Eric Quach

CREATES Analysis Template

Descriptive Study

Figure or Table Number: 1

"Official" title for this figure or table (from the caption):

Moron expression can affect bacterial growth rates

My (simplified, decoded, in regular language) title for this figure or table:

How phage morons impact bacterial growth rates

If we compare panel(s)/column(s) From the bottom left **and** The panel on the bottom right **, we learn about:**

That most of the phage moron had no impact on bacterial growth. Only moron JBD30-14 and JBD26-15 and an effect on strain PA14 while PAO1 was only affected by moron JBD44-8. JBD26-15 greatly reduced the growth rate of PA14 while JBD3014 only slightly decreased growth rate of PA14. JBD44-8 also slightly lowered the growth rate of PA01.

When we make these comparisons, we conclude from this figure:

That most phage morons have no discernible effect on bacterial growth.

Was the hypothesis supported? Why or why not?

The hypothesis was not supported as most of the phage morons did not alter bacterial growth rate with the exceptions of morons JBD26-15, JBD 30-14, and JBD44-8. All of these

reduced the growth rate.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

- Should have measured growth rate of both PA14 and PAO1 without phage infection as baseline. Instead, researchers simply stated that the phage morons had no effect on the growth curve.

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Free Response

Figure or Table Number: 2

"Official" title for this figure or table (from the caption):

Phage morons increase resistance to further phage infection

My (simplified, decoded, in regular language) title for this figure or table:

Table showing moron conferring resistance to superinfections

Analysis of the figure or table:

Figure shows a table with each strain of *P. aeruginosa* infected by a different phage. The *P. aeruginosa* is then exposed to the phage listed on the top of the table. Strains that are resistant to superinfection are dark blue, while light blue is partial resistance, yellow meaning no resistance, and gray meaning the phages were unable to infect wild-type PAO1. Phage morons JDB24-4, JBD26-61, JBD26-5 all have considerable dark squares meaning they confer resistance to superinfection to both strains (except for JBD26-5 conferring more resistance to strain PAO1).

When we analyze the figure or table, we conclude:

Almost all phage morons confer resistance to superinfections, except some provide a lot more resistance to bacteria than other phage morons. Most notably, JBD44-8 providing the least as it provided no resistance to PA14 and only partial resistance to 4 strains to PAO1.

Was the hypothesis supported? Why or why not?

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The hypothesis was supported for most of the phage moron as the majority provided both strains with resistance to subsequent infections. However, one phage moron JDB44-8 provided little to no resistance to both strains.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

- The use of a control was never stated, however it was implied. The gray squares indicated that the phages used for superinfecting *P. aeruginosa* could not infect PAO1 wild-type. This implies that they did look at these phages infecting wild-type strains of PAO1 and PA14 as a control, however since it was never explicitly stated the use of a control cannot be assumed.

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Experimental Test

Figure or Table Number: 3

"Official" title for this figure or table (from the caption):

Moron expression can cause changes in bacterial twitching motility, swimming motility, and biofilm production.

My (simplified, decoded, in regular language) title for this figure or table:

Effect of moron expression on bacterial motility and biofilm production.

The controls in this experiment are:

The strains of PAO1 and PA14 that contained knockouts of pilA, and fliC genes which results in no flagella and type IV pili.

They are represented (in which part of the chart or graph, or what figure panels?)

They are represented by the column labeled ΔpilA or ΔfliC with pilA representing the pilus gene and fliC representing the flagella gene.

The experimental conditions are:

Fig 3a: The distance traveled by twitching measured in diameter traveled by strains PAO1 and PA14 that are infected phage morons.
Fig 3b: The distance traveled by swimming measured in diameter traveled by strains PAO1 and PA14 that are infected by phage morons.
Fig 3c: The level of biofilm formation measured as OD595 by PAO1 and PA14 that are infected by phage morons

They are represented as:

In all figures, the distance or level of biofilm formation is represented by black bars for PA14 and grey bars for PAO1.

Fig 3c. I-iii: Represented as either black strands for SDS PAGE or as a black blob for Western Blot

Fig 3c i-iii: SDS-PAGE and Western Blot that assays for presence of flagella and pili within wild-type PAO1 and PAO1 that has been infected by phages.

We need to compare the controls in Fig 3a-b **with the experimentals in** Fig 3a-b **to find out:**

Infection of phage morons in both strains of *P. aeruginosa* reduces the twitching and swimming capabilities of both strains.

We need to compare the controls in Fig 3c **with the experimentals in** Fig 3c **to find out:**

Phage moron expression decreases biofilm production in most instances, with the exception of JBD23-13 which drastically increased biofilm production within PAO1 but drastically reduced it within PA14.

When we make these comparisons, we conclude from this figure:

Phage morons inhibit twitching motility by inhibiting the production of pili and flagella on the bacteria. Since biofilms often require the motility granted by flagella and pili, most of the bacteria infected from the phage demonstrated reduced biofilm formation.

Was the hypothesis supported? Why or why not?

The hypotheses were somewhat supported as most phage morons had an decreased twitching motility, but some decreased a lot more than others. Similarly, the swimming motility also supported the hypothesis in that most of the bacterial strains demonstrated a small decrease in swimming motility, but not by a huge factor except for JD44-8 and JD26-15. The Western Blot and SDS-PAGE confirmed that the reduction in motility was attributed to the loss of either pili or flagella. The biofilm experiment hypothesis was also supported in that some strains of bacteria demonstrated lower levels of biofilm formation, but some strains were mostly unaffected. The anomaly of JBD23-13 should be

noted as it drastically increased the biofilm forming capabilities of PAO1 while drastically reducing it for PA14.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

- A possible mechanism should be proposed to explain why JBD23-13 drastically increased biofilm production levels.

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Experimental Test

Figure or Table Number: 4

"Official" title for this figure or table (from the caption):

Phage morons lead to changes in phenotypes linked to virulence

My (simplified, decoded, in regular language) title for this figure or table:

Phage morons cause physical changes related to virulence

The controls in this experiment are:

The knockout of the rmlC gene, wbpM gene, and the survival rate of *D. melanogaster* when injected with wild-type PA14. Also the wild-type PAO1 for the rhamnolipid production assay. No negative control in Fig 4c.

They are represented (in which part of the chart or graph, or what figure panels?)

KO of rmlC is represented in Fig 4a by "ΔrmlC". The PAO1 wild-type control is the top left square in Fig 4a. KO of wbpM is represented by "ΔwbpM". Survival rate of *D. melanogaster* is represented by the dark blue line in Fig 4d.

The experimental conditions are:

Fig 4a: Each of the strains of PAO1 and PA14 that are infected by their respective phage moron.

Fig 4b: The PAO1 and PA14 strain that has been infected by the JBD30-9 moron.

Fig 4c: The PAO1 and PA14 strains that have been infected by different phage

They are represented as:

Fig 4a: The 3 squares from the right on the top half of the figure and the 3 squares from the left from the bottom half of the figure.

Fig 4b: The 3 columns from the left in both gels.

Fig 4c: The black bars represent PA14

morons that are measured for the production of elastase. Fig 4d: The PA14 strain that was infected by JBD44-8 and injected into *D. melanogaster*.	strains while the gray bars represent PAO1 strains. Fig 4d: The gray line represents the survival rate of flies injected with PA14 containing JBD44-8.

We need to compare the controls in Fig 4a **with the experimentals in** Fig 4a **to find out:**

That there is decreased rhamnolipid production in strains infected by phage morons.

We need to compare the controls in Fig 4b **with the experimentals in** Fig 4b **to find out:**

The long O antigen that is normally present on bacteria is altered by the phage morons.

We need to compare the controls in Fig 4d **with the experimentals in** Fig 4d **to find out:**

There is an increased killing rate in PA14 strains that are infected with the JBD44-8 moron.

When we make these comparisons, we conclude from this figure:

There is an overall decrease in rhamnolipid production, alteration to the O antigen, no change in elastase production, and an overall no change in killing rate in vitro in strains infected with phage morons with the exception of PA14 infected by JD44-8.

Was the hypothesis supported? Why or why not?

The hypothesis that involved a change in rhamnolipid production was supported as all the moron genes tested involved a decrease in rhamnolipid production. Similarly, the O antigen hypothesis was also correctly predicted as the O antigen demonstrated alteration through the SDS-PAGE missing part of a band compared to the wild-type control O antigens. The overall lack of a change in elastase production did not support the hypothesis as the levels of production did not deviate much from normal levels.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

- The researchers should have had a KO mutation of the gene that is responsible for producing elastase as a negative control. They had done this for similar prior experiments.

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Experimental Test

Figure or Table Number: 5

"Official" title for this figure or table (from the caption):

Phage morons are actively expressed from the lysogen

My (simplified, decoded, in regular language) title for this figure or table:

Morons are continuously expressed within bacteria

The controls in this experiment are:

The wild-type PA14 strain, the PA14 strain infected with JBD26.

They are represented (in which part of the chart or graph, or what figure panels?)

Top two rows of the Fig 5b.

The experimental conditions are:

PA14 infected with JBD26 with a deletion mutation at gene 61 and PA14 infected with JBD26 with a deletion mutation at gene 31 and 61.

They are represented as:

The bottom two rows of Fig 5b.

We need to compare the controls in Fig 5b **with the experimentals in** Fig 5b **to find out:**

That both genes JBD26-31 and JBD26-61 are responsible for conferring resistance to superinfection within PA14.

We need to compare the Levels of gene expression **to find out:**

Which genes are expressed while the prophage remains dormant.

When we make these comparisons, we conclude from this figure:

That JBD26-31 and JBD26-61 are expressed in high levels even with the repressor being expressed and that knockout of these genes will make the bacteria more susceptible to superinfection.

Was the hypothesis supported? Why or why not?

The hypothesis was overall supported as the phage moron genes that are expressed at a high level, indicating some kind of functionality, demonstrated that it conferred resistance to subsequent infections. This supports the hypothesis as it was predicted that moron genes expressed at high levels while the phage was a prophage most likely protected its host from other infections.

The following issues are ones of concern to me (these can be things you don't understand, or criticisms of the method, questions for the authors, or anything else that comes to mind):

- They should have performed more knockout mutations to see if they could pinpoint other phage morons that were important in resisting other phage infections.