Distinct mechanical roles for bacterial biopolymers in sensing and strength

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Short Abstract — **Biofilm bacteria are embedded in extracellular polymers (EPS). Multiple types of EPS can be produced by a single bacterial strain - the reasons for this redundancy are not well-understood. Our work suggests that different polymers may confer distinct mechanical benefits. Biofilms initiate when bacteria attach to a surface, sense the surface, and change their gene expression. The EPS PEL enhances surface sensing by increasing mechanical coupling of single bacteria to the surface. For the mature biofilm, the EPS PSL stiffens and strengthens biofilms. For bacteria in chronic infections, EPS expression evolves to combine mechanical fitness with complementary, chemical fitness benefits.**

***Keywords — P. aeruginosa*, biofilm, mechanosensing, signaling, cyclic-di-GMP, extracellular polysaccharide (EPS), motility, shear stress, rheology, evolution.**

I. BIOFILM INITIATION, MECHANICS, AND EVOLUTION

Pseudomonas aeruginosa is an opportunistic human pathogen that forms chronic infections in the form of biofilms, a phenotypic state associated with increased antibiotic resistance and evasion of the immune defense. In biofilms, sessile microbes are embedded in a matrix consisting largely of self-produced extracellular polysaccharides (EPS). PAO1 is a lab strain that, *in vitro*, produces two types of EPS, PEL and PSL.

In vitro biofilm formation initiates when bacteria encounter, and attach to, a surface. Cyclic-di-GMP, a second messenger whose intracellular levels increase upon adhesion of *P. aeruginosa* to a surface, regulates the expression of many genes for biofilm initiation. What cues notify bacteria that they are attached to a surface to increase cyclic-di-GMP production are unknown. This is a gap in our understanding of a fundamental microbiological process.

The biofilm matrix can protect bacteria chemically and mechanically. *P. aeruginosa* infections in the cystic fibrosis (CF) lung often last for decades, ample time for the infecting strain(s) to evolve. Production of a third EPS material, alginate, is well-known to tend to increase over time in CF infections and to be associated with poorer outcomes for patients. Alginate chemically protects biofilms, but also makes them softer, which seems to be a mechanical disadvantage. It was recently found that bacteria in chronic CF infections also evolve to increase PSL production [1].

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II. MECHANOSENSING OF SURFACES

We use a green fluorescent protein (GFP) reporter for intracellular cyclic-di-GMP levels [2]. With increased flow rate of liquid media, and thus increased shear stress, the intracellular cyclic-di-GMP levels increase in a dose-response fashion. Moreover, at low shear stress we find that PEL enhances the cyclic-di-GMP signaling response– populations of wild-type (WT) and Δ*pel* have indistinguishable GFP intensity distributions when in liquid suspension and at high shear rates, but at low shear the WT are brighter than the Δ*pel*. Motility measurements suggest that PEL may increase frictional interactions between the surface and the bacteria. To date, the role of PEL in PAO1 biofilms has seemed relatively minor and redundant with PSL. We infer that a major role of PEL is to enhance surface sensing by increasing the mechanical coupling.[3]

III. PSL STIFFENS AND STRENGTHENS BIOFILMS

We use oscillatory bulk rheology to determine the unique contributions of EPS materials to the mechanics of biofilms grown from isogenic PAO1 variants and from sets of chronological clinical isolates from four CF patients over decades of infection [1]. We find that PSL stiffens biofilms and PEL and alginate make biofilms more ductile. Comparing, biofilm mechanics to estimated forces exerted by phagocytosing neutrophils [4], we infer that increased PSL could confer a mechanical fitness benefit. [5]

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