The *alp* system has a key role in cell lysis of pyocin-producing *Pseudomonas aeruginosa* cells

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Abstract: *Pseudomonas aeruginosa* produces intraspecific bacteriophage-like killing complexes, called pyocins, in response to DNA damage. The pyocins are only capable of killing nearby, susceptible *P. aeruginosa* cells. However, to permit the relatively large pyocin complexes to escape the cell, producer cells must die via programmed cell lysis. The canonical pathway induced by DNA damage is dependent on *recA* and unlocks a cascade of events that activates a cluster of pyocin-encoding genes, including genes encoding auto-lysing holin and lysin enzymes. In regard to the subset of pyocin-producing cells, it was previously thought that these two enzymes were the main regulators of pyocin release via lysis. Indeed, pyocin-producing cells lyse in a wild-type background after DNA damage and also in a *xerC* mutant strain that produces pyocins without DNA damage and independently of *recA.* However, additional genes found outside of the pyocin cluster, the *alp* genes, are also involved in mediating cell lysis in the presence of DNA damage. My research is aimed at understanding if and how the *alp* system is involved in the lysis of pyocin-producing cells in the wild-type PA14 background or the Δ*xerC* mutant background. I present evidence that the *alpBCDE* genes, which include the holin-encoding *alpB* gene, have a key role in the cell lysis that releases pyocins.

Introduction: *Pseudomonas aeruginosa* is a gram-negative opportunistic pathogen that is prevalent in nosocomial settings and preys on immunocompromised individuals, specifically those with cystic fibrosis. Treating *P. aeruginosa* infections is especially difficult because of the ability to produce biofilm, which is an antibiotic resistant matrix of polysaccharides, proteins, and DNA that essentially encases the cells, protecting them from the external environment. However, when cells are exposed to DNA damaging agents, they produce intraspecific killing complexes called pyocins that kill other nearby *P. aeruginosa* cells. My research focuses on the production and release pathways of pyocins by mutating, manipulating, and deleting specific genes that are believed to be essential in these pathways, and ultimately leveraging pyocin production for therapeutic advantage.

P. aeruginosa cells can undergo DNA damage when exposed to certain agents, including commonly prescribed fluoroquinolone antibiotics such as ciprofloxacin, in turn kicking off a cascade of events that activates the RecA protein. Activated RecA causes PrtR, the repressor of the *prtN* gene, which encodes the activator of the pyocin gene cluster to auto-cleave itself. Hence, *prtN* becomes derepressed (1). The pyocin gene cluster is activated by PrtN and all genes that are involved in the production of pyocins, including those encoding the holin and lysin, are expressed. Holin, which essentially creates holes in the cell membrane, and lysin, which further degrades the peptidoglycan, were previously thought to be the principal pair of lysis genes involved in the pyocin production and release pathway.

Previous research in the Cabeen lab uncovered a pyocin-producing and release pathway that does not require DNA damage and is RecA-independent. With the deletion of a tyrosine-recombinant encoding gene called *xerC*, the cell is able to bypass the activation of RecA and activate the pyocin cluster via PrtN activation. This pathway also significantly upregulates the

production of pyocin encoding genes and we see a higher frequency of cells producing the killing complexes (2). In addition to this finding, in a *xerC* deleted background, there is a significant increase in cells' susceptibility of fluroquinolone antibiotics, including ciprofloxacin. In this background, not only are the pyocin encoding gens upregulated, but another known lysis encoding set of genes, the *alp* system, is also upregulated. *AlpA* works in a similar manner as PrtN, as it is the activator of *alpBCDE*. *AlpB* is thought to be a "holin-like" protein (3). Due to the previous data collected in the Cabeen lab, we suspect that *holin* and *lysin* are not the only genes involved in the release of pyocins by *P. aeruginosa*, but that the *alp* system, namely *alpBCDE*, are part of, if not dominating the lysis that releases pyocins.

Methods: The ideal method of testing the involvement the *alp* system is by making a deletion of the *alpBCDE* region. These genes were deleted from a PA14 Δ*xerC* mutant strain containing *holin* and *lysin* deletions by deleting the entire pyocin cluster as well. A green fluorescent protein reporter was added to this strain in the promoter region of the pyocin cluster to see, via microscopy, cells that produce pyocins. This same strain was used in a pyocin indicator experiment, in which a cell-free supernatant of this strain is spotted on a lawn of cells that are known to be susceptible to pyocins. Then, the same strain is sonicated, which artificially lyses cells, and again, the cell-free supernatants are spotted on the pyocin-susceptible lawn.

The *alpBCDE* region was deleted from PA14, and additionally the pyocin-gfp reporter was added to view via microscopy. This same deletion was made in a *xerC* deleted background, alone, without the *holin* and *lysin* deletions.

Results: When viewing the microscopy data for the PA14 Δ*xerC* Δ*pyocins* Δ*alpBCDE* strain with the pyocin-promoter gfp reporter, cells that exhibited fluorescence did not lyse at all, but they do stop growing (**Fig. 1**). Comparatively, the PA14 Δ*xerC* Δ*holin* Δ*lysin* strain with the pyocin-promoter region gfp reporter cells did lyse (though not explosively).

Additionally, this strain exhibited pyocins killing the susceptible strain as indicated by the cell-free supernatants of the sonicated cells. This strain does not exhibit pyocin killing unless sonicated (**Fig. 2**).

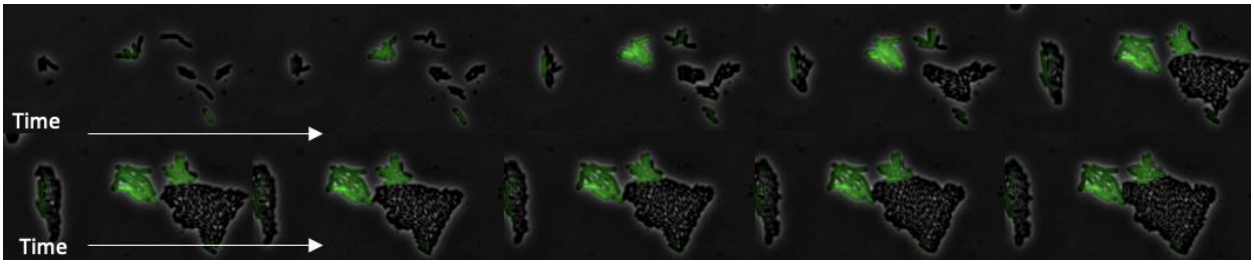

Fig. 1: PA14 Δ*xerc* Δ*pyocins* Δ*alpBCDE* p*Pyocin-gfp* frame-by-frame microscopy, with some cells exhibiting fluorescence, indicating pyocin expression, but those cells appear to stop growing nor do they lyse.

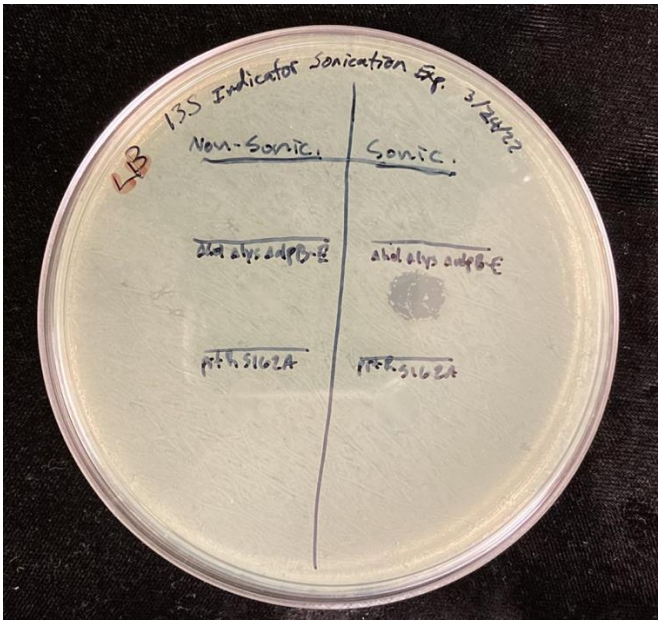

Fig. 2: PA14 Δ*xerC* Δ*holin* Δ*lysin* Δ*alpBCDE* cell free supernatants spotted on 13S clinical isolate pyocin susceptible strain, using non-sonicated and sonicated cells.

Discussion: The PA14 Δ*xerC* Δ*pyocins* Δ*alpBCDE* strain with the pyocin-promoter gfp-reporter not exhibiting lysis is indicative that the *alp* system is important and may even be dominant in lysing pyocin-producing cells; however, when artificially lysed by sonication, functional pyocin production (but pyocins are trapped within non-lysing cells) is seen as indicated bv clearing in **Fig. 2**. A PA14 *xerC holin lysin* deleted strain with the pyocin-promoter gfp-reporter does exhibit pyocin-producing cells lysing (not explosively), which is evidence that *alpBCDE* alone can mediate cell lysis in pyocin producing cells.

Current on-going work: It is suspected that PA14 Δ*alpBCDE pPyocin-gfp* and PA14 Δ*xerC* Δ*alpBCDE pPyocin-gfp* will not exhibit lysis in pyocin producing cells, solidifying that *alpBCDE* alone are substantial for cell-lysis in pyocin producing cells, and that *holin* and *lysin* are not the main lysis-encoding genes in pyocin producing *P. aeruginosa* cells.

Acknowledgements:

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2 Baggett BS, Bronson AS, Cabeen MT. 2021. SOS-independent pyocin production in Pseudomonas aeruginosa in induced by XerC recombinase deficiency. mBio. doi: 10. 1128/mBio.02893-21.

3 Pena JM, Prezioso SM, McFarland KA, Kambara TK, Ramsey KM, Deighan P, Dove SL. 2021. Control of a programmed cell death pathway in Pseudomonas aeruginosa by an antiterminator. Nature Communications. doi: 10.1038/s41467-021-21941-7