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Prophage-host interactions: Lifting the curtain on Pseudomonas' puppet masters

Prathyusha Vishwanathan

Humans have been aware of Bacteriophages as major players in the microbial world for over 100 years. Their most widely recognized feature is their ability to infect and kill specific bacteria, but they are also known to provide some beneficial characteristics to their bacterial host. Apart from a few famous examples, this aspect of phage biology has been largely neglected and the temperate phage-bacteria relationships are not fully understood. Dr.Chloe James, a senior lecturer in Medical microbiology at the University of Salford, has been very curious about this dynamic and has been working on this for a while.

Recently, Dr. James started a new project funded by the BBSRC, which aims to observe how bacteriophages affect the behaviour of their bacterial host. It will focus on a notorious opportunistic bacterium, Pseudomonas aeruginosa, a common cause of respiratory infection in Cystic Fibrosis patients.

Prathyusha Viswanathan interviews Dr Chloe James about her unique and interesting project:

It is quite a different yet interesting topic. How did it all start?

A few years back, I worked on a project examining cases of Cystic fibrosis patients who were chronically infected with the Liverpool Epidemic Strain of Pseudomonas aeruginosa. The team had previously found this stain to cause much more severe disease than other strains and to spread from patient to patient, which seemed very unusual.

After sequencing the genome, they found several never-seen-before co-existing prophages. This is where I came in. I regularly monitored phage and bacteria in the patient's sputum samples for over 2 years. The most notable finding was that these phages were always active and present in abundance. But we could not find any association between the phages and patient condition or antibiotic treatment.

So, this got me extremely curious, like wow, even though the phages are actively killing their bacterial host, the bacteria are still keeping hold of them which means that they must be helping the bacteria in some manner and this point made me determined to find out what they are actually doing. So, I worked with colleagues [Dr. Ian Goodhead and Dr. Heather Allison] to design a project that would better understand how the phages and bacteria affect eachother's biology, the hypothesis being that temperate bacteriophages do so much more than what we know and at the moment. I think that they pull all kinds of strings and regulate bacterial behavior in different ways.

The BBSRC awarded us funding for 2 postdoctoral researchers to work full time on the project, but we also have some linked projects that are being explored by research students at Salford and Liverpool University.

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What achievements have you so far?

Some key findings of our work are that for one, each of the LES phages seems to be affecting the fitness of the P. aeruginosa host differently and depending on environmental conditions; secondly, together, these temperate phages seem to facilitate rapid evolution of their bacterial host contributing to their adaptation to the CF lung environment; lastly, we think that phages may have an important role in the competitiveness of the LES in CF lungs by acting as anti-competitor weapons (killing other P. aeruginosa strains). But this new project will delve much deeper into the mechanisms of interaction between these intriguing microbial partners. We have published a lot of this work.

These are two complex creatures; you may have noticed many interactions – have any been particularly unexpected or peculiar?

Yes, that's true. We are uncovering all kinds of interesting nuggets to follow up on. So far, the most striking discovery is the evidence that the phages are interacting with each-other. This means that the bacteria behave differently depending on which phage they are infected with, and in cases when multiple prophages co-exist together, we observe a completely different behavior. Of course, we also suspect that the bacteria is affecting the biology of the phages. Seeing how the phage infection progresses differently in other P. aeruginosa host strains has helped me direct my thinking in more broader dimensions.

What has been the most recent outputs from the project?

Grace Plahe, a Salford MRes student, presented her work on how LES phages affect bacterial growth and virulence at three different conferences last year. One of her abstracts has been published, and some of her preliminary data helped us to secure the bigger project funding we have now. Since then, two postdoc researchers have been employed on the project, and they will present their preliminary findings on phage-phage interactions and phage genome annotation at the next Microbiology Society annual conference.

What are the upcoming stages of research?

So, our latest funding is to run for 3 years and there are plenty of upcoming tasks on our list. Firstly, our aim is to thoroughly profile the infection cycles of each phage under a range of environmental conditions, and monitor changes in the expression of genes that reports the key stages of the process. We will then conduct a huge transcriptomics experiment which will map global gene expression of bacteria with and without their phage partners. This will show which phages are regulating which bacterial genes and vice-versa.

We will also perform experiments by exposing the bacteria to both favorable as well as challenging conditions, so that this will help us to identify why the bacteria is keeping hold of so many elements that could so easily kill it and most importantly, we will also construct a series of mutants and perform functional assays to confirm our theories about how these phage puppet masters are pulling the strings.

It seems that your findings have a wide scope in research and could help thought processes in other fields, leading to new technologies; yet temperate phage research is rare and has not yet been given much importance. What do you have to say about this?

Very true, I agree. This aspect of phage research has not been given much attention. Most of the research is concentrated on the destructive nature of bacteriophages towards specific bacteria for developing antibacterial treatments. There is a lot of renewed excitement in that area at the moment, with real potential to improve treatment of infections caused by antibiotic resistant bacteria. Whilst the beneficial effects of prophages on bacteria have not been ignored, the scale of this has been grossly underestimated.

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There is so much more left undiscovered. There are relatively few published findings on the regulatory properties of temperate phages and yet there is a huge amount of genome evidence to show us that ~60% of the sequenced bacterial strains carry prophages in their genomes, and even more tantalisingly, over 70% of most prophage genes are of unknown function. We know they are present everywhere, and that bacteria are keeping hold of them even though they can present a considerable cost, but we don't know what they are doing!

I am sure there are many exciting crossroads for this type of research, and you were absolutely correct, this type of research would also help other research fields to understand the pathology of disease better and could inform completely novel approaches to patient treatment and management. In fact, CRISPR systems were actually invented by bacteria, in order to protect them against bacteriophage attack! So, studying phage- bacteria interactions can indeed trigger revolutionary new fields of research and I hope that some of our findings will lead to new thinking in all kinds of areas that I haven't even considered.

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