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COMMENTARY

Pseudomonas aeruginosa Alginate Benefits *Staphylococcus aureus*?

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ABSTRACT In this issue of *Journal of Bacteriology*, Price et al. show that the *Pseudomonas aeruginosa*-produced exopolysaccharide alginate protects *Staphylococcus aureus* by dampening the expression of *P. aeruginosa* virulence products that usually inhibit *S. aureus* respiration and cell membrane integrity when the two organisms compete in other environments (C. E. Price, D. G. Brown, D. H. Limoli, V. V. Phelan, and G. A. O'Toole, J Bacteriol 202:e00559-19, 2020, https://doi.org/10.1128/jb.00559-19). This is the first report that exogenously added alginate affects *P. aeruginosa* competition and provides a partial explanation for *S. aureus* and *P. aeruginosa* coinfections in cystic fibrosis.

KEYWORDS coinfection, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, cystic fibrosis

In this issue of the *Journal of Bacteriology*, Price et al. show that the *Pseudomonas aeruginosa*-produced exopolysaccharide alginate protects *Staphylococcus aureus* by dampening the expression of *P. aeruginosa* virulence genes whose products usually inhibit *S. aureus* when the two organisms compete in other environments. The authors show that exogenous alginate, regardless of the source, protects *S. aureus* from *P. aeruginosa* in both planktonic and biofilm coculture models. Furthermore, the authors demonstrate that coculture of mucoid *P. aeruginosa* with nonmucoid *P. aeruginosa* strains can mitigate *S. aureus* killing by the nonmucoid *P. aeruginosa* strain. Interestingly, the authors also show that some clinical mucoid *P. aeruginosa* isolates retain their ability to kill *S. aureus*, indicating that there are strain-specific variations for these observations. The authors thus provide at least a partial explanation for the ~30% of cystic fibrosis (CF) patients who are coinfected with both organisms and demonstrate that alginate protects not only *P. aeruginosa* but also *S. aureus*. Their results also indicate that exogenous alginate affects nonmucoid and mucoid *P. aeruginosa* gene expression (1).

The lungs of CF children are readily colonized by *S. aureus* during the early years of life, with colonization by *P. aeruginosa* during the mid- to late teenage years. *S. aureus* colonization is associated with a higher probability of secondary *P. aeruginosa* infection. *P. aeruginosa* colonization will eventually outcompete *S. aureus* and other microbes present in the CF lung to become the predominant pathogen (2, 3). Coinfection with *P. aeruginosa* and *S. aureus* ultimately results in a poor clinical outcome for the patient, including a reduced median forced expiratory volume in 1 s (FEV1) and increased rates of pulmonary exacerbation (4). During the process of coinfection and treatment, *P. aeruginosa* will eventually be pushed into a mucoid phenotype through the acquisition of *mucA* (*algN*) mutations (5, 6). Several studies have documented that the *mucA* mutation exists within several mucoid clinical CF isolates (7, 8). The result of this mutation is the release of an extracytoplasmic sigma factor (ECF) called AlgU (9), AlgT (10), or σ^{22} (11) and is the *Escherichia coli* RpoE orthologue (12). AlgU/T is required for the transcription of the *P. aeruginosa* alginate biosynthetic pathway (9, 13–15). Alginate

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is a secreted anionic exopolysaccharide composed of various proportions of 1,4-linked β-D-mannuronic acid (M) and its C-5 epimer α-L-guluronic acid (G), which is naturally produced by some bacteria and brown seaweeds (16). Significant effort to understand alginate biosynthesis has been due to its role in bacterial pathogenesis as well as the possibility to produce tailor-made alginates exhibiting material properties suitable for various medical and industrial applications. In the context of pathogenesis, alginate provides mucoid *P. aeruginosa* strains several advantages over nonmucoid strains (17, 18). These advantages include resistance to phagocytosis (19), resistance to killing by polymorphonuclear cells (20), scavenging of free radicals released by macrophages (21), and resistance to antibiotics by reducing diffusion to the organism (22). Additionally, mucoid strains grow slowly, are generally nonmotile, and express low levels of protease exotoxins and siderophores (23–28). Ultimately, *P. aeruginosa* alginate production creates a survival advantage as it mediates the formation of persistent biofilms during chronic infections (29, 30). Until very recently, the only documented advantages that alginate provided were to *P. aeruginosa*.

A few studies have described the *in vitro* and *in vivo* interactions between *S. aureus* and *P. aeruginosa* using CF lung infection models and sputum samples (31–33). These studies led to the conclusion that the microorganisms are antagonistic *in vitro* (34–36); however, *in vivo* models showed contradictory results (35, 37) despite clinical evidence that there may be synergy between them. For the *in vitro* work, Filkins et al. utilized a *P. aeruginosa-S. aureus* coinfection model on a human CF bronchial epithelial (CFBE) cell line to show that *P. aeruginosa* drove the *S. aureus* expression profile from that of aerobic respiration to that of fermentation. The fermentative respiration was dependent on *P. aeruginosa* production of both 2-heptyl-4-hydroxyquinoline *N*-oxide (HQNO) and siderophores. The authors observed that initially, *S. aureus* and *P. aeruginosa* coexisted; however, extended coculture reduced *S. aureus* viability. Additionally, the authors showed that *S. aureus* small-colony-variant (SCV) genetic mutant strains, which have defects in their electron transport chain, experience reduced killing by *P. aeruginosa* compared to their wild-type parent strains, indicating that *P. aeruginosa* HQNO and siderophores act specifically to inhibit the *S. aureus* aerobic electron transport chain (32). Nguyen and Oglesby-Sherrouse extended this further by observing that the iron-regulated antimicrobial activity of *P. aeruginosa* against *S. aureus* was due to the cumulative effects of multiple alkyl quinolone (AQ) metabolites, both the production and activity of which are modulated by environmental iron levels (28). Yang et al. utilized coculture biofilms of *S. aureus* with *P. aeruginosa* mutants in a flow chamber system and showed that wild-type *P. aeruginosa* PAO1 facilitates *S. aureus* microcolony formation (27). In contrast, *P. aeruginosa mucA* (presumably mucoid) and *rpoN* mutants did not facilitate *S. aureus* microcolony formation and tended to outcompete *S. aureus* in coculture biofilms. Interestingly, the data from Yang et al. are in agreement with those from Filkins et al. when a mucoid *P. aeruginosa* strain, FRD1, was used on a CFBE cell coculture, and both outcompeted *S. aureus*. Some additional understanding of mucoid *P. aeruginosa* interactions with *S. aureus* was provided by Baldan et al., who showed that early CF *P. aeruginosa* isolates were competitive with *S. aureus*, whereas late CF isolates were not as competitive with *S. aureus*. The authors surmised that the secondary "pathoadaptive" mutations acquired by *P. aeruginosa* made the organism less competitive (31). Interestingly, the authors did not mention that *S. aureus* found in the same lung environment and exposed to the same innate and adaptive immune responses and antibiotics likely underwent pathoadaptive mutations as well. For instance, were *S. aureus* SCVs tested? Alginate production is the best-studied pathoadaptive virulence factor made by *P. aeruginosa* and has been shown to be one result of the onslaught of antibiotics and oxidative stress that is endured by *P. aeruginosa* in the CF lung (18, 38).

Altogether, it appears that mucoid strains can both antagonize and synergize with *S. aureus*. Whether antagonism or synergism is most important remains an open question and will probably be determined by a multitude of factors, including the patient's immune status, the efficacy of antibiotic treatment, or even other organisms

that are found in the CF lung. The current work has brought to light that alginate helps *S. aureus* under certain conditions by dampening *P. aeruginosa* gene expression, with the surprise being that alginate can do this exogenously.

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