Regulation of Pyocin Genes in *Pseudomonas aeruginosa* by constitutively repressive PrtR

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Abstract

Pyocins are bacteriophage tail-like complexes that are produced by *Pseudomonas aeruginosa* under the SOS response of the cell. These molecules are used for intraspecies competition by targeting and killing susceptible *Pseudomonas* strains. The pathway of pyocin production begins with the protein RecA which is activated by the SOS response. Its function is to cleave the pyocin gene repressor, PrtR, which allows for the activation of the gene activator, PrtN. The repressor inhibits pyocin production by binding to the promoter of the gene encoding the activator, prohibiting the expression of pyocin genes. Notably, pyocin production can also be induced independently of the SOS response in strains that are deficient in the gene *xer*C. Additionally, the *xer*C deleted mutant significantly increases the number of pyocin-producing cells compared to a wild-type strain. In my lab research, we reveal the limitations of the repressive ability of PrtR as well as the potential existence of other targets of PrtR. We constructed a mutant version of PrtR in a *xer*C deficient strain, specifically PrtRS162A, which is an uncleavable repressor. Surprisingly, the uncleavable repressor is found to be insufficient in completely blocking pyocin gene expression in a pyocin-overproducing strain, but it is able to block the production of functional pyocins. Expression of at least some pyocin-encoding genes, including our reporter (*hol*), suggests that the pyocin-producing strain can somehow bypass the repressive activity of PrtR. However, the strain containing PrtRS162A did not produce any functional pyocins, suggesting that one or more genes required for pyocin function are not expressed. This study suggests that PrtR has novel targets within the pyocin gene cluster in addition to its previously known target of PrtN expression.

Introduction

Pseudomonas aeruginosa is an opportunistic pathogen commonly found within infections of patients with other illnesses such as cystic fibrosis. *P. aeruginosa* is particularly dangerous because of its ability to form biofilms and its multi-drug resistance (1). However, this bacterium can also produce intraspecific killing molecules called pyocins. These complexes target and kill susceptible strains around them by latching onto the cell and rupturing the membrane (2). In order for these pyocins to reach other bacteria cells they must be released from the producer cell. A series of cell lysis regulating genes are activated, along with the pyocin synthesizing genes, and they cause producer cells to explosively lyse and release their components, including pyocins. The efforts of this research are aimed at discovering information about pyocins, specifically the mechanism behind their production and release, that will allow for the manipulation and leveraging of this pathway to effectively treat clinical infections.

The canonical pathway of pyocin production features a cascade of genes that are activated by DNA damage and the SOS response. Specifically, RecA, a DNA repair protein, is activated during this response and initiates the process of pyocin production. The repressor, PrtR, is constantly bound to the promoter of the gene encoding an activator, PrtN, until it is cleaved through RecA activation. Once PrtR is cleaved, PrtN is produced and able to activate transcription of the pyocin gene cluster (3). A newly discovered pathway of pyocin induction is

completely independent of DNA damage and RecA activation. Deletion of the gene *xer*C was found to significantly upregulate the pyocin genes and induce their activation in the absence of the SOS response (4). The exact mechanism of this pathway is still unknown, but it is confirmed to be independent of RecA activity. It is also thought that PrtN is still responsible for activating the pyocin genes in a Δ*xerC* (deletion of *xerC*) mutant. As was previously mentioned, in order for PrtN to work its repressor, PrtR, must be cleaved and inactivated. Therefore, the alternative pathway of pyocin production must either bypass PrtR or cause its autocleavage. To determine which scenario was exhibited in a Δ*xerC* mutant, we constructed a mutant strain of *P. aeruginosa* that contained a mutated version of the *prt*R gene. The catalytic serine required for the protein's autocleavage was mutated to an alanine, ultimately removing its autocleavage ability (5). That mutated gene produces an uncleavable version of the PrtR repressor, PrtRS162A, so in the event that the alternative pathway causes cleavage of PrtR, the mutant version will remain intact. The mutant PrtR was introduced to a *xerC* deficient mutant, creating the strain PA14 Δ*xerC* PrtRS162A. We hypothesized that an uncleavable PrtR would completely repress PrtN production and inhibit the activation of all pyocin genes. Initial experiments with this mutant PrtR revealed that it blocks pyocin production, however, it does not completely inhibit pyocin gene expression. Therefore, two possibilities arise for the PrtR mutant strain. Either pyocins are produced but are not released, or the pyocin gene expression is negligible and no pyocins are produced at all.

Methods

The initial experiments previously mentioned with PrtRS162A involved multiple assays looking at pyocin production and expression. The first experiment was a pyocin indicator assay which involved a *P. aeruginosa* strain (13S) that was known to be sensitive to the killing activity of pyocins. To observe the phenotypic effects of PrtRS162A in PA14 Δ*xerC* PrtRS162A, cell-free supernatants, which would contain pyocins (if produced), were collected from the mutant and spotted onto the indicator strain. The pyocin-sensitive strain was grown into a lawn on LB plate media; four different strains of PA14 were grown in liquid LB and filtered down to cell-free supernatants to be spotted on the lawn. Interpreting results for this experiment is simply observing the amount of clearing on the location of the spotted strains. Clearing indicates killing by pyocins and no clearing suggests no pyocin production or activity. Controls of regular PA14, PA14 PrtRS162A, and PA14 Δ*xerC*, were used along with the mutant PA14 Δ*xerC* PrtRS162A. As stated before, this experiment resulted in the finding that PA14 Δ*xerC* PrtRS162A does not show evidence of pyocin production (Fig. 1) As mentioned before, the genes involved in pyocin release are located within the pyocin gene cluster, so if the PrtRS162A mutant was acting in agreement with our hypothesis, then these genes would not be active either. This result led to another experiment to investigate pyocin gene expression rather than production. For this experiment, we used a green fluorescent protein (GFP) tag that targets the beginning of the pyocin gene cluster (*hol*). This reporter allows us to observe pyocin gene expression, and also cell lysis, at the single-cell level by emitting green light once gene expression occurs. The GFP tag was added into PA14 Δ*xerC* PrtRS162A and observed using phase and fluorescent

microscopy. Unexpectedly, this experiment showed significant pyocin gene expression similar to what is seen in a PA14 Δ*xerC* strain. It also showed some cells lysing as well as other cells exhibiting a lysis defect phenotype. This result directly contradicted what was previously found in the pyocin indicator assay showing no pyocin production. These two results ultimately led us to conduct a final experiment, similar to the first pyocin indicator assay. However, this experiment involved a process called sonication in which cell membranes are artificially degraded to release the contents of the cell. We carried out the same steps as the first pyocin indicator assay, except this time using a different control. PA14 D*xer*C Dhol Dlys DalpB-E is a mutant with all cell lysis regulating genes removed, rendering it unable to release any contents produced by the cell, namely pyocins. This control as well as PA14 Δ*xerC* PrtRS162A were spotted on the pyocin indicator strain, but another sample of each was sonicated and then spotted on the lawn as well. We knew that PA14 Δ*xerC* PrtRS162A did not produce pyocins based on the first assay, however, that conclusion was refuted in the second experiment when we saw significant pyocin gene expression. Sonication of this mutant would allow us to determine if pyocins are being produced and are unable to escape the cells, or rather that pyocin production is not occurring at all.

Results

Fluorescent microscopy with PA14 Δ*xerC* PrtRS162A resulted in the observation of significant pyocin gene expression suggesting that PrtRS162A is not completely repressing pyocin genes (Fig. 2). The final pyocin indicator assay revealed that even with significant pyocin gene expression, PA14 Δ*xerC* PrtRS162A does not produce pyocins. The non-sonicated sample did not clear the indicator strain as expected, but even the sonicated sample was unable to exhibit pyocin killing as well. This indicates that no pyocins were produced and trapped within the cells of PA14 Δ*xerC* PrtRS162A (Fig. 3).

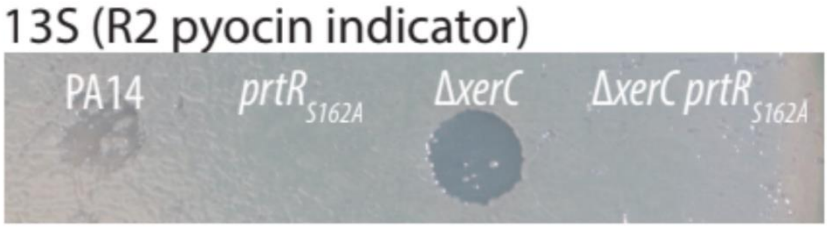
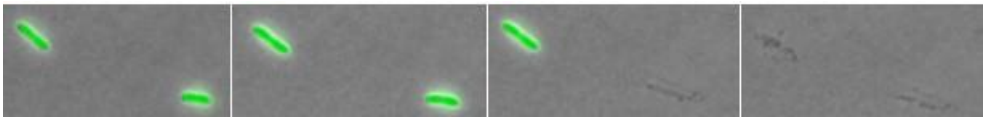


Figure 1:

Initial pyocin indicator assay conducted by former lab crew (4). Pyocin indicator strain (13S) spotted with PA14, PA14 PrtRS162A, PA14 Δ*xerC,* PA14 Δ*xerC* PrtRS162A. Clearing within the spot indicates killing activity of released pyocins while no clearing indicates no pyocin activity.



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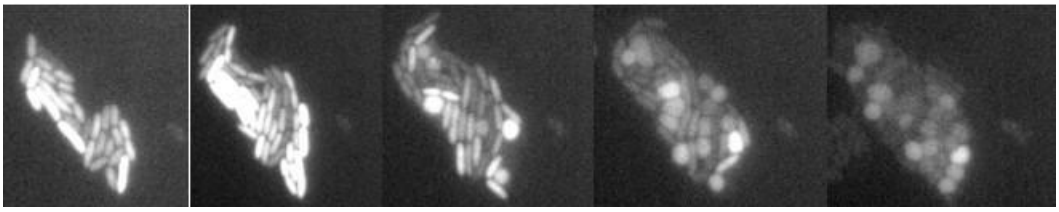

B

Figure 2:

Microscopy frame-by-frame images using a pyocin GFP tag. **A.** PA14 Δ*xerC* images, observed lysis after a short period of time. Green or bright cells have pyocin genes activated. **B.** PA14 Δ*xerC* PrtRS162A images, some cells turn on pyocin genes and lyse while others stay active and form spheroplasts. Numerous bright cells are evidence for significant pyocin gene expression.

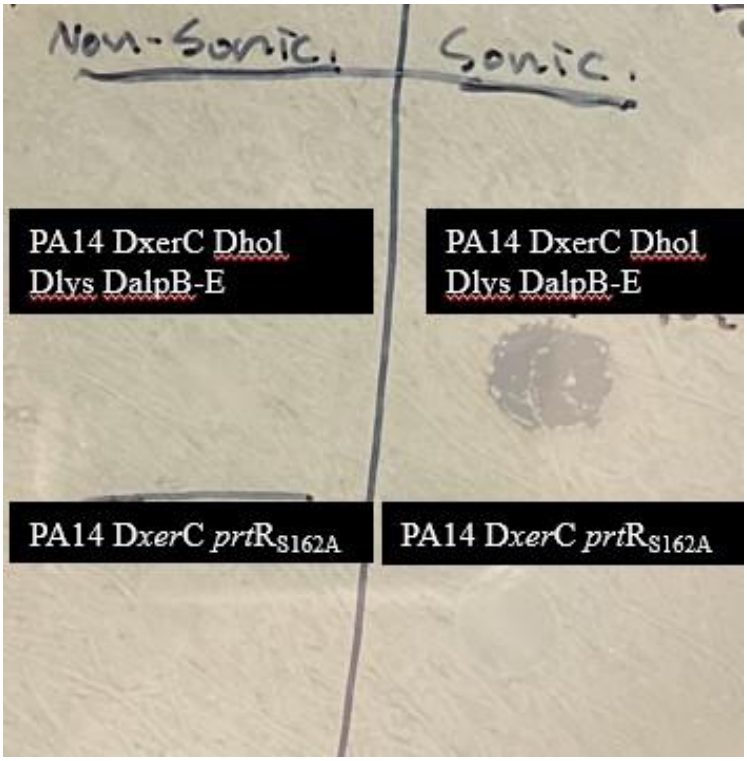

Figure 3:

Final sonication experiment using the same 13S pyocin indicator strain. Non-sonicated samples are pictured on the left and sonicated samples are on the right. (Control: PA14 D*xer*C Dhol Dlys DalpB-E. Experimental: PA14 Δ*xerC* PrtRS162A). Clearing within the spot indicates killing activity of released pyocins while no clearing indicates no pyocin activity. No clearing on the sonicated sample of PA14 Δ*xerC* PrtRS162A is evidence that this mutant strain does not have functional pyocins trapped within the cell.

Discussion:

The original goal of creating an uncleavable PrtR mutant was to determine the effects it would have on a pyocin-overproducing strain like PA14 Δ*xerC.* Initial experimentation with PA14 Δ*xerC* PrtRS162A revealed that all pyocin production was inhibited. However, through microscopy, we discovered that, although pyocins are not produced/released from the mutant, pyocin genes are still being expressed. This brought us to two conclusions regarding the effects that PrtRS162A has on pyocin expression and production. One possibility was that pyocins are being produced by mutant cells of PA14 Δ*xerC* PrtRS162A, but they are unable to escape the cell because the lysis mechanism is blocked by PrtRS162A. Our final pyocin indicator assay proved this possibility to be wrong by showing that even sonicated cells from PA14 Δ*xerC* PrtRS162A contain no pyocins sufficient to kill the indicator strain. The second possibility was that no pyocins are being made by PA14 Δ*xerC* PrtRS162A at all. This scenario is still in question considering that our microscopy data suggests that one or more pyocin genes are activated within the mutant strain. The absence of functional pyocin production by PA14 Δ*xerC* PrtRS162A, combined with the presence of pyocin gene expression in that same mutant strain, suggests that PrtR has other targets of repression besides its previously known target of PrtN. PrtR repression at multiple targets within the pyocin gene cluster, specifically genes that are required for the production of functional pyocins, would explain the inability of a PA14 Δ*xerC* PrtRS162A mutant to produce pyocins even when some pyocin genes are being expressed. Other efforts within the lab have explored electron microscopy of PA14 Δ*xerC* PrtRS162A and observed no detectable pyocin complexes. Ultimately, it can be concluded that PrtRS162A is able to block the production of pyocins, however, it is unable to completely inhibit pyocin gene expression.

References:

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