The Role of Exopolysaccharides in Oral Biofilms

C. Cugini, M. Shanmugam, N. Landge, and N. Ramasubbu

Appendix

Structural aspects of EPS synthesis and export by Gram negative bacteria –*A. actinomycetemcomitans* EPS is produced by the proteins encoded by the four-gene operon, *pgaABCD* (Izano et al. 2008). The newly synthesized EPS in *A. actinomycetemcomitans* should traverse through the export machinery made up of the PgaABCD proteins. Very few studies have been undertaken to decipher the structure of this machinery in *A. actinomycetemcomitans* or other non-oral bacteria that produce similar EPS. The 3D structures for some of the proteins involved in the production and export have been determined for *A. actinomycetemcomitans* and *E. coli* (Little et al. 2012; Parthiban et al. 2016; Wang et al. 2016). However, the architecture of the whole machinery remains undetermined. Thus, there is a void in understanding the complete 3D architecture of the EPS export machinery and other EPS-producing enzymes in oral bacteria.

Role of calcium in biofilm development - As carious lesions develop, a shift in the non-mutans streptococci species to a primarily mutans streptococci accumulation at the site is promoted, and as a result, biofilms are exposed to higher concentrations of calcium at the local level due to enamel dissolution. The role of calcium in matrix strengthening is now beginning to be understood. It should be noted that the *S. mutans* glycosyltransferases, which synthesize soluble and insoluble glucans, are calcium-binding proteins and require calcium for their enzymatic activity. *S. mutans*, through the calcium ion binding ability of EPS, has an increased tolerance for elevated levels of calcium, even higher than what is found in saliva (Astasov-Frauenhoffer et al. 2017). This calcium binding mechanism affects the rate of free calcium available for remineralization in favor of the bacterium (Astasov-Frauenhoffer et al. 2017). This calcium-binding role of EPS provides a mechanism by which cariogenic bacteria can adapt to high, toxic levels of calcium ion concentrations, and strengthen their biofilm matrix core. As a caveat, it should be noted that there is evidence that calcium can be antimicrobial against Gram-positive organisms (Xie and Yang 2016).

Matrix development in mixed species biofilms - Matrix development can also depend on interspecies interactions within the multispecies biofilm in the oral cavity. The biofilms formed by

polymicrobial interactions, especially between *S. mutans* and *C. albicans*, are structurally complex and challenging to eradicate or study. The mixed biofilm is rich in α-glucan EPS produced by S. mutans (Bowen et al. 2018). In this mixed biofilm, *C. albicans* induces *gtfB* expression in S. mutans resulting in α -glucans in situ, which in turn enhance co-adhesion (Megan et al. 2017; Hwang et al. 2017; Kim et al. 2017). Emerging studies demonstrate that *S. mutans*-derived EPSs play a critical role in enhancing *C. albicans* tolerance to topical fluconazole, thus strengthening the mixed species biofilm matrix as there is no loss of either species (Kim et al. 2018). However, the relationship between S. mutans and *C. albicans* cannot be classified as only synergistic. For instance, while in mono-culture and in co-culture of these two species, biofilm biomass increased but EPS synthesis in *S. mutans* is suppressed. Similarly, EPS synthesis in *S. mutans* is also suppressed when grown in spent culture supernatants of dual-species biofilms (Sztajer et al. 2014).

Second messenger regulation of extracellular polysaccharide production in oral bacteria - Many oral bacteria, such as *Treponema denticola* (Romling et al. 2013) and *P. gingivalis* (Chaudhuri et al. 2014), have been shown to possess genes that encode for the synthesis of the second messenger, cyclic-di-GMP. To date, though, it has not been determined how cyclic-di-GMP contributes to EPS production in these organisms. *A. actinomycetemcomitans* produces an EPS similar to *E. coli* whose EPS production is controlled by cyclic-di-GMP (Steiner et al. 2013), however, *A. actinomycetemcomitans* does not produce the second messenger. The *brp* locus, described above in *V. vulnificus*, is controlled by cyclic-di-GMP (Chodur and Rowe-Magnus 2018). It has also been suggested that EPS production might be regulated by cyclic-di-GMP in other bacteria, including *P. aeruginosa* (Perez-Mendoza and Sanjuan 2016).

Another second messenger, cyclic-di-AMP (Corrigan and Grundling 2013) has been identified in Gram-positive bacteria, including *S. mutans,* where it plays a regulatory role in the production of EPS (Peng et al. 2016). While the cyclic-di-AMP synthesis genes are present in *P. gingivalis*, their roles have not been fully characterized (Wei et al. 2015). Clearly, the area of second messenger signaling and how EPS and biofilm formation is affected by cyclic-di-nucleotides in oral bacteria is severely understudied. One possible reason for this might be the unavailability of fast and reliable methods for detection of cyclic-di-nucleotides. Recent developments of detection methods should move the research forward (Underwood et al. 2014).

Immune evasion in non-oral bacteria through EPS – The medically important bacterium *S. epidermidis* produces an EPS similar to that of *A. actinomycetemcomitans*. When comparing wild type and a mutant that lacks EPS production, the presence of EPS in *S. epidermidis* biofilms led to the prevention of complement protein C3b and IgG deposition on the bacterial surface, contributing to immune evasion by this bacterium. Notably, C3a induction was higher by the wild type cells than by either planktonic cells or the EPS mutant (Kristian et al. 2008). *P. aeruginosa* produces three kinds of exopolysaccharides, alginate, Psl, and Pel, which are suggested to be involved in the establishment of chronic biofilm infections and immune evasion. In a recent study, it was demonstrated that the neutral polysaccharide Psl and not alginate, plays a role in blocking the deposition of the complement component C3 and increasing serum resistance in mucoid *P. aeruginosa* strains (Jones and Wozniak 2017; Mishra et al. 2012)

Capsular polysaccharides of group B streptococci also confer diminished or no immune response by host cells. These bacteria produce high levels of a highly negatively charged capsular polysaccharide. Because of the presence of the charged saccharide, when in contact with the similarly charged mucopolysaccharides of the luminal mucus, these bacteria evade the mucocilliary flow and transition to the epithelial surface to access host cell surface receptors for colonization (Nelson et al. 2007). In this regard, it should be noted that the EPSs of both staphylococci and *A. actinomycetemcomitans* are partially deacetylated by a deacetylase [IcaB (Vuong et al. 2004) or PgaB (Parthiban et al. 2016)] and could play a potential role in immune evasion. In a recent study highlighting the importance of EPS in disease prevention, purified exopolysaccharide from *Bacillus subtilis* has been used to prevent disease and subsequent inflammation caused by the enteric mouse pathogen *Citrobacter rodentium*, which is similar to the human pathogen enteropathogenic *E. coli* (Jones et al. 2014).

Appendix References

Astasov-Frauenhoffer M, Varenganayil MM, Decho AW, Waltimo T, Braissant O. 2017. Exopolysaccharides regulate calcium flow in cariogenic biofilms. PloS one. 12(10):e0186256.

Bowen WH, Burne RA, Wu H, Koo H. 2018. Oral Biofilms: Pathogens, Matrix, and Polymicrobial Interactions in Microenvironments. Trends Microbiol. 26(3):229-242.

Chaudhuri S, Pratap S, Paromov V, Li Z, Mantri CK, Xie H. 2014. Identification of a diguanylate cyclase and its role in *Porphyromonas gingivalis* virulence. Infect Immun. 82(7):2728-2735.

Chodur DM, Rowe-Magnus DA. 2018. Complex control of a genomic island governing biofilm and rugose colony development in *Vibrio vulnificus*. J Bacteriol. Epub 2018/05/16. doi: 10.1128/JB.00190-18. .

Corrigan RM, Grundling A. 2013. Cyclic di-AMP: another second messenger enters the fray. Nat Rev Microbiol. 11(8):513-524.

Hwang G, Liu Y, Kim D, Li Y, Krysan DJ, Koo H. 2017. *Candida albicans* mannans mediate *Streptococcus mutans* exoenzyme GtfB binding to modulate cross-kingdom biofilm development *in vivo*. PLoS Pathog. 13(6):e1006407.

Izano EA, Sadovskaya I, Wang H, Vinogradov E, Ragunath C, Ramasubbu N, Jabbouri S, Perry MB, Kaplan JB. 2008. Poly-N-acetylglucosamine mediates biofilm formation and detergent resistance in *Aggregatibacter actinomycetemcomitans*. Microb Pathog. 44(1):52-60.

Jones CJ, Wozniak DJ. 2017. Psl Produced by Mucoid *Pseudomonas aeruginosa* Contributes to the Establishment of Biofilms and Immune Evasion. MBio. 8(3).

Jones SE, Paynich ML, Kearns DB, Knight KL. 2014. Protection from intestinal inflammation by bacterial exopolysaccharides. J Immunol. 192(10):4813-4820.

Kim D, Liu Y, Benhamou RI, Sanchez H, Simon-Soro A, Li Y, Hwang G, Fridman M, Andes DR, Koo H. 2018. Bacterial-derived exopolysaccharides enhance antifungal drug tolerance in a cross-kingdom oral biofilm. The ISME journal. 12(6):1427-1442.

Kim D, Sengupta A, Niepa THR, Lee BH, Weljie A, Freitas-Blanco VS, Murata RM, Stebe KJ, Lee D, Hyun Koo H. 2017. Scientific Reports 7:Article number: 41332

Kristian SA, Birkenstock TA, Sauder U, Mack D, Gotz F, Landmann R. 2008. Biofilm formation induces C3a release and protects *Staphylococcus epidermidis* from IgG and complement deposition and from neutrophil-dependent killing. J Infect Dis. 197(7):1028-1035.

Little DJ, Poloczek J, Whitney JC, Robinson H, Nitz M, Howell PL. 2012. The structure- and metal-dependent activity of *Escherichia coli* PgaB provides insight into the partial de-N-acetylation of poly-beta-1,6-N-acetyl-D-glucosamine. J Biol Chem. 287(37):31126-31137.

Falsetta ML, Klein MI, ColonnePM, Scott-Anne K, Gregoire S, Pai C-H, Gonzalez-Begne M, Watson G, Krysan DJ, Bowen WH, Koo H 2017. **S**ymbiotic Relationship between *Streptococcus mutans* and *Candida albicans* Synergizes Virulence of Plaque Biofilms *In Vivo*. DOI: 10.1128/IAI.00087-14

Mishra M, Byrd MS, Sergeant S, Azad AK, Parsek MR, McPhail L, Schlesinger LS, Wozniak DJ. 2012. *Pseudomonas aeruginosa* Psl polysaccharide reduces neutrophil phagocytosis and the oxidative response by limiting complement-mediated opsonization. Cell Microbiol. 14(1):95-106.

Nelson AL, Roche AM, Gould JM, Chim K, Ratner AJ, Weiser JN. 2007. Capsule enhances pneumococcal colonization by limiting mucus-mediated clearance. Infect Immun. 75(1):83-90.

Parthiban C, Varudharasu D, Shanmugam M, Gopal P, Ragunath C, Thomas L, Nitz M, Ramasubbu N. 2016. Structural and functional analysis of de-N-acetylase PgaB from periodontopathogen *Aggregatibacter actinomycetemcomitans*. Mol Oral Microbiol.

Peng X, Zhang Y, Bai G, Zhou X, Wu H. 2016. Cyclic di-AMP mediates biofilm formation. Mol Microbiol. 99(5):945-959.

Perez-Mendoza D, Sanjuan J. 2016. Exploiting the commons: cyclic diguanylate regulation of bacterial exopolysaccharide production. Curr Opin Microbiol. 30:36-43.

Romling U, Galperin MY, Gomelsky M. 2013. Cyclic di-GMP: the first 25 years of a universal bacterial second messenger. Microbiol Mol Biol Rev. 77(1):1-52.

Steiner S, Lori C, Boehm A, Jenal U. 2013. Allosteric activation of exopolysaccharide synthesis through cyclic di-GMP-stimulated protein-protein interaction. EMBO J. 32(3):354-368.

Sztajer H, Szafranski SP, Tomasch J, Reck M, Nimtz M, Rohde M, Wagner-Dobler I. 2014. Cross-feeding and interkingdom communication in dual-species biofilms of *Streptococcus mutans* and *Candida albicans*. The ISME journal. 8(11):2256-2271.

Underwood AJ, Zhang Y, Metzger DW, Bai G. 2014. Detection of cyclic di-AMP using a competitive ELISA with a unique pneumococcal cyclic di-AMP binding protein. J Microbiol Methods. 107:58-62.

Vuong C, Kocianova S, Voyich JM, Yao Y, Fischer ER, DeLeo FR, Otto M. 2004. A crucial role for exopolysaccharide modification in bacterial biofilm formation, immune evasion, and virulence. J Biol Chem. 279(52):54881-54886.

Wang Y, Andole Pannuri A, Ni D, Zhou H, Cao X, Lu X, Romeo T, Huang Y. 2016. Structural Basis for Translocation of a Biofilm-supporting Exopolysaccharide across the Bacterial Outer Membrane. J Biol Chem. 291(19):10046-10057.

Wei Q, Xingqun C, Xuedong Z, Yuqing L. 2015. Cloning, expression, and purification of c-di-AMP metabolism-related genes from *Porphyromonas gingivalis*. Hua Xi Kou Qiang Yi Xue Za Zhi. 33(6):607-612.

Xie Y, Yang L. 2016. Calcium and Magnesium Ions Are Membrane-Active against Stationary-Phase *Staphylococcus aureus* with High Specificity. Sci Rep. 6:20628.