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# A bacteriophage integrase regulates virulence factor production in Pseudomonas aeruginosa

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# A bacteriophage integrase regulates virulence factor production in *Pseudomonas aeruginosa*

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## *Pseudomonas aeruginosa (Pa)* biofilms and their clinical importance

- Pa is a bacterial pathogen common in nosocomial infections.
- Pa is resistant to antibiotics, especially if it grows as a biofilm, a community of bacteria within a protective matrix (Costerton, et al., 1999).
- Biofilms produce large amounts of Pf phage, which are viruses that infect Pa (Whiteley, et al., 2001).

## *intP* integrase

- Pf phage encode an integrase called *intP.*
- *intP* inserts Pf phage DNA into the Pa chromosome.

## Virulence

- Virulence is a measure of how acute a bacterial infection will be. Pa produces many virulence factors that damage or kill host cells.
- Pf phage increase the virulence of Pa (Rice et al., 2009), but the underlying mechanisms are unknown.
- Pyocyanin is a primary virulence factor produced by Pa (Fig. 1).

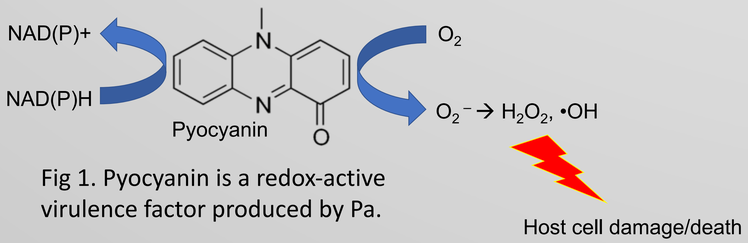


Fig 1. Pyocyanin is a redox-active virulence factor produced by Pa.

## Hypothesis

- In previous work, we used the Pf phage-null strain Δ*intP.*
- We observed that when *intP* was deleted, pyocyanin (a green pigment) production was repressed relative to wild type Pa (fig. 2 & 3).

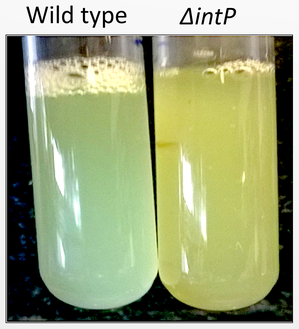


Fig. 2: Representative images showing pyocyanin production (green) in wild type Pa cultures and in Pa cultures where *intP* was deleted.

- We hypothesize that *intP* regulates pyocyanin production in Pa.

## Experiment

- We engineered a strain of Pa that overexpresses IntP (IntP+++).

## Results

- When *intP* was overexpressed, production of pyocyanin was enhanced (fig. 3).

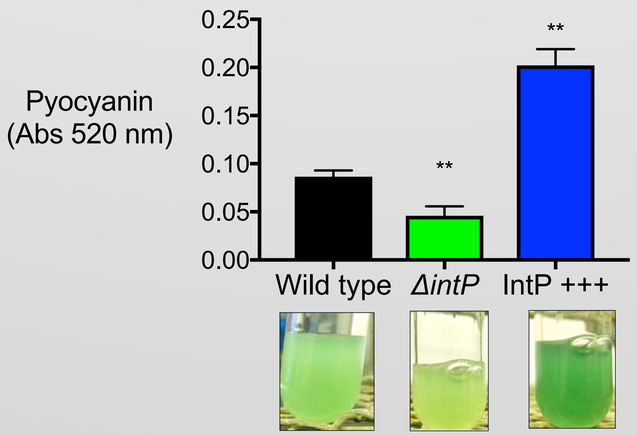


Fig. 3: Pyocyanin was chloroform extracted from the indicated cultures and quantified using absorbance. Results are mean +/- SD of three experiments. **p<0.01.

- These results suggest that *intP* regulates pyocyanin.

## Future directions

- One possible explanation for *intP*-dependent pyocyanin production is that IntP integrates Pf genetic elements into genes involved in pyocyanin production.
- To test this idea, we inactivated the integrase activity of IntP by introducing the point mutation Y380F producing $IntP^{Y\text{-}F}$.
- $IntP^{Y\text{-}F}$ over expression did not result in enhanced pyocyanin production (Fig. 4).

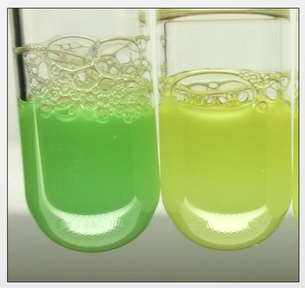


Fig. 4: Representative images showing pyocyanin production (green) in Pa strains overexpressing wild type *intP* (*intP+++)* or the *intP* point mutant ($IntP^{Y\text{-}F}$+++).

- Future work will focus on how the integrase IntP regulates pyocyanin production in Pa.

## Conclusions

- The Pf phage integrase IntP enhances production of the virulence factor pyocyanin
- The integrase activity of IntP is required for pyocyanin production.

## Why is this important?

- The World Health Organization recently categorized Pa as a priority pathogen of the greatest risk to human health.
- We need new ways to combat Pa infections.
- Understanding how Pf phage regulate virulence factor production by Pa may reveal new therapeutic strategies, which in turn could save lives.