TITLE: Investigating the impacts of bacterial respiration state on biofilm formation

Keywords: Adherence factors, biofilm formation, respiration states in biofilms

INTRODUCTION

Ubiquitous in nature, biofilms are multicellular communities formed by bacteria, fungi, and small eukaryotes. While most biofilms positively impact the environment, there are many instances in which biofilms are detrimental and are extremely difficult to eradicate [1]. Biofilms are responsible for fouling pipes and shipping hulls, as well as contaminating prostheses and indwelling medical devices including catheters. Understanding how the immediate microenvironment shapes biofilm infrastructure allows us to devise better approaches to remove detrimental biofilms. Our laboratory studies the mechanisms by which uropathogenic *Escherichia coli* (UPEC) form biofilms on surfaces. *E. coli* is a Gram-negative facultative anaerobe that typically exists as a commensal in the gut; however, horizontal gene transfer events have given rise to several different pathogenic *E. coli*, such as UPEC, with distinct infection strategies. In recent studies, we have shown that UPEC biofilms are comprised of subpopulations of bacteria that express different adhesive fibers at different depths of the biofilm in an oxygen-dependent manner [2]. Interestingly, type 1 pili, which are essential for UPEC adherence in the bladder, were only expressed in the presence of molecular oxygen. **I hypothesize that there is a mechanism that directly responds to oxygen levels to regulate expression of type 1 pili.** In this proposal, I outline experiments designed to investigate biofilm formation and identify key genetic players regulating biofilm formation under anoxic conditions.

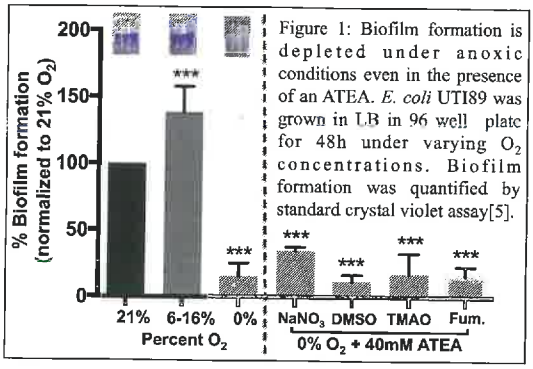

Figure 1: Biofilm formation is depleted under anoxic conditions even in the presence of an ATEA. *E. coli* UTI89 was grown in LB in 96 well plate for 48h under varying O_2 concentrations. Biofilm formation was quantified by standard crystal violet assay[5].

SPECIFIC AIMS AND RESEARCH PLAN

Aim 1: Dissect the connection between type 1 pili production, biofilm formation, and aerobic respiration. Anoxic conditions result in a significant decrease in pilus expression and biofilm formation in *E. coli*, regardless of the presence of an alternative terminal electron acceptor (ATEA) (**Fig. 1**). It is possible that altered ATP production, in the presence of different ATEAs, impacts the expression of large appendages like pili. To determine if the decrease in biofilm formation is a function of reduced energy, I will measure ATP levels in *E. coli* grown anaerobically in the presence of ATEAs used by *E. coli*: nitrate, dimethyl sulfoxide (DMSO), trimethylamine N-oxide (TMAO), and fumarate. Production of type 1 pili will be assessed by immunoblotting with well-characterized antibodies (2, 3) and by transmission electron microscopy. Simultaneously, I will determine differences in growth rate in the presence of each ATEA by counting colony-forming units (CFUs) and measuring new DNA synthesis via well-characterized methods. Collectively, these studies will determine if there are differences in energy production impacting growth rate and production of type 1 pili components.

Anticipated results/alternative approaches: If biofilm reduction is a function of reduced ATP in the anaerobic cultures, this should correlate with reduced growth rate and reduced expression of other large bacterial appendages. However, in our previous studies, we saw other fibers expressed under anoxic conditions [2], suggesting that a different mechanism controls type 1 pili expression. For example, products of ATEA-driven anaerobic respiration may specifically repress type 1 pili, which can be further investigated by analyzing metabolic products by mass spectrometry and examining the effects of adding these products in excess to growing cultures.

Aim 2: Determine the gene(s) contributing to oxygen-mediated biofilm formation in *E. coli*. To identify the sensing mechanism that responds to diminished oxygen levels and alters expression of adhesive fibers, as well other possible biofilm components, I will perform a suppressor screen via transposon mutagenesis. The generated mutant library will be screened to identify mutants that gain the ability to form biofilms under anoxic conditions. Suppressor mutants will be sequenced to identify the disrupted gene(s). Clean deletion mutants will be generated and further characterized for their role in repressing biofilm formation.

Anticipated results/alternative approaches: I anticipate that if a sensory network is responsible for actively repressing expression of type 1 pili, I will be able to identify disruptions within the corresponding gene(s) if I perform a saturating screen. However, if oxygen is sensed by a system that actively induces expression of type 1 pili, this approach may identify no hits. To bypass this possibility, I will perform a positive selection mutagenesis approach, in which I will create a bacterial suicide system: I will insert a toxin gene under the control of the type 1 pili promoter. Thus, bacteria will die in the presence of oxygen. I will then create a library in which a promoterless anti-toxin gene will be randomly inserted in the chromosome. Bacteria will be plated in aerobic conditions in search of mutants that survive. These mutants will have insertions in genes that are typically induced under aerobic conditions and positively influence type 1 pili.

BROADER IMPACTS

Parasitic bacterial biofilms are disturbing global industries including, but not limited to, shipping, food, water treatment, and healthcare. Findings from my studies have the potential to uncover mechanisms by which UPEC form biofilms in human urine. Urine has low oxygen concentrations and contains ATEAs (nitrate and TMAO); my studies will begin to elucidate mechanisms that allow the bacteria to use these resources effectively. In addition to expanding my research prowess during my graduate career, I will also communicate its impacts to the broader audience. I will continue mentoring young scientists, as well as getting involved in the community in multiple ways. For example, in February 2016, I will teach elementary- and middle school-aged girls about "the bacteria around us" with interactive experiments, while exposing them careers in the STEM fields through Girls, Inc. My lab also partakes in outreach activities together, such as interacting with toddlers at a daycare showing them that their body is like a "zoo for microbes" and volunteering at the [redacted] Science Center to explain how bacteria form biofilms in an interactive way. These outreach activities are crucial for bringing the science to the public and increasing their understanding of research.

In the future, I envision a career leading a team through the design of new diagnostic tools for infectious diseases, so that I can contribute to improving the life quality of others. This outreach and mentoring plan will prepare me to be a successful leader in research and in the community to fulfill my future goals for developing improved diagnostics and treatments.

Resources Cited

1. [redacted]
2. [redacted]
3. [redacted]
4. [redacted]
5. [redacted]