The world of biofilms

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This issue of Virulence has a special focus on microbial biofilms. Biofilms are robust communities of surface-associated microbes that are held together by extracellular matrix material. They can form on almost any surface in the environment, be it natural (e.g., plants and animals) or synthetic (e.g., medical implants and industrial surfaces). Because of their ubiquity and natural resistance to many antimicrobials and decontamination techniques, biofilms have been extensively studied in a multitude of contexts. For example, biofilms are medically relevant, since our body’s surfaces—from our skin to our teeth to our intestines—are covered in microbes that can potentially cause disease or contaminate medical devices such as catheters or other implants (see below). Similarly, contamination of surfaces is a major challenge in food processing, as microbes can increase spoilage and cause safety issues.1 However, in other circumstances, we can reap the benefits of the sturdiness of microbes within biofilm communities. Such is the case in wastewater treatment plants where biofilms are an integral part of the purification process.2 Furthermore, electro-active biofilms can be used in microbial fuel cells to produce electrical energy by oxidizing a variety of organic compounds.3 4 Thus biofilms are not simply a problem we must eliminate, but are also a promising and potentially sustainable solution to global energy and waste issues.

The collection of articles in this issue covers a broad range of biofilm-related topics including multi-species dental biofilms, potential beneficial uses of biofilms in bioremediation and energy production, and emerging biofilm eradication techniques and technologies. In addition, it includes detailed reviews on biofilm formation by specific pathogens such as Proteus mirabilis, Staphylococcus aureus and Candida albicans. Finally, there is a survey of current topics in biofilm research provided by the abstracts from a recent workshop “Biofilms: Friend or Foe?” held by the European Cooperation in Science and Technology (COST).

Commensal Biofilms

Dental biofilms are perhaps the best-studied example of complex, multi-species biofilms. After pioneer species initially colonize the tooth surface, hundreds of other organisms subsequently colonize the oral cavity. The review by Huang et al. describes the interactions between some of the best-characterized oral microbes—including the mechanisms of initial colonization of the tooth surface.5 They also highlight the numerous forms of metabolic communication occurring between different species within the community. Some interactions are mediated by nutrients, waste products and oxygen, while others are driven by small-molecule signaling. Competition between oral microbes is high and production of small molecules such as bacteriocins, which have antibiotic activities that target related species, can alter the balance of species in a biofilm. In addition, intraintratrait interspecies quorum-sensing signals are prevalent and play a role in shaping the community.

Historically, oral care has focused on eliminating microbes to prevent and control disease, but not all of the microbes in these multi-species communities are detrimental. In fact, the main determinant of oral health is not simply the absence of pathogenic bacteria, but also the presence of key beneficial species. The challenge thus lies in targeting pathogenic organisms within multi-species communities while preserving beneficial, protective organisms. This concept of maintaining beneficial microbes when treating oral disease is highlighted by the COST abstract presented by Rob Allaker.6

Battling Biofilms

Unlike the oral cavity system, in some cases there are no obvious benefits to preserving any part of a microbial biofilm, and the goal is complete prevention or eradication of biofilms. Gauthier Boels of the company REALCO, in a collaborative project with the French National Institute for Agriculture Research (INRA), describes a process of enzymatically removing biofilms after they have formed.7 By targeting the biofilm matrix using specific enzymes and detergents, the biofilm structure can be weakened. Using this technique prior to other decontamination procedures (such as using mechanical force or disinfection agents) allows for more effective removal of troublesome biofilms. This approach proved effective in a number of laboratory conditions as well as pilot tests in industrial settings.

Discovering novel inhibitors of biofilm formation is also a goal of an interdisciplinary consortium of labs based in Belgium. As detailed by Steenackers et al. in their abstract from the COST meeting, this group aimed to identify inhibitors of bacterial biofilms that do not affect bacterial growth in planktonic conditions.6 Agents that specifically target biofilms but not bacterial growth would lack the selective pressure of an antibiotic and therefore resistant strains in the bacterial population would be less likely to arise. This group has screened several small-molecule libraries for anti-biofilm activity against a handful of pathogenic bacteria. A number of promising compounds have been identified and are currently being assessed for structure-activity relationships by the consortium.

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Recently there has been great interest in the search for non-lethal anti-biofilm agents. A major focus in reaching this end has been the disruption of quorum sensing systems. Because quorum sensing and extracellular communication play pivotal roles in biofilm formation as well as in virulence, they are appealing targets as an alternative to antibiotics. Another novel target is the bacterial signaling molecule cyclic-di-GMP, which is involved in the transition between planktonic and biofilm conditions in countless bacterial systems. In addition to its role in regulating biofilm formation, cyclic-di-GMP has been implicated in virulence in both human and plant pathogens. Given the substantial recent progress in our knowledge of the molecular mechanisms regulating both quorum sensing and cyclic-di-GMP signaling, it will be interesting to see how this translates to the development of new therapeutics.

Just as understanding the molecular details of the signaling pathways that lead to biofilm formation could provide potential anti-biofilm targets, there are also efforts to harness biofilm dispersal mechanisms that were evolutionarily developed in these systems. One recent example of this is the discovery of D-amino acids as a biofilm inhibitor and disassembly factor. In this study, specific D-amino acids were shown to be produced during the late stages of biofilm formation by the constituent bacteria. While the initial study was performed using the non-pathogen Bacillus subtilis, the mixture of specific D-amino acids was effective in inhibiting biofilms by several pathogens including Pseudomonas aeruginosa and S. aureus.

In natural settings, most biofilms are multispecies aggregates. Thus, it is important to understand the functional relevance of microbial interactions in establishing and maintaining biofilms. As seen in dental biofilms, not all community members are equally important in terms of their contribution to biofilm formation. Several COST abstracts describe ongoing work to study multispecies biofilms. Giaouris et al. discuss the problem of multispecies biofilms in food processing. Roberfroid et al. also analyze biofilms related to food-borne pathogens. They focus on understanding heterogeneous gene expression within a given species (specifically Salmonella typhimurium) within the context of multispecies biofilms.

Medical-relevant biofilms are also often composed of many species, frequently including both bacterial and fungal constituents. In a COST abstract, Eleftherios Mylonakis details work studying bacterial-fungal interactions and describes a high-throughput screen for fungal inhibitors in the nematode Caenorhabditis elegans. This screening procedure identified several compounds that not only have anti-fungal activity, but also disrupt biofilm formation in the fungal pathogen C. albicans.

### Biofilm Infections

As mentioned, biofilms have important—and frequently deleterious—roles in medical contexts. A common source of nosocomial infection is biofilm-related contamination of central venous and other catheters. Once infected, the recommended treatment is often removal of the contaminated device, which is a difficult and costly process that can result in medical complications. As discussed below, three reviews in this issue specifically address different pathogens (S. aureus, P. mirabilis and C. albicans) and the roles that they play in biofilm-related infections. These are not the only organisms that develop biofilm-related infections; Escherichia coli and Enterococcus faecalis, among others, are also often found in biofilms associated with nosocomial infections. In addition, polymicrobial biofilms that form on biliary stents as well as mechanisms to disrupt them are the focus of two COST abstracts by the Donelli group.

Archer et al. present a review on S. aureus biofilms that begins with a summary of the molecular mechanisms described thus far that regulate S. aureus biofilm development. This review covers a broad range of S. aureus biofilm-related infections including osteomyelitis, indwelling device infections, periodontitis, chronic wounds and chronic rhinosinusitis, among others. They also discuss the host response to S. aureus infection, current therapies against S. aureus infections, and the development of vaccines. Notably, S. aureus is not exclusively a human pathogen. The COST abstract by Antoni Prenafeta describes the role that S. aureus plays in dairy cattle mastitis as well as efforts to vaccinate a herd against S. aureus biofilm infections. As we learn more about the specific factors that play a role in S. aureus biofilm formation, as well as the variation in the mechanisms of biofilm formation by individual strains, we should be able to better target this problematic pathogen.

P. mirabilis is responsible for a number of opportunistic nosocomial infections, including urinary tract infections (UTI) and catheter-associated UTIs. Jacobsen and Shirliff describe virulence factors that contribute to pathogenesis in P. mirabilis infections. An intriguing feature of this organism is its ability to form struvite and hydroxyapatite mineral crystals on the surface of urinary catheters. These crystals form a conditioning film that allows further colonization and leads to encrustation, which can block the catheter. Furthermore, they can be associated with bladder stones, which contribute to re-colonization of replacement catheters. P. mirabilis also displays a highly motile swarming state. This promotes the spread of Proteus along with other organisms that are found in multispecies biofilm infections.

Bacteria are not the only microbes that form biofilms on catheters. Fungi such as Candida species are commonly associated with catheter infections and studies on this organism have been conducted in vitro under static as well as flow conditions. In this issue, Chandra et al. describe a rabbit model of catheter-associated fungal biofilms. They include an in-depth protocol for assessing catheter contamination by biofilms in an in vivo, clinically-relevant system. This system was used to evaluate the effectiveness of antifungal agents in lock therapy for catheters. During lock therapy, a concentrated antimicrobial solution is introduced into the lumen of a catheter for given period of time in an effort to sterilize the catheter. The COST abstract by Christine Imbert also emphasizes the clinical importance of
fungal biofilms. Several approaches are currently being assessed for their efficacy including anti-fungal lock therapy, coating catheter surfaces with anti-biofilm agents, and long-term systemic therapy to treat ongoing infections.

Biofilms formed by pathogens do not always have to form on medically relevant surfaces to be a problem. Yersinia species cause a variety of diseases in humans. However, instead of forming on medical devices, the biofilms formed by this organism occur in its insect vector, the flea. In a COST abstract, Steve Atkinson describes a C. elegans host model to study how cell signaling via quorum sensing molecules regulates the transition to biofilm formation in Y. pseudotuberculosis. This work emphasizes the importance of intracellular signaling in biofilm formation.

**Beneficial Biofilms**

While the role that biofilms play in infection has prompted much research related to biofilm removal and disassembly, not all biofilms have a negative impact. For example, biofilm-based biofertilizers are a possible method to sustainably improve soil quality and allow for decreased use of chemical fertilizers. Seneviratne et al. explore the beneficial aspects of using such biofilm-based fertilizers in two COST abstracts. Applying well-developed microbial biofilms to soils that harbor phytotoxic allelochemicals can help degrade the phytotoxins and favor plant growth.

The article by Piet Lens discusses the increasing challenges that we face as a planet to maintain a clean water supply and environment in the face of increasing industrial development. Bioremediation is a sustainable and cost-effective option for addressing these issues. Lens pinpoints legislation as a major factor in implementing changes in environmental protection. He emphasizes that we must not only develop effective and safe technologies, but legislation must also be in effect to accelerate the process.

As the population of Earth is increasing, so are our energy requirements. For example, waste-water treatment requires a massive amount of electricity. In addition to using microbial biofilms to decontaminate waste water, we can also use them as a promising source for providing electrical energy in the form of microbial fuel cells. Relatively recent studies on electro-active microbes, which are capable of directly exchanging electrons with conductive materials, are yielding encouraging results in the development of cheaper, more useful microbial fuel cells. New techniques are being developed to characterize more cost-effective methods for analyzing electro-active biofilms. In this issue, Connolly et al. describe new methods using transparent conductive metal oxide for better characterization of electro-active biofilms.

**Concluding Thoughts**

As exemplified by the diverse array of topics addressed in this issue, biofilms are a major presence in all natural settings. There is a plethora of ongoing research ranging from understanding the basic mechanisms of biofilm development to analyzing biofilms on human or industrial surfaces. As our knowledge increases, we can now begin to explore multispecies biofilms in natural and clinically relevant settings in more detail. It is important to continue research on eradicating detrimental biofilms in nosocomial infections and industry. However, we must not overlook the potential uses of these incredible microbial communities. Biofilms have been successfully used for decades in bio-remediation of wastewater. Probiotics are generally accepted, and many bacteria are known to be beneficial to human health. Microbial fuel cells can be added to the increasing list of renewable energy sources that will be necessary in order to meet global energy requirements in a sustainable way. We are now at the stage where we can use our knowledge of the microbial world in a targeted manner, by optimizing our ongoing usage of polymicrobial communities and designing new systems to harness our understanding of bacterial signaling and communication.

**References**

1. Van Houw R, Michielis CW. Biofilm formation and the food industry, a focus on the bacterial outer surface. J Appl Microbiol 2010; 109:117-31; PMID:20522145; http://dx.doi.org/10.1111/j.1365-2672.2010.04756.x.
2. Singh R, Paul D, Jain RK. Biofilms: implications in bioremediation. Trends Microbiol 2006; 14:389-97; PMID:16857359; http://dx.doi.org/10.1016/j.timm.2006.07.001.
3. Erable B, Echeverry L, Bergel A. From microbial fuel cell (MFC) to microbial electrochemical snorkel (MES): maximizing chemical oxygen demand (COD) removal from wastewater. Biofouling 2011; 27:319-26; PMID:21409694; http://dx.doi.org/10.1080/08927044.2011.564615.
4. Logan BE. Exoelectrogenic bacteria that power microbial fuel cells. Nat Rev Microbiol 2009; 7:735-81; PMID:19350018; http://dx.doi.org/10.1038/nrmicro20113.
5. Huang R, Li M, Gregory RL. Bacterial interactions in dental biofilm. Virulence 2011; 2:437-46; PMID:21778817; http://dx.doi.org/10.4161/viru.2.5.16140.
6. Abstracts from the COST workshop: Biofilms: Friend or foe? Virulence 2011; 2:484-91; PMID:21921681; http://dx.doi.org/10.4161/viru.2.5.17531.
7. Boels G. Enzymatic removal of biofilms. Virulence 2011; 2:478-88; PMID:21921679; http://dx.doi.org/10.4161/viru.2.5.17317.
8. Sintim HO, Smith JA, Wang J, Nakayama S, Yan L. Paradigm shift in discovering next-generation anti-infective agents targeting quorum sensing, c-di-GMP signaling and biofilm formation in bacteria with small molecules. Funtne Med Chem 2010; 2:1005-35; PMID:21426116; http://dx.doi.org/10.4155/fmc.10.185.
9. Njorge J, Spandrio V. Jamming bacterial communication: new approaches for the treatment of infectious diseases. EMBO Mol Med 2009; 1:201-10; PMID:20949722; http://dx.doi.org/10.1002/emmm.200900392.
10. Hengge R. Principles of c-di-GMP signalling in bacteria. Nat Rev Microbiol 2009; 7:263-73; PMID:19287449; http://dx.doi.org/10.1038/nrmicro2109.
11. Ryan RP Dow JM. Intermolecular interactions between HD-GYP and GGDEF domain proteins mediate virulence-related signal transduction in Xanthomonas campestris. Virulence 2010; 1:404-8; PMID:21778479; http://dx.doi.org/10.4161/viru.1.5.12704.
12. Kaplan JB. Biofilm dispersal: mechanisms, clinical implications and potential therapeutic uses. J Dent Res 2010; 89:205-18; PMID:20393399; http://dx.doi.org/10.1177/00220345103959403.
13. Kolodkin-Gal I, Romero D, Cao S, Clardy J, Kolter R, Losic R. D-amino acids trigger biofilm disassembly. Science 2010; 328:627-9; PMID:20431016; http://dx.doi.org/10.1126/science.1186628.
14. Franzolini I, Donelli G. Prevention and control of biofilm-based medical-device-related infections. FEMS Immunol Med Microbiol 2010; 59:227-38; PMID:20412380.
15. Garson DA, Willems RJ. Insights into the biofilm lifestyle of enterococci. Virulence 2010; 1:219-21; PMID:21178446; http://dx.doi.org/10.4161/viru.1.4.12388.
16. Archer NK, Mazaitis MJ, Costerton JW, Leids JG, Powers ME, Shirliff ME. Staphylococcus aureus biofilms: Properties, regulation and roles in human disease. Virulence 2011; 2:447-62; PMID:21921685; http://dx.doi.org/10.4161/viru.2.5.17724.
18. Otto M. Staphylococcal biofilms. In: Romeo T, Ed. Bacterial Biofilms. Heidelberg: Springer 2008; 207-28.
19. Jacobsen SM, Shirluff ME. Proteus mirabilis biofilms and catheter associated urinary tract infections. Virulence 2011; 2:462-7; PMID:21921687; http://dx.doi.org/10.4161/viru.2.5.17783.
20. Uppuluri P, Lopez-Ribot JL. An easy and economical in vitro method for the formation of Candida albicans biofilms under continuous conditions of flow. Virulence 2010; 1:685-7; PMID:21178492; http://dx.doi.org/10.4161/viru.1.6.13186.
21. Chandra J, Long L, Ghanoum MA, Mukherjee PK. A rabbit model for evaluation of catheter-associated fungal biofilms. Virulence 2011; 2:468-76; PMID:21921676; http://dx.doi.org/10.4161/viru.2.5.16341.
22. Hinnebusch BJ, Erickson DL. Yersinia pestis biofilm in the flea vector and its role in the transmission of plague. Curr Top Microbiol Immunol 2008; 322:229-48; PMID:18453279; http://dx.doi.org/10.1007/978-3-540-75418-3_11.
23. Lens P. Biofilms for environmental biotechnology in support of sustainable development. Virulence 2011; 2:480-1; PMID:21921686; http://dx.doi.org/10.4161/viru.2.5.17758.
24. Erable B, Duteanu NM, Ghangrekar MM, Dumas C, Scott K. Application of electro-active biofilms. Biofouling 2010; 26:57-71; PMID:20390557; http://dx.doi.org/10.1080/08927010903161281.
25. Connolly J, Jain A, Pastorella G, Krishnamurthy S, Mosnier JP, Marsili E. Zinc oxide and indium tin oxide thin films for the growth and characterization of Shewanella loihica PV-4 electroactive biofilms. Virulence 2011; 2:481-4; PMID:21921690; http://dx.doi.org/10.4161/viru.2.5.17912.