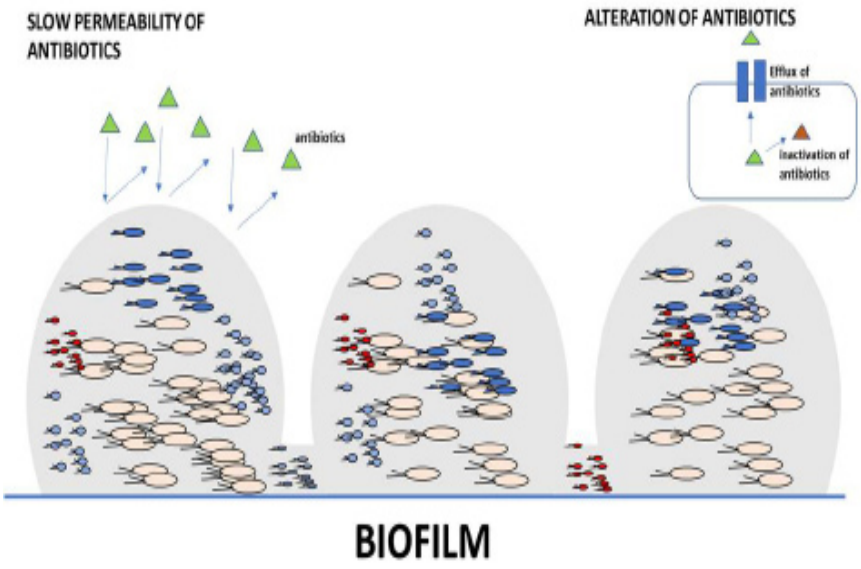

Figure 2: Antibiotic resistance exhibited by biofilm

5. Quorum Sensing and Biofilm Formation

The regulation of cell relying on its mass is termed as “Quorum Sensing” [63], the way bacteria communicate among themselves. This signalling is believed to be responsible for

growth, virulence, biofilm formation [2], sporulation [64], pigment production [52], antibiotic resistance and symbiosis and increases the pathogenicity of the microorganism [65]. Gram negative bacteria utilise N-acyl homoserine lactones for the signalling, which is produced by acyl carrier protein (ACP) [66,67], where Gram positive bacteria use peptides for quorum sensing [68]. *Pseudomonas aeruginosa* uses rhl genes for signalling [69] because lasI gene is responsible for production of N-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL), and rhl is responsible for the production of *N*-butyryl-L-homoserine lactone (C4-HSL).In *Pseudomonas aeruginosa* approximately 4% of the genes out of 6000 genes function by the mechanism of quorum sensing [70]. The expression of the exoproducts in *P. aeruginosa* like elastase Las A, elastase Las B, exotoxin A and alkaline protease was initially regulated by Las RI system [70]. In *P. aeruginosa* quorum sensing is also controlled by the LuxRI homologues and VsmRI. The synthesis of N-Butanoyl-Lhomoserine lactone (BHL) is directed by RhlI [69]. The expression of rhlAB, an operon encoding rhamnosyltransferase essential for the production of rhamnolipid is due to the interaction of acyl HSL with RhlR. Rhamnolipids are bio surfactants which help in reducing the surface tension [71]. Sigma S encoding RpoS protein helps in the expression of many activities that are known to be regulated by the Las and Rhl regulons [72].

Staphylococcus aureus causes nosocomial infections worldwide. Biofilm formation in *Staphylococcus aureus* allows the attachment of cells to a biotic or abiotic surface with the help of adhesions. Multiplication of the cells in the adhesive matrix gives rise to many layers which are associated with the production of extracellular factors, as well as the polysaccharide intercellular adhesion component [73]. The Quorum sensing system of *S.aureus* is different from that of *Pseudomonas aeruginosa* acyl homoserine lactone system. The accessory gene regulator (agr) locus is responsible for the quorum sensing system in *S.aureus* [74]. The virulence contributed by the agr system varies with the type of infection model used [75]. The virulence associated with agr is due to four proteins AgrB, AgrA, AgrC, and AgrD which are encoded by RNAII [76]. Agr can up-regulate 104 genes and down-regulate 34 genes that are involved in quorum sensing [77]. After exponential phase the agr locus directs the expression of RNAII and RNAIII transcripts through two promoters P2 and P3 [76]. At stationary phase, the agr prevents the expression of cell surface proteins and activates expression of the genes involved in the secretion of exotoxins and tissue degrading mechanism [78]. The agr locus seemingly affects several extracellular and cell wall associated protein when a transpose on (Tn551) is inserted [79]. An octapeptide is generated by AgrD and AgrB which at extracellular threshold concentration activates AgrC and AgrA responsible for the regulation of a two- component regulatory pathway [76,78].

In *Escherichia coli*, two major components of cpx signalling system are Cpx A and Cpx R. Among these, CpxA is a sensor kinase, phosphatase, involves in bacterial conjugation and

also stabilises cell surface interactions [80]. NIpE, an outer membrane lipoprotein initiates Cpx signalling system after interaction with surface and upregulates pili mediated surface adherence mostly to hydrophobic environment and regulates OmpF and OmpC [80,81]. Increased osmolarity activates EnvZ/OmpR signalling system which further produces phosphorylated OmpR and results in better adherence of the cells to the surface [82]. Phosphorylated OmpR indirectly regulates csAb operon and it codes for the structural subunits of curli, which is specialised form of pili [83]. Phosphorylated OmpR also positively regulates transcription of adrA gene which is involved in production of cellulose, which is a part of EPS in *E.coli* and *Salmonella typhirium* [84]. The EnvZ/OmpR signalling system has been found to be conserved among various bacterial species [85]. It has been observed that the EnvZ/OmpR signalling system induces surface adherence only in response to moderate increase in osmolarity while drastic rise in osmolarity impedes biofilm formation in a few species like *E. coli, Pseudomonas fluorescens* and *Streptococcus gordonii* [82]. Vibrio fischeri is a gram negative bioluminescent marine bacterium which is considered to be the finest model to understand the process of Quorum sensing. Bioluminescence is a cell population density based mechanism. The multifactorial mechanisms which are responsible for bioluminescence is well understood [86]. In *Vibrio fischeri*, the genes responsible for bioluminescence contain two chromosomes out of which the luxCDABEG gene present on the second chromosome is an integral part of the operon which is responsible for all the structural components necessary for bioluminescence [87]. The enzyme luciferase encoded by luxA and luxB is responsible for bioluminescence; it coordinates simultaneous oxidation of a long chain aldehyde and reduction of flavin mononucleotide. The fatty acids required for luminescence is derived by the diversion of fatty acyl groups from the fatty acid biosynthesis pathway by luxD [88]. LuxI and LuxR control the luciferase operon. In order to initiate luminescence, Acyl-homoserine lactones (AHLs) produced by LuxI and AHL coinducers produced by LuxR (DNA binding transcriptional activator) is required [89].The produced AHL molecules constantly diffuse in and out of the cell membrane increasing the concentration of cell population, once the threshold concentration is reached the AHL bound to LuxR activates thereby transcribing the luciferase operon which results in the emission of light [90,91].

6. Conclusion

Over the years bacteria have evolved beyond our imagination. The impact of bacterial evolution on humans is vivid from the increasing number of untreatable diseases. Bacterial communication systems have advanced creating a new era for bacteria. But we have grasped the evolution pattern and the signalling involved in communication systems. Present day advances in various fields of science and medicine has extended our knowledge on quorum sensing systems and technology has given us limitless opportunities to explore. Therefore, our aim is to develop alternatives to antibiotics (supplements which act on biofilm formation) or

discovering new antibiotics will help us to overcome the impact of Quorum sensing.

7. References

1. Moradigaravand D, Engelstädter J. The impact of natural transformation on adaptation in spatially structured bacterial populations. BMC evolutionary biology. 2014; 14:141.

2. Costerton. JW, Stewart. PS, Greenberg. EP. Bacterial Biofilms: A Common Cause of Persistent Infections. Journal of Science. 1999; 284: 1318–1322.

3. Chen L, Wen YM. The role of bacterial biofilm in persistent infections and control strategies. International journal of oral science. 2011; 3: 66.

4. Cook LC, Dunny GM. The influence of biofilms in the biology of plasmids. Microbiology spectrum. 2014; 2: 0012.

5. Deepigaa M. Antibacterial Resistance of Bacteria in Biofilms. Research Journal of Pharmacy and Technology. 2017; 10: 4019-4023.

6. Gibbs EP. The evolution of One Health: a decade of progress and challenges for the future. Veterinary Record. 2014;174(4):85-91.

7. Dias C, Borges A, Oliveira D, Martinez-Murcia A, Saavedra MJ, Simões M. Biofilms and antibiotic susceptibility of multidrug-resistant bacteria from wild animals. PeerJ. 2018; 6: e4974.

8. Tenke P, Köves B, Nagy K, Hultgren SJ, Mendling W, Wullt B, Grabe M, Wagenlehner FM, Cek M, Pickard R, Botto H. Update on biofilm infections in the urinary tract. World journal of urology. 2012; 30: 51-57.

9. Aparna MS, Yadav S. Biofilms: microbes and disease. Braz J Infect Dis. 2008; 12: 526–30.

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

11. Bjarnsholt T, Ciofu O, Molin S, Givskov M, Høiby N. Applying insights from biofilm biology to drug development—can a new approach be developed?. Nature Reviews Drug Discovery. 2013; 12: 791.

12. Jefferson K K. What drives bacteria to produce a biofilm? FEMS Microbiol Lett. 2004; 236: 163–73.

13. Barbra V, Miao C, Russel J, Crawford and Elena I. Bacterial Extracellular Polysaccharide involved in Biofilm Formation. Molecules. 2009; 14: 2535-2554.

14. van Hullebusch ED, Zandvoort MH, Lens PNL. Metal immobilisation by biofilms: mechanisms and analytical tools. Rev. Environ. Sci. Biotechnol. 2003; 2: 9-33.

15. Ruiz LM, Valenzuela S, Castro M, Gonzalez A, Frezza M, Soulère L, et al. AHL communication is a widespread phenomenon in biomining bacteria and seems to be involved in mineral-adhesion efficiency. Hydrometallurgy. 2008; 94: 133-137.

16. Mayer C, Moritz R, Kirschner C, Borchard W, Maibaum R, Wingender J, Flemming H.-C. The role of intermolecular interactions: studies on model systems for bacterial biofilms. Int. J. Biol. Macromol. 1999; 26: 3-16.

17. Ferrero MA, Martínez-Blanco H, Lopez-Velasco FF, Ezquerro-Sáenz C, Navasa N, Lozano S, Aparicio RLB. Purification and characterization of GlcNAc-6-P 2-epimerase from Escherichia coli K92. Acta. Biochim. Pol. 2007; 54: 387-399.

18. Sutherland IW, Kennedy L. Polysaccharide lyases from gellan-producing Sphingomonas spp. Microbiology. 1996; 142: 867–872

19. Veiga MC, Mahendra KJ, Wu WM, Hollingsworth RI, Zeikus JG. Composition and role of extracellular polymers in

methanogenic granules. Appl. Environ. Microbiol. 1997; 63: 403–407.

20. Zhang XQ, Bishop PL, Kinkle BK. Comparison of extraction methods for quantifying extracellular polymers in biofilms. Water Sci. Tech. 1999; 39 : 211–218.

21. FlemmingHC, WingenderJ. Relevance of microbial extra cellularpolymeric substances (EPSs). Part II. Technical aspects. Water Sci. Technol. 2001; 43: 9–16.

22. Tsuneda S, Park S, Hayashi H, Jung J, Hirate A. Enhancement of nitrifying biofilm formation using selected EPS produced by heterotrophic bacteria. Water Sci. Tech. 2001; 43: 197–204.

23. Jiao Y, D'haeseller P, Dill BD, Shah M, Ver Berkmoes NC, Hettich RL, Banfield JF, Thelen MP. Identification of biofilm matrix associated proteins from an acid mine drainage microbial community. Applied and environmental microbiology. 2011; 77: 5230-5237.

24. Pal A, Paul A.K. Microbial extracellular polymeric substances: central elements in heavy metal bioremediation. Indian J. Microbiol. 2008; 48: 49-64.

25. Sand W, Gehrke T. Extracellular polymeric substances mediate bioleaching/biocorrosion via interfacial processes involving iron (III) ions and acidophilic bacteria. Res. Microbiol. 2006; 157: 49-56.

26. Rehm, B. Microbial exopolysaccharides: Variety and potential applications. In Microbial production of biopolymers and polymer precursors: applications and perspectives; Caister Academic: Norfolk, UK. 2009; 229-254.

27. Sheng G, Yu H, Yu Z. Extraction of extracellular polymeric substances from the photosynthetic bacterium Rhodopseudomonasacidophila. ApplMicrobiolBiotechnol. 2005; 67: 125–130.

28. Gehrke T, Telegdi J, Thierry D, Sand W. Importance of extracellular polymeric substances from Thiobacillus ferro oxidans for bioleaching. Appl. Environ. Microbiol. 1998;64: 2743-2747.

29. Limoli DH, Jones CJ, Wozniak DJ. Bacterial extracellular polysaccharides in biofilm formation and function. Microbiology spectrum. 2015; 3.

30. Rodríguez-Martínez JM, Ballesta S, Garcia I, Conejo MC, Pascual A. Activity and penetration of linezolid and vancomycin against Staphylococcus epidermidis biofilms. Enfermedadesinfecciosas y microbiologiaclinica. 2007; 25: 425-428.

31. Maira-Litrán T, Kropec A, Abeygunawardana C, Joyce J, Mark G, Goldmann DA, Pier GB. Immunochemical properties of the staphylococcal poly-N-acetylglucosamine surface polysaccharide. Infection and immunity. 2002; 70: 4433-4440.

32. Sadovskaya I, Vinogradov E, Flahaut S, Kogan G, Jabbouri S. Extracellular carbohydrate-containing polymers of a model biofilm-producing strain, Staphylococcus epidermidis RP62A. Infection and immunity. 2005 May 1;73: 3007-17.

33. Gadd, G.M. Microbial influence on metal mobility and application for bioremediation. Geoderma. 2004; 122: 109-119.

34. Bhaskar PV, Bhosle NB. Bacterial extracellular polymeric substance (EPS): A carrier of heavy metals in the marine food-chain. Environ. Int. 2005; 32: 191-198.

35. Janaki V, Vijayaraghavan K, Ramasamy AK, Lee KJ, Oh BT, Kamala-Kannan S. Competitive adsorption of Reactive Orange 16 and Reactive Brilliant Blue R on polyaniline/bacterial extracellular polysaccharides composite—A novel eco-friendly polymer. Journal of hazardous materials. 2012; 241: 110-117.

36. Sun F, Qu F , Ling Y, Mao P, Xia P , Chen H & Zhou D. Biofilm-associated infections: antibiotic resistance and novel therapeutic strategies. Future Microbiol. 2013; 8: 877–886

37. Aslam S. Effect of antibacterials on biofilms. American journal of infection control. 2008;36: 175-179.

38. Zuroff TR, Bernstein H, Lloyd-Randolfi J, Jimenez-Taracido L, Stewart PS, Carlson RP. Robustness analysis of culturing perturbations on Escherichia coli colony biofilm beta-lactam and aminoglycoside antibiotic tolerance. BMC microbiology. 201; 10: 185.

39. Potera C. Forging a link between biofilms and disease. Science. 1999; 283: 1837-1839.

40. Donlan RM. Biofilms and device-associated infections. Emerging infectious diseases. 2001; 7: 277.

41. Dhar N, McKinney JD. Microbial phenotypic heterogeneity and antibiotic tolerance. Current opinion in microbiology. 2007; 10: 30-38.

42. Hunt SM, Werner EM, Huang B, Hamilton MA, Stewart PS. Hypothesis for the role of nutrient starvation in biofilm detachment. Applied and environmental microbiology. 2004;70: 7418-7425.

43. Sri kumar R, Li XZ, Poole K. Inner membrane efflux components are responsible for beta-lactam specificity of multidrug efflux pumps in Pseudomonas aeruginosa. Journal of bacteriology. 1997; 179: 7875-7881.

44. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nature reviews microbiology. 2004;2: 95-108.

45. Souli M, Giamarellou H. Effects of slime produced by clinical isolates of coagulase-negative staphylococci on activities of various antimicrobial agents. Antimicrobial agents and chemotherapy. 1998; 42: 939-941.

46. Farber BF, Kaplan MH, Clogston AG. Staphylococcusepidermidis extracted slime inhibits the antimicrobial action of glycopeptide antibiotics. Journal of Infectious Diseases. 1990; 161: 37-40.

47. Mathur T, Singhal S, Khan S, Upadhyay D, Fatma T, Rattan A. Adverse effect of staphylococci slime on in vitro activity of glycopeptides. Japanese journal of infectious diseases. 2005 Dec 1;58: 353.

48. De Beer D, Stoodley P, Roe F, Lewandowski Z. Effects of biofilm structures on oxygen distribution and mass transport. Biotechnology and bioengineering. 1994; 43: 1131-1138.

49. Stewart PS, Franklin MJ. Physiological heterogeneity in biofilms. Nature Reviews Microbiology. 2008; 6: 199.

50. Hirai K, Suzue S, Irikura T, Iyobe S, Mitsuhashi S. Mutations producing resistance to norfloxacin in Pseudomonas aeruginosa. Antimicrobial agents and chemotherapy. 1987;31: 582-6.

51. Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature. 2000;407: 762.

52. Cha C, Gao P, Chen YC, Shaw PD, Farrand SK. Production of acyl-homoserine lactone quorum-sensing signals by gram-negative plant-associated bacteria. Molecular Plant-Microbe Interactions. 1998; 11:1119-1129.

53. Gram L, Christensen AB, Ravn L, Molin S, Givskov M. Production of acylated homoserine lactones by psychrotrophic members of the Enterobacteriaceae isolated from foods. Applied and Environmental Microbiology. 1999; 65: 3458-3463.

54. Gambello MJ, Iglewski BH. Cloning and characterization of the Pseudomonas aeruginosa lasR gene, a transcriptional activator of elastase expression. Journal of Bacteriology. 1991;173: 3000-3009.

55. Livermore DM, Woodford N. Carbapenemases: A problem in waiting?. Current opinion in Microbiology. 2000; 3: 489-495.

56. Piddock LJ. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. Clinical microbiology reviews. 2006;19: 382-402.

57. Masuda N, Gotoh N, Ohya S, Nishino T. Quantitative correlation between susceptibility and OprJ production in NfxB mutants of Pseudomonas aeruginosa. Antimicrobial agents and chemotherapy. 1996;40: 909-913.

58. Lambert PA. Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. Journal of the royal society of medicine. 2002; 95: 22.

59. Campbell EA, Korzheva N, Mustaev A, Murakami K, Nair S, Goldfarb A, Darst SA. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell. 2001; 104: 901-912.

60. Thirumurugan R, Kathirvel M, Vallayyachari K, Surendar K, Samrot AV, Muthaiah M. Molecular analysis of rpoB gene mutations in rifampicin resistant Mycobacterium tuberculosis isolates by multiple allele specific polymerase chain reaction in Puducherry, South India. Journal of Infection and Public health. 2015; 8: 619-625.

61. Davies J. Inactivation of antibiotics and the dissemination of resistance genes. Science. 1994; 264: 375-382.

62. Munita JM, Bayer AS, Arias CA. Evolving resistance among Gram-positive pathogens. Clinical Infectious Diseases. 2015;61: S48-57.

63. Fuqua C, Greenberg EP. Listening in on bacteria: Acyl homoserine lactone signalling. Nat Rev Mol Cell Biol. 2002; 3: 685–695.

64. De Kievit TR, Iglewski BH. Bacterial quorum sensing in pathogenic relationships. Infection and immunity. 2000; 68: 4839-49.

65. Fux CA, Costerton JW, Stewart PS, Stoodley P. Survival strategies of infectious biofilms. Trends in microbiology. 2005; 13: 34-40.

66. Schaber JA, Triffo WJ, Suh SJ, Oliver JW, Hastert MC, Griswold JA, Auer M, Hamood AN, Rumbaugh KP. Pseudomonas aeruginosa forms biofilms in acute infection independent of cell-to-cell signaling. Infection and immunity. 2007;75: 3715-3721.

67. Parsek MR, Val DL, Hanzelka BL, Cronan JE, Greenberg EP. Acyl homoserine-lactone quorum-sensing signal generation. Proceedings of the National Academy of Sciences. 1999; 96: 4360-4365.

68. Rutherford ST, Bassler BL. Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harbor perspectives in medicine. 2012; 2: a012427.

69. Pearson JP, Gray, KM, Passador, L, Tucker, K.D, Eberhard, A, Iglewski, B.H, Greenberg E.P. Structure of the autoinducer required for expression of Pseudomonas aeruginosa virulence genes. Proc. Natl. Acad. Sci. USA. 1994; 91: 197-201.

70. Ochsner UA, Fiechter A, Reiser J. Isolation, characterization, and expression in Escherichia coli of the Pseudomonas aeruginosarhlAB genes encoding a rhamnosyltransferase involved in rhamnolipid biosurfactant synthesis. Journal of Biological Chemistry. 1994; 269:19787-19795.

71. Köhler T, Curty LK, Barja F, Van Delden C, Pechère JC. Swarming of Pseudomonas aeruginosa is dependent on cell-to-cell signaling and requires flagella and pili. Journal of bacteriology. 2000; 182: 5990-5996.

72. Winzer K, Falconer C, Garber NC, Diggle SP, Camara M, Williams P. The Pseudomonas aeruginosa lectins PA-IL and PA-IIL are controlled by quorum sensing and by RpoS. Journal of bacteriology. 2000; 182: 6401-6411.

73. Kitahara T, Koyama N, Matsuda J, Aoyama Y, Hirakata Y, Kamihira S, Kohno S, Nakashima M, Sasaki H. Antimicrobial activity of saturated fatty acids and fatty amines against methicillin-resistant Staphylococcus aureus. Biological and Pharmaceutical Bulletin. 2004; 27: 1321-1326.

74. Projan SJ, Novick RP. The molecular basis of pathogenicity, In KB. Crossley and GL. Archer (ed.), the staphylococci in human disease. Churchill Livingstone Inc, New York NY. 1997; 55-81.

75. Goerke CU, Fluckiger A, Steinhuber W, Zimmerli C, Wolz. Impact of the regulatory loci agr, sarA and sae of Staphylococcus aureus on the induction of alpha-toxin during device-related infection resolved by direct quantitative transcript analysis. Mol. Microbiol. 2001; 40: 1439-1447.

76. Kornblum J, Kreiswirth B., Projan SJ, Ross H, Novick RP. Agr: A polycistronic locus regulating exoprotein synthesis in Staphylococcus aureus. VCH Publishers, New York NY. 1990.

77. Dunman PÁ, Murphy E, Haney S, Palacios D, Tucker-Kellogg G, Wu S, Brown EL, Zagursky RJ, Shlaes D, Projan SJ. Transcription Profiling-Based Identification of Staphylococcus aureus Genes Regulated by the agrand/or sarA Loci. Journal of bacteriology. 2001; 183: 7341-7453.

78. Novick RJ, Schäfers HJ, Stitt L, Andréassian B, Duchatelle JP, Klepetko W, Hardesty RL, Frost A, Patterson GA. Recurrence of obliterative bronchiolitis and determinants of outcome in 139 pulmonary retransplant recipients. The Journal of thoracic and cardiovascular surgery. 1995; 110: 1402-1414.

79. Recsei P, Kreiswirth B, O'reilly M, Schlievert PM, Gruss A, Novick RP. Regulation of exoprotein gene expression in Staphylococcusaureus by agr. Molecular and General Genetics MGG. 1986; 202: 58-61.

80. Raivio TL, Silhavy TJ. Transduction of envelope stress in Escherichia coli by the Cpx two-component system. Journal of Bacteriology. 1997;179(24):7724-33.

81. Batchelor E, Walthers D, Kenney LJ, Goulian M. The Escherichia coli CpxA-CpxR envelope stress response system regulates expression of the porins ompF and ompC. Journal of Bacteriology. 2005; 187: 5723-5731.

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

83. Vidal O, Longin R, Prigent-Combaret C, Dorel C, Hooreman M, Lejeune P. Isolation of an Escherichiacoli K-12 mutant strain able to form biofilms on inert surfaces: involvement of a new ompR allele that increases curli expression. Journal of Bacteriology. 1998; 180: 2442-2449.

84. Chirwa NT, Herrington MB. CsgD, a regulator of curli and cellulose synthesis, also regulates serine hydroxymethyltransferase synthesis in Escherichia coli K-12. Microbiology. 2003; 149: 525-535.

85. Dziejman M, Mekalanos JJ. Two-component signal transduction and its role in the expression of bacterial virulence factors. In Two-component signal transduction.. American Society of Microbiology. 1995; 305-317.

86. Meighen EA. Bacterial bioluminescence: organization, regulation, and application of the lux genes. The FASEB Journal. 1993; 7: 1016-1022.

87. Engebrecht J, Nealson K, Silverman M. Bacterial bioluminescence: isolation and genetic analysis of functions from Vibrio fischeri. Cell. 1983; 32: 773-781.

88. Boylan M, Miyamoto C, Wall L, Graham A, Meighen E. Lux C, D and E genes of the Vibrio fischeri luminescence operon code for the reductase, transferase, and synthetase enzymes involved in aldehyde biosynthesis. Photochemistry and photobiology. 1989; 49: 681-688.

89. Hao Y, Winans SC, Glick BR, Charles TC. Identification and characterization of new LuxR/LuxI-type quorum sensing systems from metagenomic libraries. Environmental microbiology. 2010; 12: 105-17.

90. Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. Annu. Rev. Cell Dev. Biol.. 2005 Nov 10; 21: 319-346.

91. Von Bodman SB, Majerczak DR, Coplin DL. A negative regulator mediates quorum-sensing control of exopolysaccharide production in Pantoeastewartii subsp. stewartii. Proceedings of the National Academy of Sciences. 1998; 95: 7687-7692.