Physical Disruption and Antibiotic Treatment of *Pseudomonas aeruginosa* with Gentamicin and Ciprofloxacin Biofilms has No Effect on the Proportion of Rough Small Colony Variants

Cynthia Gunaratnam, Lara Robertson, Caroline So, and Maya Tong  
Department of Microbiology & Immunology, University of British Columbia

*Pseudomonas aeruginosa* is an opportunistic pathogen, armed with multiple resistance mechanisms that render it resistant to many antibiotics. One such mechanism is the ability to form biofilms. A previous study assessed the viability of *P. aeruginosa* cells within biofilms after physical disruption and antibiotic treatment, and found that there was no difference in cell viability despite physical disruption. However, an increase in green color, thought to be pyocyanin, was observed at higher amounts in the biofilms that had been exposed to both stresses. The purpose of this study was to determine if the lack of change in cell viability during antibiotic treatment and physical disruption was due to an increase in rough small colony variants of *P. aeruginosa*. The relative proportions of these variants within biofilms were assessed by quantitatively measuring changes in pyocyanin and qualitatively measuring pilin production over a 4-hour antibiotic treatment period following disruption compared to undisrupted biofilms. Antibiotic treatment caused an equal decrease in cell viability, regardless of biofilm disruption, indicating that physical disruption of biofilms was not sufficient to increase susceptibility to antibiotic treatment. Additionally, the results of the pyocyanin and pilin assays showed no relative change between the antibiotic treatment and/or biofilm disruption conditions over the course of treatment. Therefore, no supporting evidence was generated for the hypothesis that *P. aeruginosa* within biofilms respond to physical disruption and antibiotic stress by increasing the proportion of rough small colony variants.

*Pseudomonas aeruginosa* is an opportunistic pathogen that can cause severe infections in immunocompromised people, such as patients with cystic fibrosis (25). These bacteria are innately resistant to many antibiotics and have acquired additional resistance mechanisms. The overall result is that antibiotic treatment against *P. aeruginosa* is largely ineffective, preventing infected patients from being able to completely clear this pathogen. One component of *P. aeruginosa*’s innate resistance is its ability to make biofilms (2). Biofilms are produced by a community of phenotypically heterogenous bacteria and serve as a protective layer between the bacteria and the outside world (4). Furthermore, they tend to form on solid or still surfaces and their production can be triggered by numerous environmental factors (1). Currently, there are two known biofilm-associated mechanisms of antibiotic resistance in *P. aeruginosa*. First, bacteria are able to produce nascent enzymes, some of which can degrade antibiotics (2). Second, the dense matrix of the biofilms makes molecular diffusion extremely slow so that antibiotics are physically blocked from reaching most of the bacteria within biofilms (2). It has also been shown that a transient phenotypic variation occurs when *P. aeruginosa* form biofilms. More specifically, an increase in the proportional presence of a minority population, referred to as rough small colony variants (RSCV) occurs (9). RSCVs were first identified based on their colony morphology (9). Since then, the cause of this phenotypic variation has been attributed to changes in the regulation of at least 500 genes (23). Further details regarding the regulation of this phenotypic variation are still being uncovered, but it is clear that phenotypic variation is extensive and plays an important role in biofilm resistance to antibiotics.

Since formation of RSCVs is dependent on an accumulation of phenotypic changes, RSCVs can be difficult to identify within biofilms. Because of this, two specific phenotypic changes that could be both identified and quantified were used to measure RSCVs in this experiment: that they tend to produce higher levels of pyocyanin and higher levels of type IV pili while in biofilms (6,13). Pyocyanin is a blue-green phenezine produced by several strains of *P. aeruginosa*, including PAO1(7). It is a signaling molecule that is regulated by quorum sensing, synthesized at high cell densities, and associated with biofilm formation (4). Pili are multimers formed from the protein subunit pilin and they make up small appendages on the bacteria that are responsible for motility(4). Pili appear to be
required for proper biofilm formation as well as microcolony formation. Microcolonies, which are normally associated with RSCVs, are described as bacterial colonies that initiate formation of the biofilm and can subsequently be found within the biofilms (24). Previous studies have shown that pilin deficient mutants strains of *P. aeruginosa* are unable to form microcolonies or normal biofilms (4,19). In addition, pilin production was found to be upregulated during biofilm formation and subsequently down regulated in mature biofilms (6,8).

A previous study done by Charlesworth et al. assessed the viability of cells within biofilms after physical disruption and brief exposure to antibiotics (3). They found that there was no difference in cell viability, whether the biofilms had been exposed to both, one, or neither of these stresses (3). They did, however, observe an increased green colour in biofilms that had been exposed to both stresses compared to those that had been exposed to one stress or no stresses (3). They proposed that the difference in colour might be due to an increase in pyocyanin levels. We hypothesized that the lack of decrease in cell viability after stress, such as biofilm disruption and antibiotic treatment, was due to an increase in the proportion of RSCVs. To investigate this, we repeated the viability test and measured the pyocyanin levels as well as pilin levels, which we have used as indicators of the proportion of RSCVs present.

**MATERIALS AND METHODS**

**Organization of experiments.** There were two experiments within this study. Whole cultures were used in the first experiment, while only biofilms were used in the second experiment.

**Bacterial strains, media and chemicals.** The *P. aeruginosa* PAO1 strain and *P. aeruginosa pilB* knock-out mutant strain were both supplied by the University of Washington (Department of Medicine, University of Washington, Seattle, Washington). All liquid cultures that were used whole were grown in tryptic soy broth (TSB) purchased from BD Biosciences (Franklin Lakes, NJ, Cat # 211824) and all the spread plate cultures were plated on tryptic soy agar (TSA) made from the same TSB and agar purchased from Invitrogen (Carlsbad, CA, Cat # 30391-023). All liquid cultures from which biofilms were isolated were grown in TSB purchased from EMD Chemicals (Gibbstown, NJ, Cat # 1.05459.0000) and all the spread plate cultures were plated on TSA made from the EMD Chemicals TSB and the original agar. A combination of two antibiotics, gentamicin (Oakville, ON, Cat # G-3632) and ciprofloxacin (Oakville, ON, Cat # 17850) purchased from Sigma-Aldrich was used.

**Biofilm preparation and isolation.** Overnight cultures of the PAO1 strain and the *pilB* mutant strain were prepared by inoculating 3 ml of TSB with a loop-full of bacteria. These were grown with aeration for 24 hours at 37°C on a shaking platform set at 100 rpm. For the PAO1 strain overnight culture, 30 μl was added to each of twenty 16 x 125 mm test tubes containing 3 ml of TSB. For the *pilB* mutant strain overnight culture, 30 μl was added to a single 16 x 125 mm test tube containing 3 ml of TSB. All 21 tubes were grown with aeration for 5 days at 37°C on a shaking platform set at 100 rpm to form mature biofilms. No isolation step was required when whole cultures were used. For cultures requiring biofilm isolation, each mature biofilm culture was placed on a 45 mm, 5.0 micron Millipore membrane filter (Billerica, MA, Cat # SVPPO700) and vacuum filtered until excess liquid was removed. After filtration, the filter was placed onto a sterile petri dish and the biofilm on top was removed using a glass spreader. The biofilms were transferred into 1.5 ml microfuge tubes to be standardized by volume. Each biofilm was then washed back into a 16 x 125 mm test tube with 3 ml of TSB.

**Physical homogenization and antibiotic treatment.** Biofilms in the physically disrupted conditions were homogenized using a 7 ml Dounce tissue homogenizer and pestle A (Wheaton, Millville, NJ) for 3 minutes using a push/twist motion. Doses of 100 μl of 23.2 μg/ml gentamicin and 100 μl of 17.4 μg/ml ciprofloxacin were used for the biofilms in the antibiotic treated conditions. The test tubes were placed on a 37°C shaking platform set at 100 rpm.

**Quantifying viable cells.** At each hour interval, one test tube representing each of the 4 treatment conditions (a total of 4 test tubes) was removed from the shaking platform. The 4 biofilms were physically homogenized as described previously. They were then homogenized a second time in 1.5 ml microfuge tubes using disposable pestles (Nalge Nunc International, Rochester, NY) using a push/twist motion for 30 times prior to the preparation of serial dilutions of 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵ in TSB. 100 μl of each were spread-plated in duplicate on TSA plates, incubated at 37°C for approximately 17 hours and the colonies were then counted.

**Isolating and quantifying pyocyanin.** At each hour interval, 300 μl was removed from each of the 4 homogenized biofilms and put into 1.5 ml microfuge tubes. Pyocyanin was then isolated in duplicate using a modified protocol from Palumbo (20). First, 600 μl of chloroform was added to each 1.5 ml microfuge tube. After vortexing for 5 seconds, they were centrifuged at 12,000 x g for 15 minutes. Then, 400 μl of the bottom layer was removed into a new 1.5 ml microfuge tube and 200 μl of 0.2 N HCl was added. After vortexing for 5 seconds, the microfuge tubes were then centrifuged again at 12,000 x g for 15 minutes. 300 μl from the top layer was removed and absorbance was measured at 520 nm using 0.2 N HCl to zero the machine.

**Isolating and qualitatively analyzing pilin.** At each hour interval, 900 μl was removed from each of the 4 homogenized biofilms and put into 1.5 ml microfuge tubes and pilin was isolated in duplicate using a modified protocol from Hsieh *et al.* (12). After each sample was vortexed at maximum speed for 15 seconds and left on ice for 15 seconds 6 times, they were centrifuged at 12,000 x g for 30 mins. The supernatant was removed into a new 1.5 ml microfuge tube. The Lowry assay (21) was used to measure total protein concentration prior to gel electrophoresis. Both the samples and standards (chicken egg albumin) were diluted with 100 mM NaOH and absorbance was measured at 520 nm. For proteins isolated from whole cultures, the gel electrophoresis protocol using SDS polyacrylamide gels and Coomassie staining from Frost *et al.* were used (11)). MultiMark multi-colored standard purchased from Invitrogen (Burlington, ON, Cat # LC5725) was used as the molecular ladder. For proteins isolated from biofilms, ProPure Next 10% gels (Solon, OH, Cat # M256) with 1X Next Gel Running Buffer (Solon, OH, Cat # M259) and silver staining were used according to the Silver Stain Plus BioRad product insert (Hercules, CA, Cat # 161-0303) was used as the molecular ladder. The pilB mutant was used as a negative control. All gels were run at 90 V for approximately 2 hours.

**RESULTS**

**Cell viability.** Results measuring cell viability following treatment (with or without biofilm disruption and/or antibiotic treatment) are depicted in Table 1 and Table 2. The standard deviation of this data, where 30-300 colonies were counted, was less than 10%. Cell viability did not differ greatly when the whole cultures
or the isolated biofilms were left untreated with antibiotics. More specifically, all cell concentrations under these conditions (no antibiotic treatment) were within 1 log of each other. However, this was not observed with cells that were antibiotic treated. Whole cultures that were treated with antibiotics showed a decrease in cell viability exceeding 1 log, which was evident within 1 to 2 hours after treatment (Table 1). This effect was apparent in both the undisrupted and disrupted conditions. Furthermore, although the trends showing a decrease in cell viability were almost equal in both of these conditions, the cell concentration at the 1-hour time point was slightly higher in the disrupted condition. Since this difference was only observed within one time point, it is unclear whether this is a true effect of disruption or an anomaly. In isolated biofilms, cell viability in equivalent experimental conditions decreased by more than 1 log by the 1-hour time point for both the undisrupted and disrupted conditions (Table 2).

In the conditions with antibiotic treatment, the bacteriostatic activity of the antibiotics resulted in less than 30 colonies being observed on the plates of the lowest dilution. The corresponding reported values were estimates and must be interpreted with caution. Similar effects yielded some time points with no observed colonies on plates of all dilutions (denoted as NC). No estimate of viability can be made in these situations, except with relation to the trends of surrounding time points.

Quantification of pyocyanin. Results from the pyocyanin assay from whole cultures showed that total pyocyanin levels had ranged from 0.20 to 0.29 A420 and that pyocyanin levels did not substantially change regardless of antibiotic treatment or physical disruption conditions (Table 3). The standard deviations for this data were all below 0.05 CFU/ml. Results from the pyocyanin assay from isolated biofilms showed no pyocyanin production, as absorbance readings were consistently zero (data not shown). Overall, the relative pyocyanin levels were consistent across both whole cultures and isolated biofilms, but the absolute levels observed could not be reproduced.

Qualitative analysis of pilin. The pilin protein of interest is approximately 15 to 17 kDa in size (17). For protein isolated from whole cultures, the protein band representing pilin was expected to be seen at the level of the 17 kDa molecular marker on the standard protein ladder. Results showed that the 17 kDa marker of interest, as well as any possible pilin protein, had run off the gel (Fig. 1A and Fig. 1B). For proteins isolated from biofilms, the smallest molecular marker on the standard protein ladder was 45kDa. Thus, a standard curve was constructed to estimate the distance any pilin protein may have travelled (data not shown). However, no bands were visualized at this estimated distance (as indicated by ⬇ in Fig. 1C and Fig. 1D).

When a protein sample from the pilB knock-out mutant was run on the gels, the lane was expected to lack the PilA protein, as well as the pilin (PilA) protein. PilB is required for proper production of pili and a knock out of this gene prevents the production of PilA. The PilB protein has a molecular weight of approximately 62 kDa (18). For both the whole cultures and the isolated biofilms (Fig. 1A), bands were observed at 65kDa for all lanes, including the lanes with pilB mutant proteins. Since the band appeared in the lanes containing samples from the pilB mutant strain, it likely represents an alternate protein that was present in the unpurified samples, making the pilB mutant an unsuitable negative control in further data analysis. On the other hand, when TSB media alone

---

### TABLE 1. Cell viability of *P. aeruginosa* whole cultures. NC indicates the absence of colonies at the lowest tested dilution.

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Viability (10⁶ CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>Undisrupted and untreated</td>
<td>535</td>
</tr>
<tr>
<td>Undisrupted and treated</td>
<td>62</td>
</tr>
<tr>
<td>Disrupted and untreated</td>
<td>121</td>
</tr>
<tr>
<td>Disrupted and treated</td>
<td>202</td>
</tr>
</tbody>
</table>

### TABLE 2. Cell viability of *P. aeruginosa* isolated biofilms. NC indicates the absence of colonies at the lowest tested dilution.

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Viability (10⁶ CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>Undisrupted and untreated</td>
<td>75</td>
</tr>
<tr>
<td>Undisrupted and treated</td>
<td>41</td>
</tr>
<tr>
<td>Disrupted and untreated</td>
<td>140</td>
</tr>
<tr>
<td>Disrupted and treated</td>
<td>90</td>
</tr>
</tbody>
</table>
was run on the gels, no protein bands were observed (lane 13 in Fig. 1C and lane 13 in Fig. 1D). We are able to eliminate the possibility of having contaminating proteins within the media that could lead to the false identification of bands.

**DISCUSSION**

We observed that the susceptibility of *P. aeruginosa* to combined treatment with gentamycin and ciprofloxacin did not differ greatly depending on the physical integrity of the biofilm layer. This was demonstrated where the effect on cells within the whole culture was investigated (Table 1) and where the effect on cells within the biofilm alone was investigated (Table 2). Table 1 and Table 2 show that the addition of antibiotics seemed to equally kill all cells, regardless of biofilm integrity, by 2 hours post-treatment. Our results are supported by previous studies, which have also demonstrated that physical disruption did not greatly increase the susceptibility of *P. aeruginosa* to gentamycin and ciprofloxacin (3,5). Although these results seem to suggest that the physical structure of biofilms has nearly no protective effects against gentamycin and ciprofloxacin treatment, previous studies suggest that physical biofilm disruption is unable to effectively disrupt the alginate in biofilms (3). Cotton et al. have shown that complete disruption of alginate, which is better established by alginate lyase treatment, is necessary for a greater susceptibility effect (5). These observations are consistent with the multifactorial protective effects biofilms, which include preventing the diffusion of antibiotics as well as the presence of other intrinsic physiological properties of the biofilm (10).

The reliability of the results shown in Table 1 and Table 2 must be considered with caution. Direct comparisons between different conditions and across different time points cannot be done since the biofilms for each condition and time point were grown individually. Although specific measures were taken in attempt to standardize the biofilms (using the same volume of inoculum in the four whole cultures and visually equalizing volumes of mature biofilms using a 1.5 ml microfuge tube for isolated biofilms), these methods were not adequate to confirm equal cell concentrations across treatment conditions and time points. Furthermore, the standard deviations for conditions where death was observed were relatively large. This was most likely due to having less than 30 colonies at the lowest dilution plated. Plating an undiluted sample would have most likely prevented the large standard deviations. However, the trend that both disturbed and undisturbed biofilms are equally susceptible to antibiotics was seen in both whole cultures and isolated biofilms, giving more confidence in these trends. Additional biological replicates would have also increased reliability.

Charlesworth et al. observed a qualitative increase in pyocyanin production after physical disruption, which they suggested to be an adaptive response to the stress of biofilm disruption (3). In addition to increased pyocyanin production, a general change in expression patterns producing the RSCV phenotype has also been shown to have a slightly protective effect on antibiotic susceptibility (6). Diezel et al. have also suggested that RSCVs have increased pilin expression (6). Given these observations, we attempted to use the relative concentrations of pyocyanin and pilin as indicators of the relative amounts of the RSCV phenotype present to investigate the suggested protective effect of RSCVs against certain stresses (such as biofilm disruption and antibiotic treatment) (3,6).

Our results show that total pyocyanin levels did not change in response to antibiotic treatment and/or physical disruption of the biofilm layer. This observation was consistent for cells within the whole culture (Table 3) and cells within the isolated biofilm (data not shown). Therefore, our results do not support the phase variation or adaptive response hypotheses. We did not observe an increase in pyocyanin levels, which is indicative of increasing proportions of RSCV cells (6). However, limitations to the pyocyanin quantification method used here make our results inconclusive. Since pyocyanin is secreted and not used up by *P. aeruginosa* grown in media with adequate nutrients (like TSB) (16), it accumulated during the 5-day biofilm formation. Therefore, the relative and absolute pyocyanin concentrations observed in Table 1, could be more of a reflection of the pyocyanin produced during the 5-day biofilm growth than of the

<table>
<thead>
<tr>
<th>Treatment condition</th>
<th>Absorbance at 520nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr</td>
</tr>
<tr>
<td>Undisrupted and untreated</td>
<td>0.29</td>
</tr>
<tr>
<td>Undisrupted and treated</td>
<td>0.20</td>
</tr>
<tr>
<td>Disrupted and untreated</td>
<td>0.26</td>
</tr>
<tr>
<td>Disrupted and treated</td>
<td>0.25</td>
</tr>
</tbody>
</table>
pyocyanin produced during the 4-hour treatment period. Qualitative observations of biofilm growth before and after antibiotic treatment suggest that the green-blue color of the culture appeared in day 2 of biofilm formation and did not change drastically during the four hours post-treatment. This suggests that a large majority of pyocyanin production occurred before treatment. Additionally, we could not definitively determine whether the lack of change in pyocyanin concentrations was due to a lack of pyocyanin production in the treatment conditions or due to the presence of pyocyanin production being masked by different levels of viability across the conditions. It is difficult to predict, based on previous data, which of these two options are more likely. Pyocyanin is known to be regulated by the global regulatory quorum sensing (QS) system, which can be affected by nutritional conditions and environmental signals, as well as two global regulatory sytems: GacA-GacS and Vfr (4,16). In addition to a complex, poorly understood regulation mechanism, the importance of pyocyanin is also unclear. It has been shown to contribute to virulence by protecting against reactive oxygen species and host phagocytic cells (22,26). Pyocyanin also contributes to the fitness of P. aeruginosa by functioning as an oxidizing agent and an antimicrobial agent against competitors (16). Since pyocyanin production is regulated by global regulatory systems, its production may or may not be affected by gentamycin and ciprofloxacin treatment and/or the stress of physical disruption. Therefore, it would be important to identify the pyocyanin production per cell in order to draw a more definitive conclusion from our results. Since

FIG. 1. P. aeruginosa cellular proteins separated by gel electrophoresis after pilin isolation. Sample treatment conditions and time points are indicated above each lane (where UD = undisrupted, UT = untreated, D = physically disrupted, T = antibiotic treated, and TSB = tryptic soy broth). (A) and (B) show Coomassie-stained gels with the whole culture samples, while (C) and (D) show silver-stained gels with the isolated biofilm samples. Pilin was expected to be visualised on (C) and (D) as indicated by (←).
pyocyanin production was observed to remain constant after antibiotic treatment, despite a decrease in cell viability, identifying pyocyanin production per cell may provide evidence for determining whether pyocyanin production had completely stopped following physical disruption and/or antibiotic treatment, or if each viable cell had up-regulated its pyocyanin production after these stresses were applied. It should be noted that the cultures from which biofilms were isolated failed to turn green-blue in colour, even after 5 days of biofilm formation, and the absorbance was observed to be zero across all the conditions and time points. This suggests that little to no pyocyanin was produced. There are three potential explanations for this observation. First, an older starting colony may have been used. Older colonies were observed to lose their green-blue colour. However, no difference in total pyocyanin was observed across the different treatment conditions for both fresh colonies and two-week old colonies (data not shown). Second, the manufacturers for the TSB used in the two experiments were different. However, it was found that both TSBs were made using the same ingredients at equivalent concentrations. Third, the TSB stock used for isolated biofilms may have been less concentrated and thus less nutrient-rich compared to that used for whole cultures. For the whole cultures, the TSB stock was made approximately 2 weeks prior to use, allowing more evaporation to occur. For the isolated biofilms, the TSB stock was made 1 day prior to use.

No pilin could be identified on the SDS-PAGE gel or the ProPure gel (Fig. 1). Again, our results do not support the phase variation or adaptive response hypotheses. We did not observe an increase in pilin levels, which is also indicative of increasing proportions of RSCV cells (6). However, due to limitations in our pilin isolation method, our results remain inconclusive. In protein samples from whole cultures, no pilin protein was observed because the gel was unfortunately left running longer than required and the lower molecular weight bands, including pilin, ran off the SDS-PAGE gel. However, in protein samples from isolated biofilms, there could be two possible explanations, other than the absence of pilin, as to why no pilin protein was observed. It could be due to the pilin isolation method insufficiently breaking the pilin down into the 17 kDa PilA monomers needed for identification. Since pilin is made up of thousands of PilA protein subunits (18) it is possible that the pili were sheared in such a way that a multitude of pili fragments of different lengths accumulated in the supernatants collected. However, the fragments of higher molecular weights (in multiples of 17 kDa) were not observed. Since all of the cellular protein content was collected and pili was neither concentrated nor purified, it is likely that the pili fragments were not at a high enough concentration to be visualized on the gel. Alternatively, our pilin isolation method was able to shear all the pili into pilin monomers. Again, due to the pili not being concentrated or purified prior to visualization on the gel, there is a possibility that the pili was not at a high enough concentration. In past experiments, after mechanically shearing the pilin off the bacteria by vortexing, additional isolation and purification steps were taken (12). Protein containing supernatants were dialyzed against 20 mM Tris-HCl and 1 mM EDTA, and subsequent column chromatography using A Sepharose HP and Sephadex 200 was performed (12). Due to a lack of funding, we were unable to isolate and purify pilin as stringently.

In conclusion, our results indicate that treatment with gentamycin and ciprofloxacin leads to a decrease in survival of P. aeruginosa over a 4-hour period and that physical disruption of biofilms had no effect on survival after antibiotic treatment. Minimal changes in the levels of pyocyanin were observed. The media used may have restricted changes in pyocyanin production. No conclusions about pilin levels could be drawn since the assay used failed to detect any pilin. Overall, due to the limitations in the technical methods used, definitive data was not produced. In addition, since colony phenotype was not directly measured, the production of pilin and pyocyanin may not be a true reflection of the proportion of RSCVs. As a result, our hypothesis that applied stresses (biofilm disruption and antibiotic treatment) lead to an increase in RSCVs is left unsupported.

**FUTURE DIRECTIONS**

Additional investigation must be done in order to more confidently reject our hypothesis. Our results were inconclusive mainly due to an inadequate method of pilin analysis. Pili quantification could be done more accurately by using Sepharose A beads to isolate the pili and subsequently using a Lowry assay, against a standard curve created with purified pili, to quantify the amount of pili present (12) In addition to improving technical methods for pilin isolation and detection, experiments to study other characteristics that distinguish RSCVs can be carried out. Some of these defining characteristics include, but are not limited to, a lack of swarming, swimming and twitching motilities (6). If our hypothesis that there is an increase in RSCVs is correct, then future experiments should show a decrease in swarming, swimming and twitching motilities.

Our results suggested that RSCVs did not confer increased protection against the external stresses of biofilm disruption and antibiotic treatment. Therefore, it would be beneficial to investigate other protective
components of biofilms. Focussing on alternate biofilm components, such as the roles of alginate and Psl polysaccharide, may shed more light onto which aspects of biofilms play more crucial roles in resistance to external stresses, such as antibiotics. The effects of alginate could be tested using alginate lyase with antibiotic treatment and Psl polysaccharide could be tested using a psl knock-out mutant with antibiotic treatment.

ACKNOWLEDGEMENTS

This study was funded by the Department of Microbiology & Immunology at the University of British Columbia. We would like to extend our gratitude to Shaan Gellatly and Dr. William Ramey for their guidance and advice. We would also like to thank the media staff room for their invaluable contribution to this study.

REFERENCES