Evaluating pathogenicity of *Pseudomonas aeruginosa* by manipulating genes associated with motility and biofilm formation

Makayla Hicks and Regina McGrane
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Abstract

Pseudomonas aeruginosa **is an opportunistic pathogen and causes infections in the lungs of cystic fibrosis patients. Motility and biofilm formation are large contributing factors to the ability of *P. aeruginosa* to cause infection, and these processes have been found to be modulated by multiple biological molecules including rhamnolipid, a biosurfactant produced by RhlA, WspA, a protein predicted to have a role in surface sensing, and MotB and MotD, proteins that contribute to the flagella stator complex. In this work, we evaluated the impact of the deletion genes encoding for expression of these factors on the pathogenicity of *P. aeruginosa*. We hypothesize that loss of these motility and biofilm factors will cause *P. aeruginosa* to be less pathogenic. This hypothesis was tested by inoculating *Galleria mellonella l*arvae, a wax moth recently developed as a model for understanding bacterial pathogenicity, with either wild type or mutant cultures. The survivability, coloration, movement, and silk formation of the larvae were evaluated each day until all the larvae died or for seven days, whichever occurred first. This required optimization of bacterial concentrations to ensure that the bacteria did not kill the larvae immediately and so that differences between wild type and mutant cultures could be detected. Differences between the wild type and mutants with deletions in *rhlA*, *wspA*, *motB* or *motD* were detected in the silk cocoon formation and movement of the larvae, suggesting these mutations impair the ability of *P. aeruginosa* to induce disease. Understanding the role of motility and biofilm formation in the pathogenicity of *P. aeruginosa* in wax moth larvae could lead to a greater understanding of how this bacterium causes infection in humans and ultimately could lead to a solution for problematic infections.**

Pseudomonas aeruginosa motility and biofilm formation

Pseudomonas aeruginosa **is abundantly found in nature but is also an opportunistic pathogen that causes nosocomial infections by colonizing the epithelial cells of the respiratory tract through flagella, pili and lipopolysaccharides (Kipnis et al., 2006). In this study, the role of motility and biofilm formation factors were evaluated for a role in pathogenicity. Motility is possible through flagella and pili; these structures also aid in the initial attachment of *P. aeruginosa* to the host cell. Biofilm formation is important because it acts as a shield for the bacteria from the immune system and antibiotics (Fig. 1A). Mutants the following genes were studied: *rhlA, wspA, motB and motD.***

- ***rhlA:*** **encodes RhlA which works with RhlB to produce rhamnolipids which aid in shielding biofilms and in swarming motility (Soberón-Chávez et al., 2005).**
- ***wspA:*** **endcodes WspA a membrane–bound surface sensor that is involved in initiating the Wsp complex involved in regulating biofilm formation (O'Conner et al., 2012).**
- ***motB:*** **encodes for MotB which associates with MotA to form a flagellar stator complex that regulates flagella torque and anchors to the cell wall (Fig. 1B) (Doyle et al., 2004).**
- ***motD:*** **encodes for MotD which forms second stator complex with MotC. (Doyle et al., 2004).**

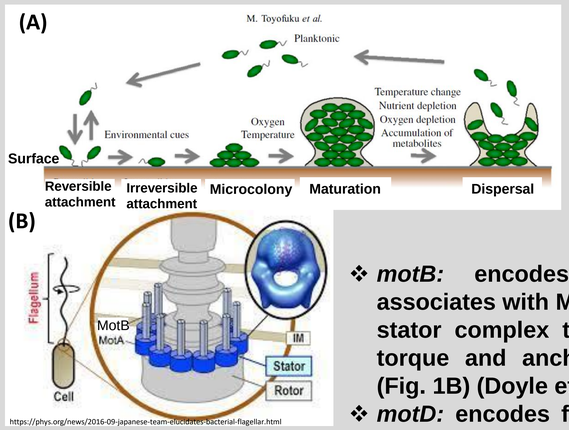


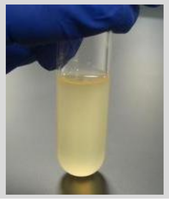
Figure 1. Biofilm and flagellar stator complex. (A) Formation of a biofilm and (B) structure of the flagella showing the stator complex.

Galleria mellonella as a model for studying human pathogens

Figure 2. *Galleria mellonella* larva.

Galleria mellonella **is a useful model organism for studying bacterial pathogenesis. This is due to the relatively large size of the larvae, the ability of the larvae to live in temperatures that represent a human environment, and the presence of an innate immune system (Ramarao et al., 2012). This organism is also inexpensive and easy to handle. These organisms do not pose as much of an ethical threat compared to mammalian and other vertebrate models.**

Experimental Methods for investigating the role of RhlA, WspA, MotB, and MotD in *P. aeruginosa* pathogenicity



Step 1: Cultures of wild type and mutant strains were inoculated in 5 ml of King's B media and incubated with shaking overnight @ 37°C.

Step 2: 5-10 wax moth larvae each were transferred into 3 square petri plates for each strain 24 h prior to injection.

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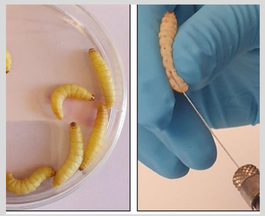
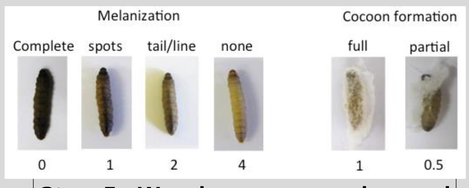
Step 3: Cultures were washed twice with 1 ml of sterile DI water. Optical density was determined using a spectrophotometer, and cultures were normalized to consistent concentrations



Step 4: Starved wax moth larvae were injected with 10 μl of wild type or mutant culture. Cultures containing 10^8, 10^7, and 10^6 cells/ml were tested. Cultures containing 10^8 and 10^7 cells/ml killed all the moths within 24 h following injections, so cultures were normalized to concentrations of 10^6 cells/ml for all future studies. Picture from Jemel *et al*, 2020.



Step 5: Wax larvae were observed each day until all the larvae died or for seven days, whichever occurred first. The number of dead and discolored larvae were recorded for each plate. The number of moving larvae and those able to form a silk cocoon were also recorded. Picture from: Champion *et al*, 2018.

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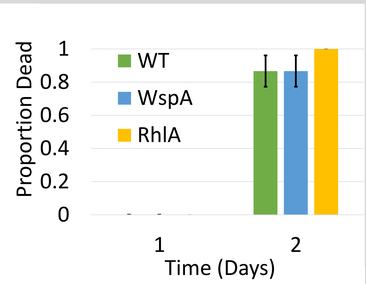
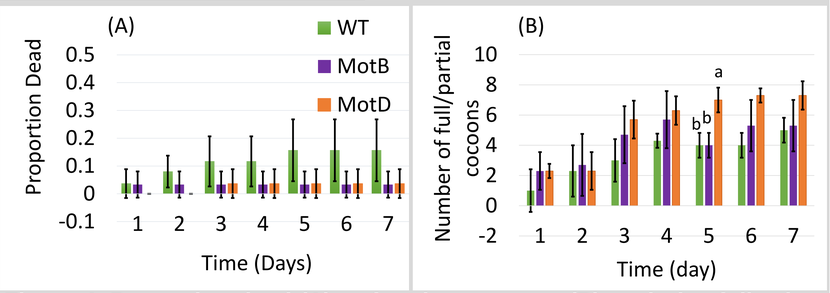


- **None of the mutants differed significantly (Single Factor ANOVA) from the wild-type in their ability to kill wax moth larvae (Fig 3 and 4A).**

Figure 3. Proportion dead each day following injection with wild type or *wspA* or *rhlA* mutants. Error bars represent standard deviation (n=3).



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- **Overall, some differences in the behavior of the larvae were observed. The larvae infected with the *motD* mutant appeared to produce more full cocoons and other silk than any of the other strains (Fig. 4B). The larvae infected with the *motB* mutant had more movement compared to the other groups (data not shown).**
- **Wax larvae inoculated with wild type *P. aeruginosa* appeared to be more lethargic and the most discolored compared to any of the mutant groups.**

Discussion and Future Directions

So far, this study has yielded some promising results. Based on observations about cocoon formation and motility, the deletion of *motB* and *motD* genes may decrease the pathogenicity of *P. aeruginosa.* Quantitative studies will be done to confirm these observations. Studies on other genes related to motility and biofilm Collectively, these experiments contributes our understanding of how *P. aeruginosa* infects, which may lead to the development of methods for preventing and treating these deadly infections.

Bibliography

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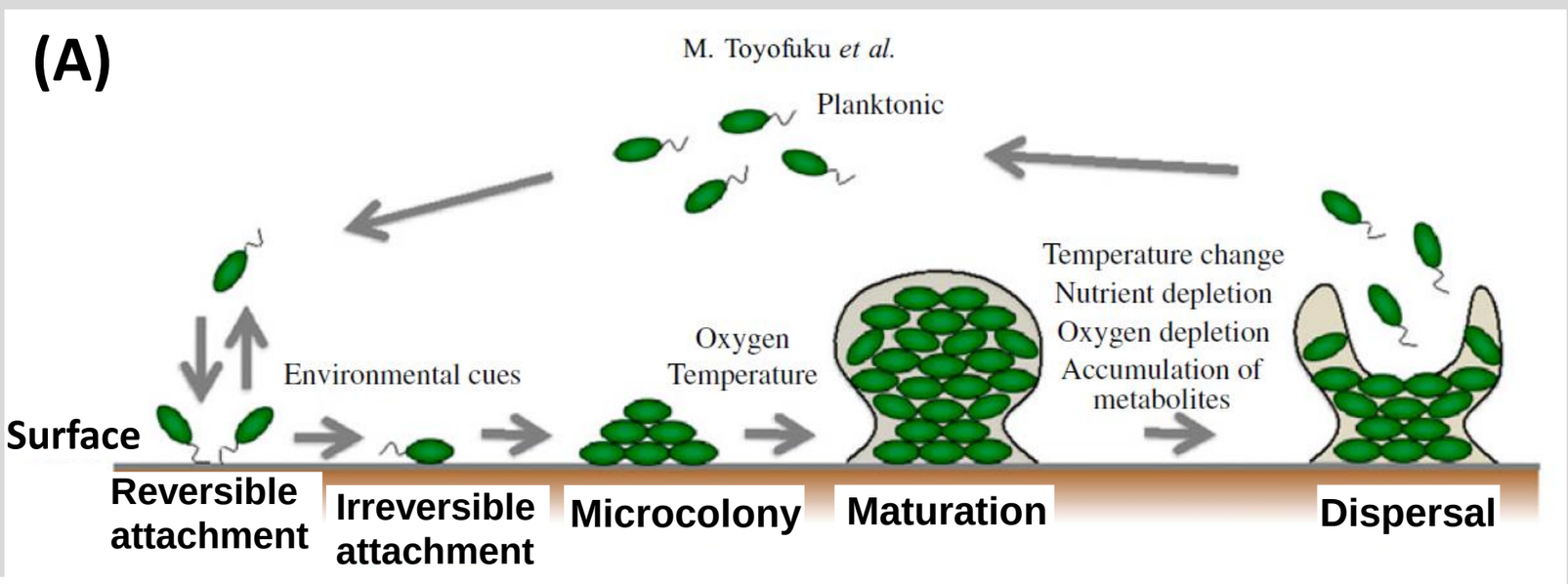

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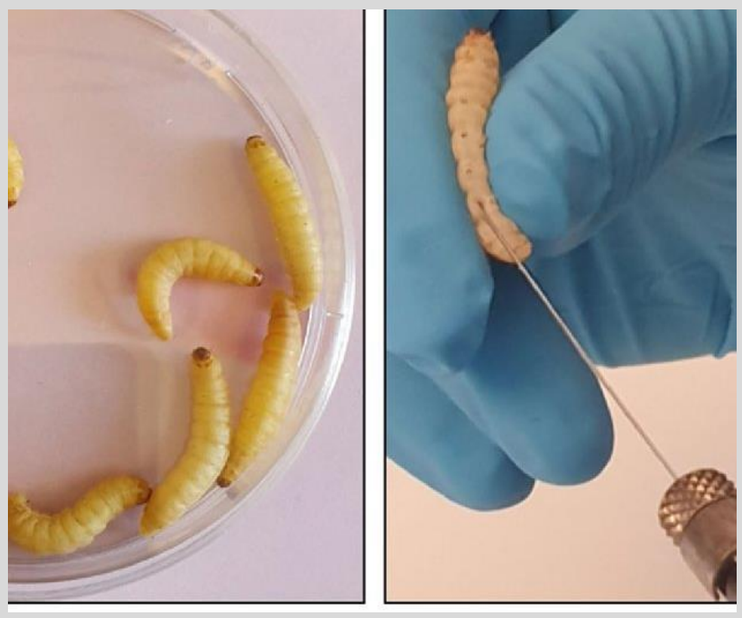

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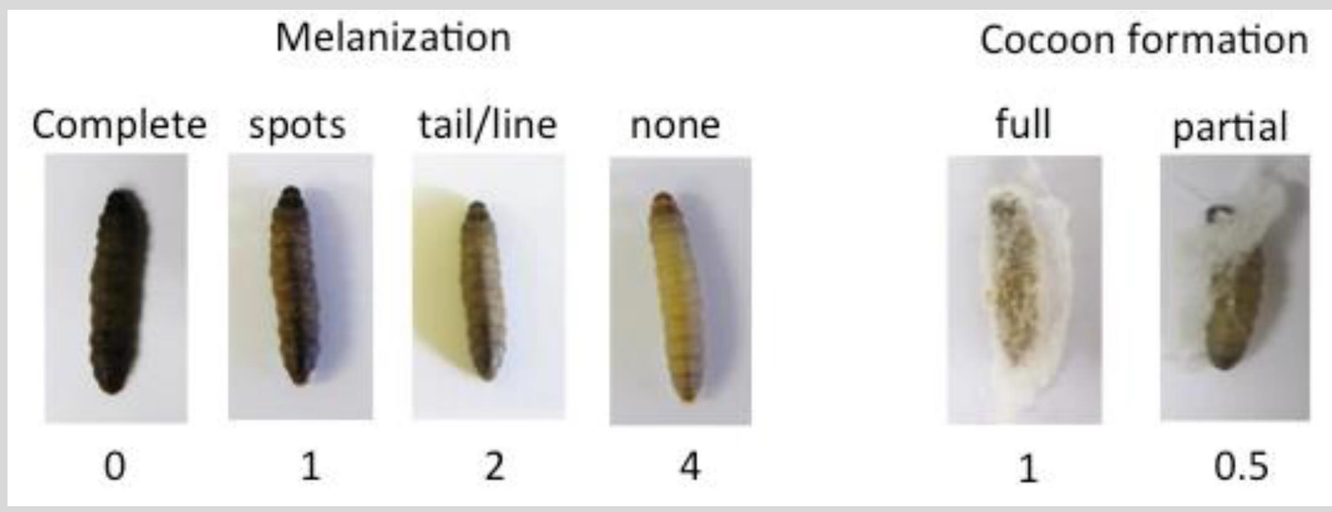

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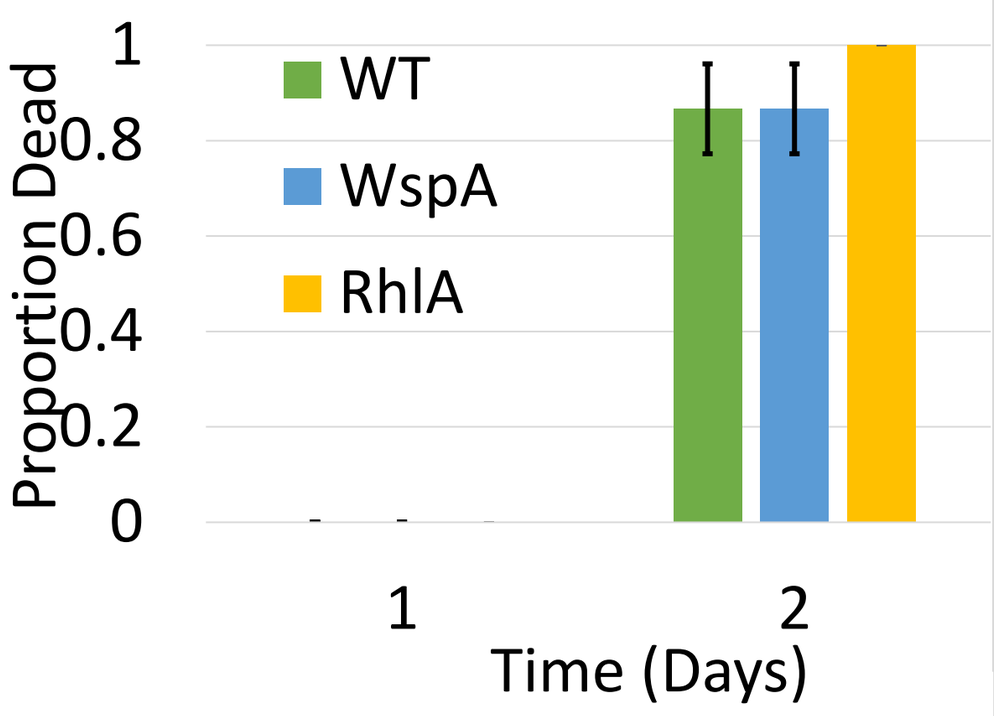

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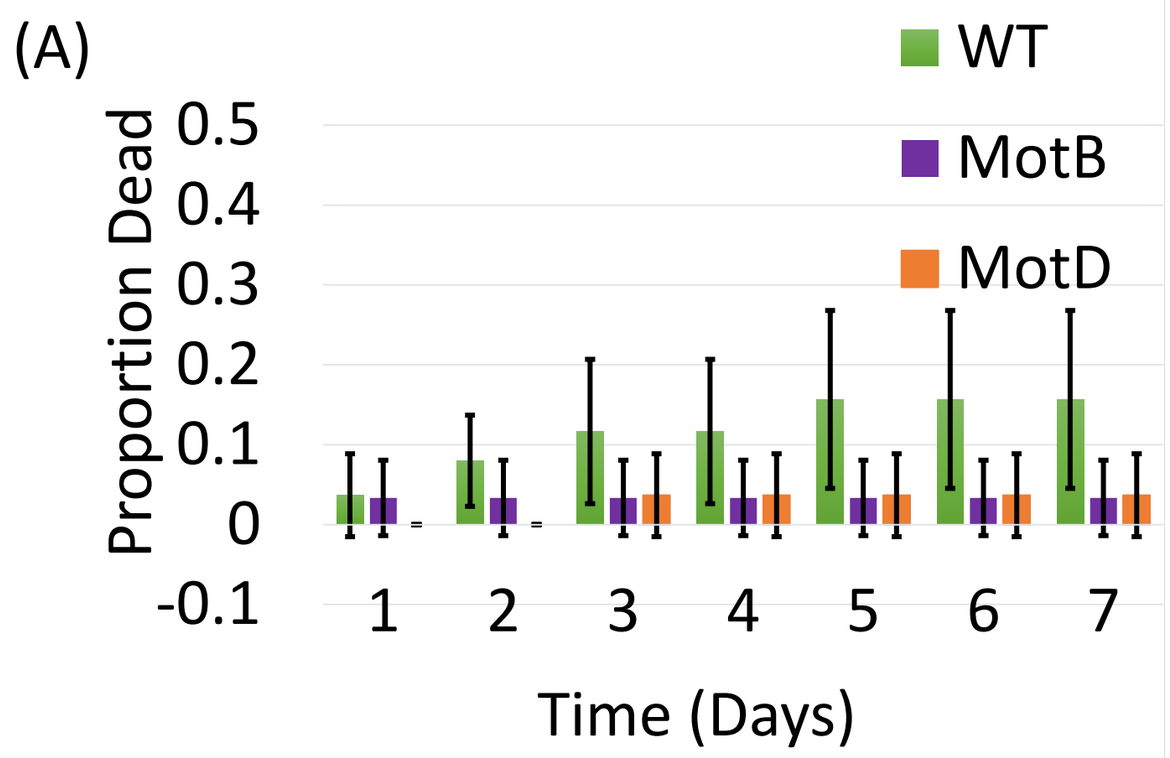

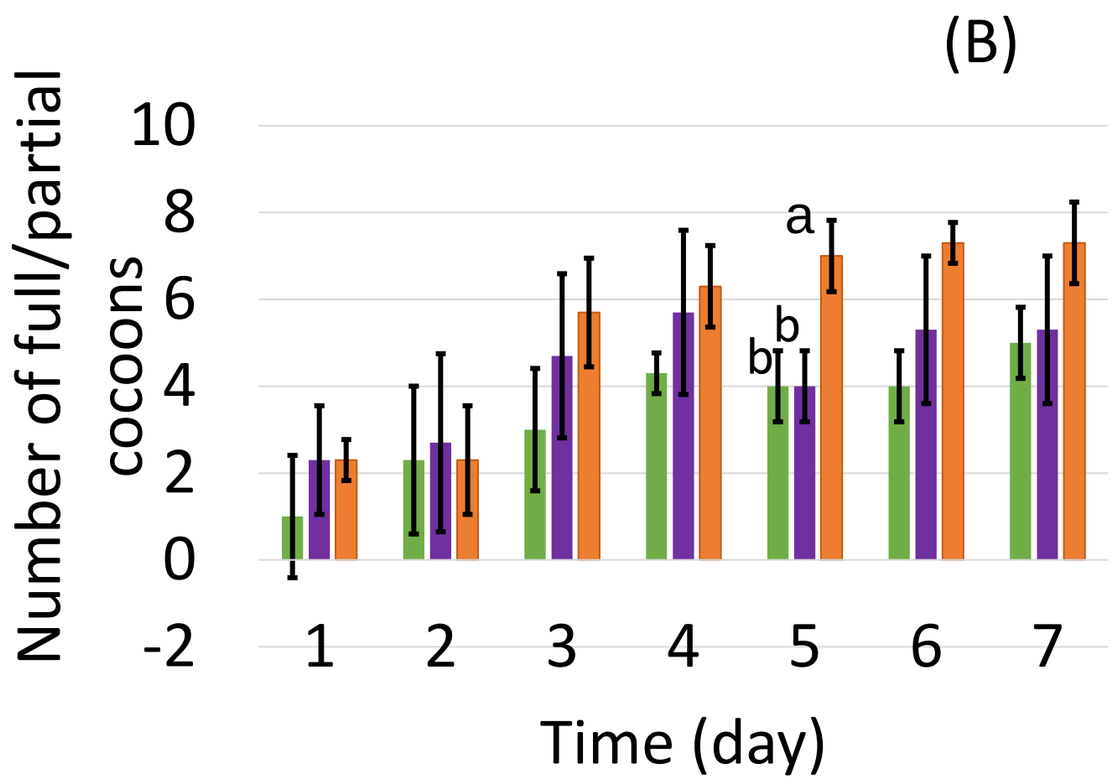

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