MirA's Impact on Motility and Biofilm Formation in Agrobacterium Tumefaciens

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Introduction

After adhering to both biotic or abiotic surfaces, microorganisms, most frequently bacteria, and their secretions come together to create social groups called biofilms. Biofilms are pervasive and can be located across various ecosystems.

Although biofilms serve different purposes, many biofilms are very destructive, and account for nearly $4,000 billion per year. Very common examples of biofilms, include plaque that grows on teeth, which leads to causing tooth decay and periodontal disease, and bacteria that causes lung infections in Cystic Fibrosis patients, which require antibiotics.

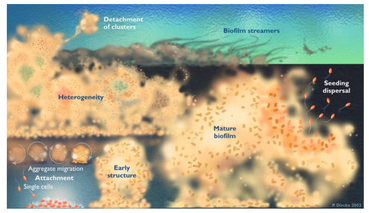


Figure 1: Clusters in Biofilm Formation

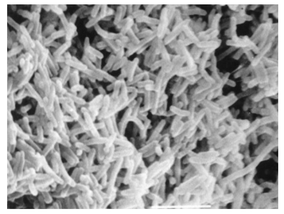


Figure 2: Picture of biofilm bacteria cells

Flagella Motility

- Flagella are hairlike structures that enable movement and chemotaxis in a bacterium cell
- The foundation of the flagella serves as a rotary motor, allowing the flagella to revolve and drive the bacterium forward
- Flagella mobility is relevant in biofilm formation, because biofilms need to swim out to find the optimal conditions for survival

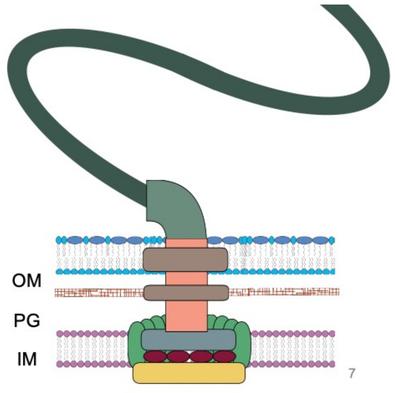


Figure 3: Model of flagella structure

Agrobacterium tumefaciens

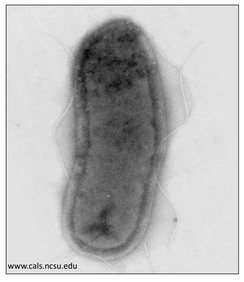


Figure 4: Agrobacterium cell

Figure 5: Tree with crown gall disease

- Gram negative rod-shaped plant pathogen
- Soil microbe and discretionary pathogen
- Causative agent of Crown Gall disease
- Model organism for biofilm formation

Motility and Regulation in Agrobacterium

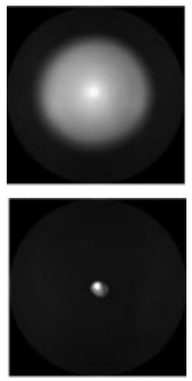


Figure 8: Comparison of increased vs. decreased bacterial mobility

Aim: examining how the assertion of MirA in Agrobacterium Tumefaciens affects the bacteria's ability to swim

-Over a gradient of time, the diameter of the rings the bacteria forms while swimming will be quantified

-It is hypothesized that increasing MirA will lead to a decrease in swimming mobility, due to it binding with Rem, and inhibiting mobility gene expression, as shown in figure 6 below.

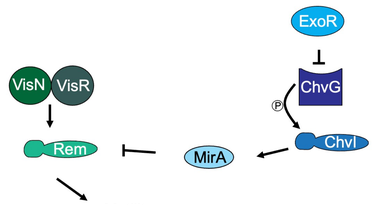
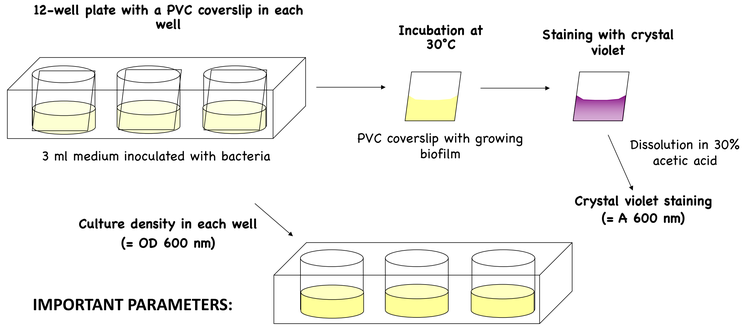


Figure 6: flagella pathway

Biofilm Assay Method

Aim: examining how the assertion of MirA and IPTG onto on Agrobacterium Tumefaciens affects the bacteria's ability to form a biofilm



IMPORTANT PARAMETERS:

1. Cell density of planktonic culture (in the wells) = $OD_{600\ nm}$
2. Crystal violet staining (biofilm formation on the coverslips) = $A_{600\ nm}$
3. Normalized biofilm quantification = $A_{600\ nm}/OD_{600\ nm}$ (Biofilm score)

The Effect of MirA on Biofilm Formation

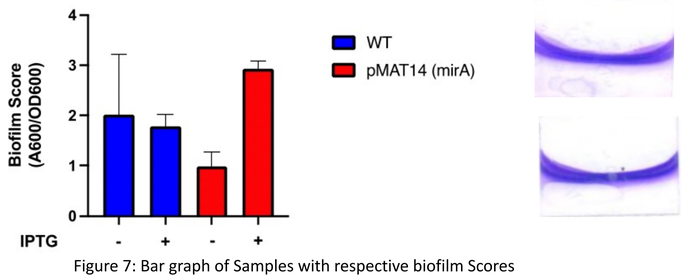
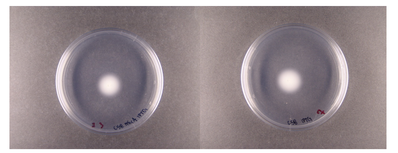


Figure 7: Bar graph of Samples with respective biofilm Scores

- WT does not show a significant difference in biofilm score compared to WT IPTG, suggesting that IPTG is not important for biofilm formation, without the assertion of MirA
- Expression of pMAT14 (MirA) results in poor biofilm formation.
- Addition of IPTG increases MirA expression, resulting in an increase of biofilm formation for pMAT14 (MirA)

The Effect of MirA on Swimming Mobility

WT pMAT14(MirA)



WT* pMAT14(MirA) *

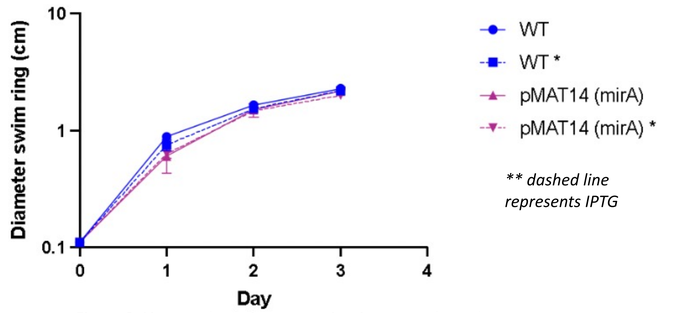


Figure 8: Line graph of diameter swim rings over three days

- WT swam further than pMAT14 (MirA), suggesting MirA decelerates swimming mobility in Agrobacterium.

Conclusion and Future Directions

Conclusion:

- MirA inhibits swimming mobility, but over time a suppressor can occur and mask the effect of MirA
- Low expression of MirA causes a decrease in biofilm formation; however, high expression of MirA stimulates biofilm formation.
- MirA is more complex, than previously realized, and there is more to be discovered about the effects.

Future Directions:

- Testing the effect of expressing a gradient amount of MirA on biofilm formation and mobility in Agrobacterium Tumefaciens, to determine the type of correlation between the amount of MirA expressed and the biofilm score.

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References

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