Alternative roles for *Pseudomonas aeruginosa* bacteriocins

Devin Hunt, Autumn Robinson, Lia Michaels, Mary Ellenbecker, Alison Coluccio, Patrick Secor, and Laura Jennings

Division of Biological Sciences, University of Montana, Missoula, MT

BACKGROUND

Bacteriocins are multi-protein assemblies that bear striking resemblance to the tails of bacteriophage (viruses that infect bacteria). Bacteriocins are an extracellular contractile injection system that kill closely related bacteria by puncturing their cell membrane.

Pseudomonas aeruginosa is an opportunistic pathogen that produces bacteriocins called **pyocins** which lyse susceptible bacteria.

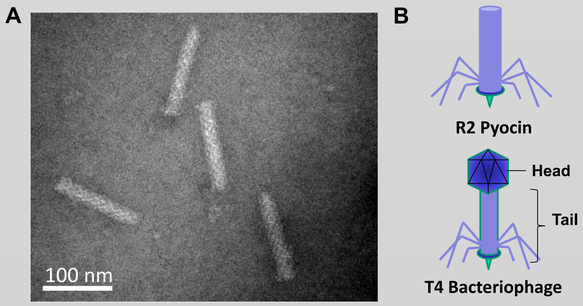


Fig. 1. *P. aeruginosa* pyocins resemble bacteriophage tails. (A) Electron microscopy imaging of type R2 pyocins from *P. aeruginosa*. (B) Structure of an R2 pyocin compared to T4 bacteriophage.

Mounting evidence suggests that besides interbacterial competition, bacteriocins also mediate interactions between bacteria and diverse eukaryotic hosts by assembling extracellular hexagonal-bacteriocin arrays composed of numerous bacteriocin particles.

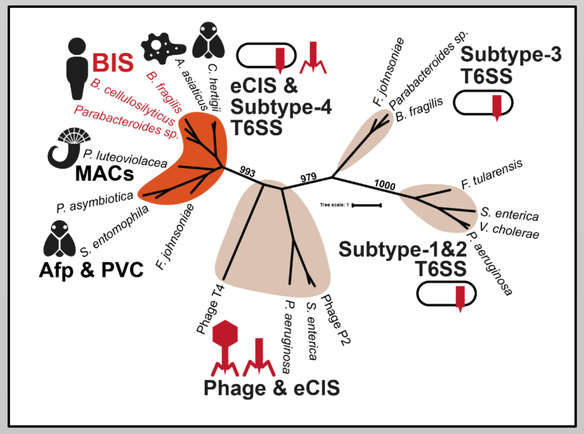
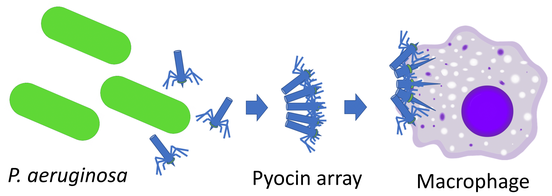


Fig. 2. *P. aeruginosa* pyocins share sequence similarity with extracellular contractile injection systems (eCIS) that form hexagonal arrays and interact with eukaryotes. Figure from[1].

Rationale:

Bacteriophage (viruses that infect bacteria) trigger an inappropriate anti-viral immune response in mice that promotes bacterial infection[2]. The bacteriophage-like tails of pyocins may also influence immune responses in a similar manner.

HYPOTHESIS



We hypothesize that *P. aeruginosa* produces extracellular pyocin arrays that modulate host responses during infection.

OBJECTIVES

Develop a method for the detection and quantification of extracellular bacteriocin arrays in *P. aeruginosa*

- **Generate fluorescently tagged pyocins**
- **Optimize plaque assay for quantification of pyocins**

FLUORESCENT PYOCINS

Fluorescent pyocin arrays were not detected in uninduced *P. aeruginosa* cultures.

Methods: To detect extracellular pyocin arrays, we fused a cyan fluorescent protein (CFP) to the baseplate of the *P. aeruginosa* R2 pyocin. We measured fluorescence intensity over time in the CFP-pyocin fusion compared to wild-type *P. aeruginosa* PAO1 using a plate reader.

Fluorescently-labelled Pyocin

Results

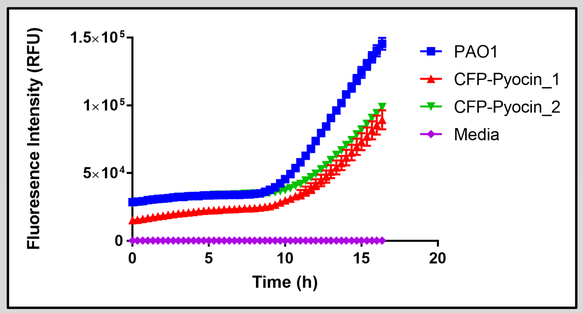


Fig. 4. Wild-type *P. aeruginosa* PAO1 and CFP-labeled R2 pyocins exhibit similar fluorescence intensity over time suggesting that fluorescence is due to autofluorescence of the cells and not pyocin production.

Discussion

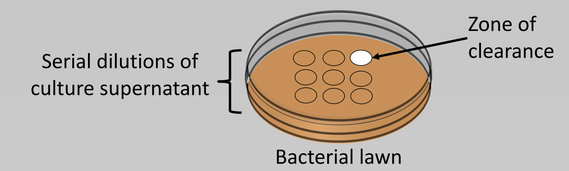
Uninduced cultures do not show evidence of pyocin array production.

- Are pyocins being produced under these conditions?
- Can we induce pyocins?

PLAQUE ASSAY

Quantification of *P. aeruginosa* R2 pyocins in culture supernatant with plaque assay.

Methods: Wild-type PAO1 can produce pyocins and bacteriophage. Pyocins, but not bacteriophage, can lyse strain PAK creating a plaque or zone of clearance. The mutagen mitomycin C (MMC) induces pyocins.



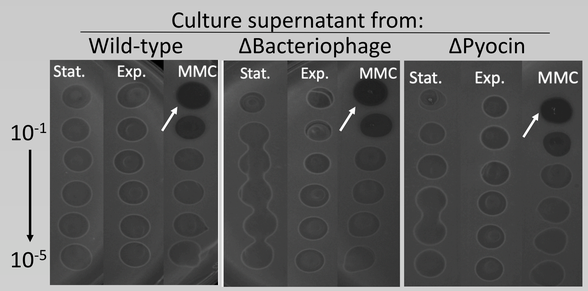


Fig. 5. Plaque assays on lawns of pyocin indicator strain PAK. Serial dilutions of culture supernatant from stationary and exponential growth phase and cultures treated with mitomycin C (MMC) to induce pyocins were plated.

Results/Discussion

The lack of plaquing in exponential or stationary cultures indicates that pyocins are not naturally produced under the conditions tested.

Induction of pyocins with MMC resulted in plaques in all strains including the pyocin deletion mutant suggesting that mutagen itself is lysing the bacterial cells. MMC needs to be removed from supernatant prior to quantification of pyocins by a plaque assay.

P. aeruginosa R2 pyocins are induced by bacteriophage infection.

Methods: To determine if bacteriophage interfered with pyocin quantification, half of the cultures were infected with Pf bacteriophage. Pyocins were precipitated from culture supernatant with polyethylene glycol (PEG) prior to plaque assay.

Results

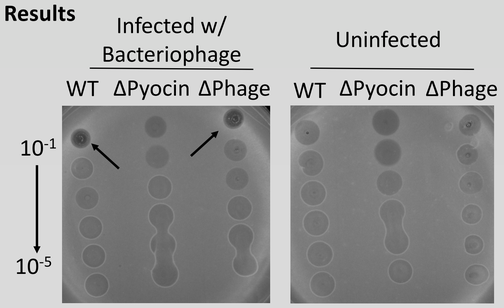
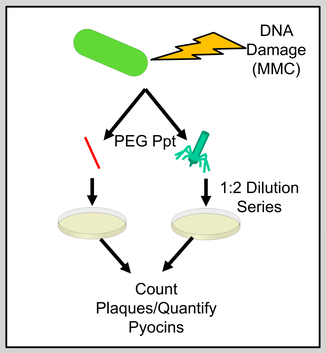


Fig. 6. Plaques in wild-type PAO1 and bacteriophage deletion mutant (ΔPhage) in cultures infected with bacteriophage suggest pyocins are induced by phage infection.

FUTURE DIRECTIONS

1) Optimize induction of *P. aeruginosa* pyocins with MMC, UV, ciprofloxacin, or genetically inducible strain.
2) Compare plaque assay to quantification of pyocin RNA with qRT-PCR.
3) Induce fluorescent pyocins and detect with fluorescence microscopy

Fig. 7. Alternative methods of inducing and quantifying pyocins using DNA mutagens (MMC) and plaque assays with suitable indicator strains.



CONCLUSIONS

- **Plaque assays on lawns of susceptible bacteria can be used to quantify pyocins.**
- **Precipitation of pyocins with polyethylene glycol is an effective method of purification.**
- **Pyocins are induced by infection with bacteriophage.**

IMPLICATIONS & LONG-TERM GOALS

Our work provides a foundation for future study of *P. aeruginosa* pyocins and their potential interactions with eukaryotic cells.

The development of an assay to purify and quantify pyocins is essential to carry out the following experiments to evaluate the effect of *P. aeruginosa* bacteriocins on:

1. Bacterial pathogenesis by measuring *C. elegans* survivability and reproduction
2. Immune cell function by measuring cytokine production and apoptosis in macrophages

REFERENCES

1. Rojas et al. 2019. A Distinct Contractile Injection System Found in a Majority of Adult Human Microbiomes. BioRxiv.

2. Sweere et al. 2019. Bacteriophage trigger antiviral immunity and prevent clearance of bacterial infection. Science.

ACKNOWLEDGEMENTS

This project was supported in part by grant P20GM103546 to P.R.S. We thank Jim Driver in the UM Electron Microscopy Facility.