Apr 27th, 11:00 AM - 12:00 PM

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).

**Hypothesis**

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

**Experiment**

- We hypothesize that intP regulates pyocyanin production in Pa.

**Results**

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

**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<sup>Y-F</sup>.
- IntP<sup>Y-F</sup> over expression did not result in enhanced pyocyanin production (Fig. 4).

**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.

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

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

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.