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Biofilm Formation by Escherichia coli csgA and fimA mutants

Abstract

Biofilms are a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface. These structures and the organisms that cause them can pose a very serious problem if they colonize on medical devices. This is because biofilms have the ability to communicate within the colony and with other organisms that might attach to the surface, acting like a community working together. Biofilms allow the organism to be resistant to harsh and unfavorable conditions allowing them to survive longer and spread. Several genes in *Escherichia coli* (*E. coli)* have been associated with biofilm formation by that organism. Many of those genes encode surface appendages such as flagella, fimbriae, and pili. We created mutations in genes encoding curli (*csgA*) and fimbriae (*fimA*) with the aim of comparing their ability to form biofilms. The respective genes were selected on kanamycin-containing agar and disrupted with a kanamycin resistance gene. Biofilm formation in nutrient-rich medium and nutrient-poor medium is currently in progress, and the ability of the mutant *E. coli* strains to form biofilms will be compared with that of the parent wild type strain using a crystal violet microplate assay.

Introduction

Biofilms are structured communities of bacteria that live in a self-produced polymeric matrix that adhere to a living or nonliving surface. By doing so, they can allow the organisms to be more resistant to harsh conditions, enabling them to survive longer and spread. These structures and the organisms that are able to form them can cause serious problems if they colonize on certain objects, such as medical devices. *Escherichia coli* (*E. coli)* is one of these organisms. Several genes in *E. coli* have been associated with biofilm formation, including the genes for curli (*csgA)* and fimbriae (*fimA*). Mutations in these two genes were created and the organism grown in two different types of media to: 1) compare the genes effect on biofilm formation, and 2) determine the effect on nutrient availability has on biofilm formation. The hypotheses of the experiment were that *E. coli* carrying mutations in *csgA* and *fimA* will not be able to form biofilm as effectively as the wild type *E. coli* and that organisms grown in nutrient rich environments will produce more robust biofilms than organisms grown in nutrient poor environments.

Methods

Formation of Mutants

The organisms used in the experiment were isogenic, meaning that the only difference in the strains were the mutations we introduced. To obtain the mutants, a single-step inactivation procedure was followed (1). A strain of *Escherichia coli* BW 25113 carrying the plasmid PKD-4, which contained a kanamycin resistance gene, was obtained and the plasmid isolated. Gene disruption cassettes were formed by Polymerase Chain Reaction (PCR) using primers designed to amplify the kanamycin resistance cassette and introduce sequences from *csgA* (primers CsgA-

1 and -2) and *fimA* (primers fimA-1 and -2). The PCR product was purified and electroporated into wild *E. coli* containing the helper plasmid pKD64, which encoded ampicillin resistance and a bacteriophage recombinase complex. Transformants were obtained by plating the sample onto nutrient agar containing kanamycin and ampicillin to select for the strains containing both the PCR product and the helper plasmid. Confirmation of the target genes was verified by PCR to confirm that the mutations were in the desired location using specific primers.

Biofilm Assay

To do the biofilm assay, the procedure suggested by George O'Toole (2) was followed. Once the mutants were confirmed, overnight cultures of the organisms (both mutant *csgA* and *fimA* strains as well as the wild type) were prepared in either LB (nutrient-rich broth) or SMB (nutrient-poor) broth. The optical densities of the cultures were adjusted to $OD_{550\ nm}$= 1.0 and the cultures were seeded onto microtiter plates to test for their ability to form biofilms. The biofilms formed were stained with 0.1% crystal violet and then the biofilms were quantified by dissolving in 30% acetic acid and measuring the absorbance at 550 nm with a microplate spectrophotometer.

Results

Based on the data collected in the assay, it was shown that when comparing the strains of mutant *E. coli* with the wild type, the amount of biofilm formed by the mutant types was less than the amount formed by the wild strain. For each environment (*csgA* in LB broth or *fimA* in SMB for example) there were 192 replicates taken. These values were then analyzed using various statistical tests and then put into graphs for a more visual representation. In Figures 1 and 2, it is shown that the wild type strains, which did not have the resistance gene formed greater

amounts of biofilm than the mutant types, which did have the resistance gene. Meaning the wild type strain produced more biofilm formation in the individual wells of the microtiter plates leading to greater statistical data. This supports one of the hypotheses for the experiment, that *E. coli* with the mutations in either *csgA* or *fimA* are not able to form as robust biofilms as the wild type *E. coli*.

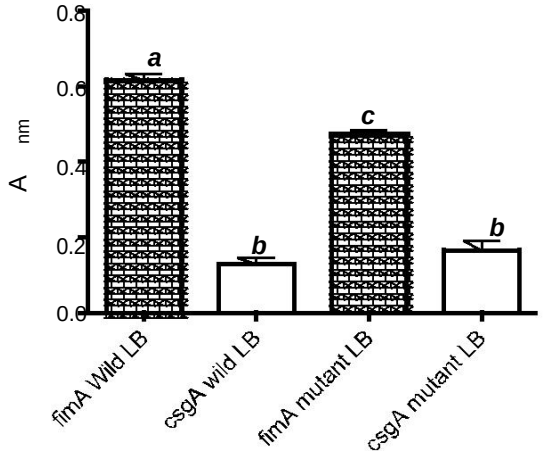

Fig. 1

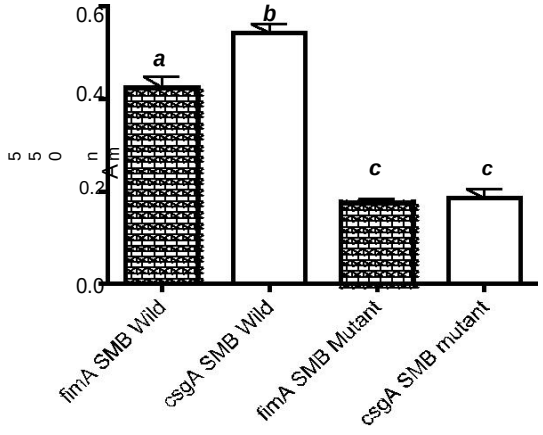

Fig. 2

Another variable in biofilm formation tested in this experiment was to determine how the amount of nutrients present as the organism is growing has an effect on the amount of biofilm formed. It was hypothesized that the organisms grown in nutrient rich environments (where LB media was used) would produce a greater amount of biofilm than organisms grown in nutrient poor environments (where SMB media was used). Figures 3 and 4 show the data collected displayed in the form of bar graphs. The patterned columns represent the wild strains while the non-patterned columns represent the mutant strains.

Figure 3 is the data from the assays with the *csgA* mutant grown in either LB or SMB media. When simply comparing the media, the organisms grown in the SMB media formed

greater amounts of biofilm. This did not support the hypothesis that organisms grown in LB would form more biofilm than the organisms grown in SMB. When looking at the difference between the wild strain and mutant strain on this graph, support of the first hypothesis was reinforced. The wild strain of *E. coli* formed over twice the amount of biofilm as was seen in the mutant strain. The same observations were seen in the assays run with the *fimA* mutant (data shown in Figure 4).

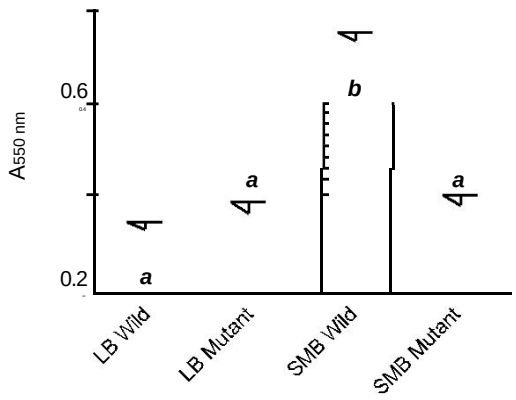

Fig. 3

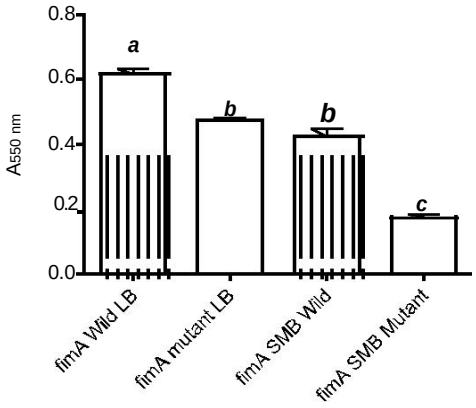

Fig. 4

Discussion

Our hypotheses were that mutant *E. coli* would not be able to form biofilms as well as wild type *E. coli* and that organisms grown in nutrient-rich environments will produce more biofilm growth than organisms frown in nutrient poor environments. More robust biofilms were produced by the wild type *E. coli* in both LB and SMB, which supported our first hypothesis. However, our second hypothesis was not supported because the mutant strains produced more biofilm in SMB than in LB in the csgA and the opposite for the fimA strain. One reason for the discrepancy is simply there could have been a small amount of contamination. One of the

reasons an organism forms a biofilm is to help it survive difficult conditions, and it can be argued that in LB, conditions become stressful only as the organisms begin to reach stationary phase. In contrast, nutrient limitations present in cultures in SMB would be expected to be comparatively stressful throughout the organisms' time in culture. Consequently, one might expect an increase in biofilm production for cells grown in minimal media due to the unfavorable conditions. Further research should be attempted to test this hypothesis, as well as the ability of *csgA-fimA* double mutants to form biofilms. Our work shows that csgA and fimA are both resposible for the formation of biofilms. With this information we maybe able to produce products such as catheters which are resistant to either of these genes. Doing so would help reduce the formation of biofilms and directly reducing the occurrence of infections. With further research into a double gene knock out we could possible discover yet another gene that may be responsible for the production of biofilms. Again knowing which genes are responsible for the formation of biofilms would allow us to produce products that are resistant to biofilms or even slow the formation.

References

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Professional Biographies

Nicole Snyder

I am from Tracy, Minnesota. I am currently an undergraduate student at Minnesota State University, Mankato majoring in Microbiology. I will be graduating in May 2014 with a BS in Microbiology. I presented this research with my partner at the 2014 Minnesota Undergraduate Scholars Conference and the 2014 Undergraduate Research Symposium. It is my goal to acquire a job in quality assurance or research.

Sean Willaert

I am from Mankato, Minnesota. I am an undergraduate student at Minnesota State University, Mankato. I will graduate in the spring 2016 with a degree in Human Biology. I presented this research with my partner at the 2014 Minnesota Undergraduate Scholars Conference and the 2014 Undergraduate Research Symposium. I would like to attend medical school and pursue a career in the medical field.

Dr. Timothy Secott

Dr. Secott received his BS in Biology from Millersville University in Pennsylvania. After working as a research technician in the Department of Microbiology at Penn State University's Hershey Medical Center, he supervised the activities of the Bacteriology and Molecular Diagnostic Sections of the Pennsylvania State Veterinary Laboratory for more than 10 years before starting PhD studies at Purdue University. He joined the Department of Biological Sciences faculty at Minnesota State University, Mankato in 2003.