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COMMENTARY

Regulating Antibiotic Tolerance within Biofilm Microcolonies

Thien-Fah Mah

Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada

Despite decades of research on antibiotic resistance in bacteria, a comprehensive understanding of biofilm-specific antibiotic resistance is lacking. When bacteria are presented with a surface and adequate nutrients, they grow within complex communities, called biofilms, which display an increased resistance to antimicrobial agents (5, 6). Given the heterogeneous nature of biofilms (13), it is likely that multiple mechanisms of resistance and/or tolerance act together to provide an overall high level of protection against natural and synthetic antimicrobial agents. Several studies, however, are beginning to address the complexity of biofilm-specific antibiotic resistance. For instance, components of the biofilm matrix, which consists of polysaccharides, DNA, and proteins (10), can contribute to antibiotic resistance in biofilms. The extracellular DNA chelates cations and induces the expression of a cationic antimicrobial resistance operon (11). Furthermore, the presence of "persister" cells in a biofilm can contribute to the survival of biofilms (7). Since cell metabolism is slowed or shut down in persisters, antibiotics are less effective because their targets are not active (7). Finally, several genes with diverse functions have been identified as important for biofilm-specific antibiotic resistance (3, 9, 14, 15). In this issue of the *Journal of Bacteriology*, Liao and Sauer identify a DNA-binding regulatory protein that is involved in the biofilm-specific antibiotic tolerance of the opportunistic pathogen *Pseudomonas aeruginosa* (8). This is the first DNA regulator shown to be involved in biofilm-specific resistance/tolerance. This discovery opens the door to a more detailed study of how this regulator itself is regulated and what its downstream targets are.

The starting point for this study was based on previous work where Sauer's group analyzed proteins that were preferentially expressed in biofilms (12). PA4878, which encodes a probable transcriptional regulator, one of the proteins identified, and this gene was confirmed to exhibit a biofilm-specific expression pattern. On the basis of subsequent experiments, PA4878 was renamed *brlR* (for biofilm resistance locus regulator). The central observation of this report is that when *brlR* was deleted from the genome, biofilms formed by the resulting Δ*brlR* deletion strain were more susceptible to tobramycin, norfloxacin, trimethoprim, tetracycline, kanamycin, and hydrogen peroxide. Overexpression of *brlR* in the biofilm formed by the Δ*brlR* deletion strain restored a wild-type level of susceptibility. This susceptibility phenotype was limited to biofilms; deletion of *brlR* had no effect on the susceptibility of planktonically grown cultures, compared to that of the wild-type strain. However, overexpression of *brlR* in wild-type and Δ*brlR* mutant planktonic cultures increased their resistance level, suggesting that the mechanism of resistance is specific to biofilms because *brlR* is normally expressed only in biofilms.

The clinical importance of BrlR was also addressed by Liao and Sauer. They obtained three *P. aeruginosa* isolates, CF1-2, CF1-8, and CF1-13, from one patient suffering from cystic fibrosis (CF). Using quantitative reverse transcriptase PCR, they determined that all three isolates expressed *brlR* when grown as biofilms, at a higher level than the lab strain. In contrast to the lab strain, all three isolates also expressed *brlR* when they grew as planktonic cultures. When the authors assayed the planktonic susceptibility of these strains to tobramycin, they found that CF1-2 and CF1-13 were less susceptible than the wild-type strain, and similar results were displayed when biofilm susceptibility was assessed. However, despite expressing *brlR*, CF1-8 was as sensitive to tobramycin as the wild-type strain was. Further investigation by the authors revealed that CF1-8 possesses a mutation that results in a nonfunctional BrlR peptide. Thus, Liao and Sauer demonstrated that the expression of *brlR* correlates with increased resistance in the real-world setting of a *P. aeruginosa* airway infection. It will be interesting to learn why the CF isolates express *brlR* under planktonic conditions. Is there a mutation that has been selected for? Is there a regulator that is active in the CF isolates that is not in the wild-type strain?

As stated earlier, BrlR is the first regulator to be linked to antibiotic resistance in biofilms. BrlR is a member of the MerR family of transcriptional regulators, named after the regulator of mercury resistance (*mer*) operons (4). MerR family members have similar N-terminal DNA-binding domains but differ in their C-terminal inducer-binding domains. While there is a subset of the family that is responsive to metals (including MerR itself), there is another subset that responds to other types of compounds and induces the expression of efflux pumps (4). BrlR is most similar to the latter group of MerR regulators. BmrR is a well-studied member of this subgroup from *Bacillus subtilis*. This regulator activates the expression of the Bmr multidrug efflux pump in the presence of this pump's substrates (1, 2), suggesting some tantalizing questions to pursue. The most obvious question is: does BrlR affect the expression of a biofilm-specific efflux pump? The targets of BrlR have not been identified. However, there is a candidate locus to consider: PA1875-77 is preferentially expressed in biofilms and does have efflux activity (15). Additionally, do specific antimicrobial agents, such as the ones tested by Liao and Sauer, alter the affinity of BrlR for its target promoters?

Liao and Sauer turned to confocal scanning laser microscopy to beautifully illustrate the effect of losing BrlR activity in mature biofilms that have been exposed to tobramycin (see Fig. 5F in reference 8). Wild-type and Δ*brlR* biofilms were grown in flow cell growth chambers and subsequently exposed to tobramycin. After tobramycin exposure, live cells were differentiated from dead cells by the use of *Bac*Light viability staining. Under these conditions,

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Address correspondence to Thien-Fah Mah, tmah@uottawa.ca.

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propidium iodide staining (dead cells) was more prominent in Δ*brlR* mutant biofilms than in the wild-type biofilm. An interesting observation was that the propidium iodide staining in the Δ*brlR* mutant biofilms was localized to the interior of the microcolonies, suggesting that the BrlR-specific resistance mechanism acts primarily within this subpopulation of the biofilm. This is a powerful demonstration of the concept that biofilms are not uniform cultures of physiologically identical cells and suggests that different mechanisms of resistance can protect different subpopulations of cells. Future experiments will undoubtedly reveal more mechanisms that are important for biofilm-specific antibiotic resistance. Our challenge will be to develop a better understanding of the interplay among these different mechanisms.

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