Journal of Bacteriology and Parasitology

Chadha, J Bacteriol Parasitol 2014, 5:3
DOI: 10.4172/2155-9597.1000190

Research Article Open Access

Bacterial Biofilms: Survival Mechanisms and Antibiotic Resistance

Tejpreet Chadha[1]

[1]*Department of Biological and Environmental Sciences, Troy University, Troy, Alabama, USA*

[*]**Corresponding author:** Tejpreet Chadha, Department of Biological & Environmental Sciences, Troy University, Troy, Alabama, USA 36082 ,Tel : +1-650-268-9744; Fax: +1-650-618-1414; E-mail: tchadha@troy.edu

Rec date: June 20, 2014; **Acc date:** July 15, 2014; **Pub date:** July 18, 2014

Abstract

Biofilm represents single species or multi-species communities that interact and cooperate with each other and their environment to carry out complex processes. One of the most important challenges is to understand intercellular communications that exist within the community that promote biofilm formation. Biofilm currently represents a major health problem as it play an important role in device-related infections such as prosthetic valves, catheters and contact lenses. The present review will focus on the mechanisms that lead to biofilm formation on surfaces and highlight several medically important pathogens.

Keywords: Biofilm; Extracellular polymeric substances; Antibiotic resistance; Horizontal gene transfer; Nosocomial infections

Introduction

In nature prokaryotes occupy diverse habitat as they have ability to attach and form communities. Once in a community, bacterial species cooperate, compete and interact with each other and carry out complex processes. Biofilm represents single species of bacteria or multispecies communities. They can be found in nature as well as in industrial and clinical environment. For instance, dental plaque are known to contain as many as 700 species [1-10] growing in Extracellular Polymeric Substances (EPS).

Biofilm Formation

The formation of biofilm begins in stages. In stage one, there is transient binding of planktonic bacteria to a solid surface with characteristic adhesion. In stage two, there is aggregation and formation of micro colonies surrounded by protective secreted molecules known as extra polymeric substance (EPS) matrix. Finally, there is dispersal that involves shedding from the mature biofilm as planktonic bacteria or as micro colonies. This dispersal stage may promote further colonizing the host with biofilms. This may ultimately benefit the organisms due to limited nutrient availability and waste accumulation.

Depending on ecological niche, bacteria are exposed to different stresses such as UV radiation, pH shifts, osmolarity, iron availability, oxygen tension, temperature, nutrient availability and desiccation [3] that may obstruct their basic activities such as ability to grow and survive. These environment signals trigger the transition from planktonic growth to life on a surface. However, the environmental cues differ greatly among organisms. For instance, *Pseudomonas aeruginosa* will form biofilms under most conditions that allow growth [11,12]. However, *Escherichia coli* O157:H7 has been reported to make a biofilm only under low-nutrient conditions[13]. The genetic analysis of biofilm formation by many organisms has revealed that they may utilize multiple genetic pathways to initiate biofilm development [14]. For instance, *Vibrio cholera* may utilize different pathways for initial attachment depending on the surface to which the organism attaches. For example, the study in vivo has shown that Tcp pilus is required for colonization of the intestine[15]. However, Tcp pilus is not important in attachment to abiotic surfaces. Although, environmental signals may trigger biofilm development, they may vary from organism to organism. In order to gain stability and ecological success, bacterial species has developed adaptive strategies. Thus, bacterial species come together and form biofilm to enhance survival especially under adverse conditions.

Biofilm Matrix

The major component in the biofilm matrix is water that may measure up to 97% [16]. The secretion of EPS is linked with the genes that are up-regulated in biofilms [17]. The EPS may vary in their composition, chemical and physical properties [16]. The phenotype of mature film depends on the environment in which it develops. The studies have shown that the changes in environment results in phenotypic changes in the biofilm formation [16,18,19]. EPS has also been reported to provide protection from a variety of environmental stresses. For instance, the protective role of EPS was demonstrated as it provided resistance to desiccation in mucoid strains of bacteria such as *E. coli* when compared to non mucoid variants of the same [20]. The EPS helped bacterial species to adapt to stressful and changeable environmental conditions. The slower growth of bacteria has been observed in biofilm to enhance EPS production for adaptation. The mutants that are unable to synthesize the EPS are usually unable to form biofilms [15]. For instance, *E. coli* strain that cannot develop normal biofilm is also defective in colonic acid production. The colonic acid is a major EPS synthesized by this organism. . However, in a mixed population, one species producing EPS may provide the stability to mutant type that are unable to synthesize EPS [16,21].

Intercellular Communication

The biofilm enables cells to live close to each other to facilitate exchange of plasmids and free DNA that enable them to overcome different environmental stresses. The bacteria in a biofilm uses chemical communication known as quorum sensing that help them to coordinated their metabolism and other complex processes and adapt

to the ongoing changes in the environment. For instance, *Bacillus subtilis* uses intercellular communication during its metamorphosis into spores to better adapt to changing environmental parameters [22]. The mutant of *P. aeruginosa* that is unable to synthesize the major quorum-sensing molecules acylhomoserine lactones (acyl-HSLs) was able to produce altered biofilm when compared to its wild type. This demonstrated that these molecules regulate the formation of biofilm structures in this organism. This data strongly suggest that cell-cell communication is essential for this bacterium to establish a well ordered surface community [23,24].

The genetic analysis of Streptococcus gordonii, an oral microbe suggested that cell-to-cell communication may also be important for biofilm development in these gram-positive organisms. The maturation of the biofilm relies on cell-to-cell interactions called co aggregation. The structural and spatial organization can have a profound impact on biofilm ecology. The three dimensional organization of biofilm allow cells to fix their locations with respect to each other [4] and help in release of distinct environmental signals within a biofilm that provides additional benefits for metabolic cooperation and niches. For instance, cells that are situated near the center of a micro colony are more likely to experience low oxygen tensions. This may provide better environment for strictly anaerobic methanogens that are embedded in EPS [11].

Implications of Biofilm Formation

In nature, microorganisms are exposed to harsh environment such as hydrothermal vents, deep sea vents, acid mine drainage. The physiological adaption to challenging conditions has many benefits. Interestingly, biofilms are involved in the processing of sewage, treatment of groundwater contaminated with petroleum products [25]. The surface-attached biofilms in form trickling filters are used in some waste-water treatment plants[17]. Biofilms are able to accumulate metals and may help in transfer of metals through an ecosystem. For instance, biofilms in acid mine drainage may contribute to the cycling of sulfur [26].

Syntrophic Relationships

Biofilms provide an ideal environment for the establishment of syntrophic relationships that enables two metabolically distinct types of bacteria to depend on each other to utilize certain substrates for energy production [27,28]. The study done by Bryant et al. showed that two different organisms interacted syntrophically to convert ethanol to acetate and methane by interspecies hydrogen transfer [27]. These relationships have gained more importance as they may promote pathogenicity of virulent organisms and promote their colonization and survival [29].

Antibiotic resistance

Biofilms are associated with an emergence of antibiotic resistant bacteria. Horizontal gene transfer promotes evolution and genetic diversity of natural microbial communities. The study of gene transfer in natural environments has gained importance by emergence of multidrug-resistant bacteria [5,30-32]. The EPS matrix prevents access of certain antimicrobial agents restricting diffusion of compounds from the surrounding into the biofilm. The classes of antibiotics that are hydrophilic and positively charged, such as aminoglycosides are more obstructed than others.

There may be inactivation of the antibiotics by extracellular polymers or modifying enzymes. The bacteria in a biofilm are 1,000-fold more resistant to antibiotic treatment than the same organism that are grown planktonically [19,33]. The extensive use of antibiotics to promote growth in domestic animals, livestock and agriculture has resulted in selection of antibiotic resistant bacteria [6,34-39]. The prevalence of plasmids in bacteria from diverse habitats and gene transfer by conjugation has resulted in dissemination of genetic information [40,41]. As most of the bacteria in natural settings reside within biofilms, conjugation is one of the most likely mechanisms by which bacteria in biofilms transfer genes within or between populations [42-46]. The study of microcosm dental plaque have shown that *Bacillus subtilis* strain that harbored a conjugative transposon with tetracycline resistant cassette was able to transfer conjugative transposon to *Streptococcus species* in biofilm bacteria [47]. These results proved that non oral bacteria have the potential to transfer genes to oral commensals [47]. Clinical biofilm infections have shown that treatment with antibiotics is not a complete solution as symptoms usually recur even after repeated treatments. The antibiotic therapy eliminates the planktonic cells, but the sessile forms are resistant and continue to propagate within the biofilm [19]. However, there is continuous release of antigens and production of antibodies that eventually causes more damage to the surrounding tissue [19,48].

Biofilm and Nosocomial Infections

Biofilms play a prominent role in the contamination of medical implants by residing on abiotic surfaces [49,50] such as prosthetic valves, catheters and contact lenses. The bacterial biofilms on prosthetic valves are the leading cause of endocarditis in patients that have undergone heart valve replacement [51,52]. The biofilm formation on urinary catheters is also reported as a leading cause of urinary tract infections [53,54]. Biofilm formation can also occur on contact lenses that may lead to keratitis[55-57].

Biofilm plays a remarkable role in cystic fibrosis (CF) patients that are infected by *Pseudomonas aeruginosa*. The inherited genetic disorder increases the susceptibility to chronic *P. aeruginosa* infections although the basis is not yet known. The infection causes hyperactive inflammatory response in the lung that may eventually destroy the functioning of the lung and leads to the death of the patient [12,58,59]. *P. aeruginosa* species isolated from the CF patients were mucoid with overexpression of EPS called alginate. The aligate may promote biofilm formation and enhance resistance to antibiotics.

The chronic ear infections are also related to biofilm bacterial species [60,61]. However, biofilm bacteria can be difficult to culture by routine methods [11,62].

Periodontitis is an important case of a biofilm-mediated disease. The main bacterium associated with this disease is *Porphyromonas gingivalis* [63] that colonizes in the oral cavity to invade mucosal cells and release toxins. The chronic inflammation may even lead to tooth loss. The bacterium may colonize mucosal and tooth surfaces directly or via interactions with primary colonizers. The primary colonizers are *S. gordonii*, *Streptococcus sanguis,* and *Streptococcus parasanguis* that add up to 60-80% of the early bacterial population [64,65].

Can Antibiotic Stimulate Biofilm Formation?

The current antibiotic treatment guidelines do not consider the difference in the ecological dynamics that exist between different

bacterial species [66]. Antibiotics when administered at concentrations below the minimum inhibitory concentration can induce biofilm formation in a variety of bacterial species [48,67]. This is of major concern as cells that are deep inside the biofilm may be exposed to sub-MIC level of antibiotic. Instead of inhibiting the biofilm, the antibiotic may promote biofilm formation [68]. The other concern is dosing regimen as bacteria are exposed to sub-MIC concentrations of antibiotics at the beginning and end of a dosing regimen [69]. The extensive use and misuse of antibiotics in agriculture, livestock and aquaculture may further exposure of bacteria to low levels of the drugs [5,70,71].

Discussion

The discovery of surface-attached bacteria happened almost 70 years ago [72]. However, we are still trying to understand the significance of biofilm communities. Interestingly, to understand bacteria as a community takes us away from our traditional view of microbiology. The major challenge is to understand intercellular communications that promote stability in biofilms and usage of models that can mimic natural communities in the laboratory. However, there is some success in this area such as development of model to study catheter- induced bladder infections [73]. The discovery of confocal scanning laser microscopes (CSLM) has further helped to examine the three-dimensional structure and function of biofilms. However, application of modern techniques with the collaborative efforts from scientists from various fields will help to better understand this continuous evolving dynamic world of biofilms.

Acknowledgement

I would like to thank everyone that has helped me in this work. Troy University, Department of Biological & Environmental Sciences.

References

1. Bjarnsholt T (2013) The role of bacterial biofilms in chronic infections. APMIS Suppl : 1-51.
2. Danielsen KA, Eskeland O, Fridrich-Aas K, Orszagh VC, Bachmann-Harildstad G, et al. (2014) Bacterial biofilms in patients with chronic rhinosinusitis: a confocal scanning laser microscopy study. Rhinology 52: 150-155.
3. Staley C, Dunny GM, Sadowsky MJ (2014) Environmental and animal-associated enterococci. Adv Appl Microbiol 87: 147-186.
4. Lupo A, Coyne S, Berendonk TU (2012) Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. Front Microbiol 3: 18.
5. Martínez JL (2012) Natural antibiotic resistance and contamination by antibiotic resistance determinants: the two ages in the evolution of resistance to antimicrobials. Front Microbiol 3: 1.
6. Sarika, Iquebal MA, Rai A (2012) Biotic stress resistance in agriculture through antimicrobial peptides. Peptides 36: 322-330.
7. Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, et al. (2010) Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbiol 8: 251-259.
8. Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74: 417-433.
9. Laskaris P, Tolba S, Calvo-Bado L, Wellington EM (2010) Coevolution of antibiotic production and counter-resistance in soil bacteria. Environ Microbiol 12: 783-796.
10. Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, et al. (2001) Bacterial diversity in human subgingival plaque. J Bacteriol 183: 3770-3783.
11. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM (1995) Microbial biofilms. Annu Rev Microbiol 49: 711-745.
12. May TB, Shinabarger D, Maharaj R, Kato J, Chu L, et al. (1991) Alginate synthesis by Pseudomonas aeruginosa: a key pathogenic factor in chronic pulmonary infections of cystic fibrosis patients. Clin Microbiol Rev 4: 191-206.
13. Danese PN, Pratt LA, Kolter R (2000) Exopolysaccharide production is required for development of Escherichia coli K-12 biofilm architecture. J Bacteriol 182: 3593-3596.
14. Pratt LA, Kolter R (1998) Genetic analysis of Escherichia coli biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol Microbiol 30: 285-293.
15. Watnick PI, Kolter R (1999) Steps in the development of a Vibrio cholerae El Tor biofilm. Mol Microbiol 34: 586-595.
16. Sutherland I (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147: 3-9.
17. Ikuma K, Decho AW, Lau BLT (2013) The Extracellular Bastions of Bacteria - A Biofilm Way of Life. Nature Education Knowledge 4: 2.
18. Davey ME, O'toole GA (2000) Microbial biofilms: from ecology to molecular genetics. Microbiol Mol Biol Rev 64: 847-867.
19. Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284: 1318-1322.
20. Ophir T, Gutnick DL (1994) A role for exopolysaccharides in the protection of microorganisms from desiccation. Appl Environ Microbiol 60: 740-745.
21. Yao ES, Lamont RJ, Leu SP, Weinberg A (1996) Interbacterial binding among strains of pathogenic and commensal oral bacterial species. Oral Microbiol Immunol 11: 35-41.
22. Mielich-Süss B, D Lopez (2014) Molecular mechanisms involved in Bacillus subtilis biofilm formation. Environ Microbiol.
23. Solano C, Echeverz M1, Lasa I2 (2014) Biofilm dispersion and quorum sensing. Curr Opin Microbiol 18: 96-104.
24. Garg N, Manchanda G, Kumar A (2014) Bacterial quorum sensing: circuits and applications. Antonie Van Leeuwenhoek 105: 289-305.
25. Flemming HC (2002) Biofouling in water systems--cases, causes and countermeasures. Appl Microbiol Biotechnol 59: 629-640.
26. Farag AM, Woodward DF, Goldstein JN, Brumbaugh W, Meyer JS (1998) Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d'Alene River basin, Idaho. Arch Environ Contam Toxicol 34: 119-127.
27. Schink B (1997) Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Mol Biol Rev 61: 262-280.
28. Angelidaki I, Karakashev D, Batstone DJ, Plugge CM, Stams AJ (2011) Biomethanation and its potential. Methods Enzymol 494: 327-351.
29. Tolker-Nielsen T, Molin S (2000) Spatial Organization of Microbial Biofilm Communities. Microb Ecol 40: 75-84.
30. Wright GD (2007) The antibiotic resistome: the nexus of chemical and genetic diversity. Nat Rev Microbiol 5: 175-186.
31. Martinez JL (2009) The role of natural environments in the evolution of resistance traits in pathogenic bacteria. Proc Biol Sci 276: 2521-2530.
32. Chadha T (2012) Antibiotic Resistant Genes in Natural Environment. Agrotechnol 1.
33. Mah TF, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol 9: 34-39.
34. McManus PS, Stockwell VO, Sundin GW, Jones AL (2002) Antibiotic use in plant agriculture. Annu Rev Phytopathol 40: 443-465.
35. Cabello FC (2006) Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbiol 8: 1137-1144.
36. Bates J (1997) Epidemiology of vancomycin-resistant enterococci in the community and the relevance of farm animals to human infection. J Hosp Infect 37: 89-101.

37. Chang CC, Chomel BB, Kasten RW, Heller RM, Ueno H, et al. (2000) Bartonella spp. isolated from wild and domestic ruminants in North America. Emerg Infect Dis 6: 306-311.
38. Alekshun MN, Levy SB (2006) Commensals upon us. Biochem Pharmacol 71: 893-900.
39. Bager F, Madsen M, Christensen J, Aarestrup FM (1997) Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant Enterococcus faecium on Danish poultry and pig farms. Prev Vet Med 31: 95-112.
40. Chadha T, Trindade AA (2013) Phylogenetic analysis of pbp genes in treponemes. Infect Ecol Epidemiol 3.
41. Chadha T, Alexandre AT (2012) Phylogenetic Analysis of Genetic Diversity of Hemolysins in Leptospira. J Proteomics Bioinform 5: 152-154.
42. Anderson JD (1975) Factors that may prevent transfer of anti-biotic resistance between gram-negative bacteria in the gut. J Med Microbiol 8: 83-88.
43. Salyers AA, Shoemaker NB (1996) Resistance gene transfer in anaerobes: new insights, new problems. Clin Infect Dis 23 Suppl 1: S36-43.
44. Ghigo JM (2001) Natural conjugative plasmids induce bacterial biofilm development. Nature 412: 442-445.
45. Juhas M, van der Meer JR, Gaillard M, Harding RM, Hood DW, et al. (2009) Genomic islands: tools of bacterial horizontal gene transfer and evolution. FEMS Microbiol Rev 33: 376-393.
46. Chadha T, John CZ (2013) Motility and ß-Lactamases: Occurrences of Antibiotic Resistance in Nosocomial Infections. J Data Mining Genomics Proteomics 4: 126.
47. Roberts AP, Pratten J, Wilson M, Mullany P et al. (1999) Transfer of a conjugative transposon, Tn5397 in a model oral biofilm. FEMS Microbiol Lett 177: 63-6.
48. Stewart PS, Costerton JW (2001) Antibiotic resistance of bacteria in biofilms. Lancet 358: 135-138.
49. Wojtyczka RD, Orlewska K, KÄ™pa M, Idzik D, Dziedzic A, et al. (2014) Biofilm formation and antimicrobial susceptibility of Staphylococcus epidermidis strains from a hospital environment. Int J Environ Res Public Health 11: 4619-4633.
50. Abdallah M, Benoliel C, Drider D, Dhulster P, Chihib NE (2014) Biofilm formation and persistence on abiotic surfaces in the context of food and medical environments. Arch Microbiol 196: 453-472.
51. Busscher HJ, Bruinsma G, van Weissenbruch R, Leunisse C, van der Mei HC, et al. (1998) The effect of buttermilk consumption on biofilm formation on silicone rubber voice prostheses in an artificial throat. Eur Arch Otorhinolaryngol 255: 410-413.
52. Gristina AG, Dobbins JJ, Giammara B, Lewis JC, DeVries WC (1988) Biomaterial-centered sepsis and the total artificial heart. Microbial adhesion vs tissue integration. JAMA 259: 870-874.
53. Nickel JC, Ruseska I, Wright JB, Costerton JW (1985) Tobramycin resistance of Pseudomonas aeruginosa cells growing as a biofilm on urinary catheter material. Antimicrob Agents Chemother 27: 619-624.
54. Nickel JC, Downey JA, Costerton JW (1989) Ultrastructural study of microbiologic colonization of urinary catheters. Urology 34: 284-291.
55. McLaughlin-Borlace L, Stapleton F, Matheson M, Dart JK (1998) Bacterial biofilm on contact lenses and lens storage cases in wearers with microbial keratitis. J Appl Microbiol 84: 827-838.
56. Kalishwaralal K, BarathManiKanth S, Pandian SR, Deepak V, Gurunathan S (2010) Silver nanoparticles impede the biofilm formation by Pseudomonas aeruginosa and Staphylococcus epidermidis. Colloids Surf B Biointerfaces 79: 340-344.
57. Wiley L, Bridge DR, Wiley LA, Odom JV, Elliott T, et al. (2012) Bacterial biofilm diversity in contact lens-related disease: emerging role of Achromobacter, Stenotrophomonas, and Delftia. Invest Ophthalmol Vis Sci 53: 3896-3905.
58. Rodríguez-Rojas A, Oliver A, Blázquez J (2012) Intrinsic and environmental mutagenesis drive diversification and persistence of Pseudomonas aeruginosa in chronic lung infections. J Infect Dis 205: 121-127.
59. Wei Q, Ma LZ (2013) Biofilm matrix and its regulation in Pseudomonas aeruginosa. Int J Mol Sci 14: 20983-21005.
60. Dingman JR, Rayner MG, Mishra S, Zhang Y, Ehrlich MD, et al. (1998) Correlation between presence of viable bacteria and presence of endotoxin in middle-ear effusions. J Clin Microbiol 36: 3417-3419.
61. Akyıldız I, Take G, Uygur K, Kızıl Y, Aydil U (2013) Bacterial biofilm formation in the middle-ear mucosa of chronic otitis media patients. Indian J Otolaryngol Head Neck Surg 65: 557-561.
62. Wessman M, Bjarnsholt T, Eickhardt-Sørensen SR, Johansen HK, Homøe P (2014) Mucosal biofilm detection in chronic otitis media: a study of middle ear biopsies from Greenlandic patients. Eur Arch Otorhinolaryngol .
63. Lamont RJ, Jenkinson HF (1998) Life below the gum line: pathogenic mechanisms of Porphyromonas gingivalis. Microbiol Mol Biol Rev 62: 1244-1263.
64. Cook GS, Costerton JW, Lamont RJ (1998) Biofilm formation by Porphyromonas gingivalis and Streptococcus gordonii. J Periodontal Res 33: 323-327.
65. Fletcher J, Nair S, Poole S, Henderson B, Wilson M (1998) Cytokine degradation by biofilms of Porphyromonas gingivalis. Curr Microbiol 36: 216-219.
66. Geli P, Laxminarayan R, Dunne M, Smith DL (2012) "One-size-fits-all"? Optimizing treatment duration for bacterial infections. PLoS One 7: e29838.
67. Strelkova EA, Zhurina MV, Plakunov VK, Beliaev SS (2012) [Antibiotics stimulation of biofilm formation]. Mikrobiologiia 81: 282-285.
68. Lewis K (2005) Persister cells and the riddle of biofilm survival. Biochemistry (Mosc) 70: 267-274.
69. Lewis K (2001) Riddle of biofilm resistance. Antimicrob Agents Chemother 45: 999-1007.
70. Martínez JL (2008) Antibiotics and antibiotic resistance genes in natural environments. Science 321: 365-367.
71. Martínez JL, Baquero F, Andersson DI (2007) Predicting antibiotic resistance. Nat Rev Microbiol 5: 958-965.
72. Zobell CE, Allen EC (1935) The Significance of Marine Bacteria in the Fouling of Submerged Surfaces. J Bacteriol 29: 239-251.
73. Stickler DJ, King JB, Winters C, Morris SL (1993) Blockage of urethral catheters by bacterial biofilms. J Infect 27: 133-135.