Investigating a novel antibiotic in plant stomata

This summer I had the opportunity to work in the lab of Professor Julie Gray at the University of Sheffield. Here I investigated the role of an enzyme that is almost exclusively expressed in guard cells. Previous studies have reported that plants lacking the enzyme are compromised in the ability to defend themselves from bacterial pathogens. The focus of my project was to understand how this enzyme is acting to increase resistance to bacterial infection.

The first aim of the project was to understand whether the gene restricts bacterial entry by helping the stomata to shut, or whether the gene acts to restrict bacterial growth once bacteria have entered the leaf. To do this we compared wild type plants with knockout plants under two distinct styles of bacterial inoculation. The first style of inoculation was a spray inoculation to measure the number of bacteria naturally entering the leaf. The second was a syringe inoculation where a known amount of bacteria is forced into the leaf, thus bypassing the stomata and the natural entry process. During the infection assays the leaf bacteria was extracted and colony counts were at made 4 and 24 hours for spray inoculation and 0, 24 and 72 hours for syringe inoculation. Results showed no significant differences in leaf bacteria between wild type plants and knockout plants for spray inoculations suggesting that the enzyme does not restrict bacterial entry into the leaf. Syringe inoculation experiments showed a small increase in the number of bacteria in the knockout plants at later time points, suggesting that these plants are partially more susceptible to infection. Interestingly although the number of bacteria extracted from knockouts was only slightly higher than what was extracted from wildtype plants, the knockouts showed significantly more infection symptoms 5 days post infection.

To understand how the enzyme is regulated during plant bacterial interaction we performed RT-qPCR across a time series following plant infection (0, 4 and 24 hours) using both virulent and avirulent pathogens. Our results conclusively showed that gene transcription was induced at 4 hours upon infection with both the virulent and avirulent pathogen. After 24 hours the results were more interesting. Gene expression levels of plants exposed to the virulent pathogens were significantly lower than basal gene expression levels, whilst gene expression levels in the avirulent pathogen treatment were significantly higher than basal gene expression levels, and significantly higher than the gene expression levels seen in the bacterial treatment at 4 hours post infection. Our results show the plant upregulates enzyme transcription upon detection of bacteria. At 24 hours the virulent bacteria supress the gene activity whereas the avirulent bacteria are not capable of suppressing gene activity. These results imply that the enzyme plays a direct role in pathogen defence as the plant upregulates it upon infection and the bacteria target the gene to suppress its activity.

Further work will focus on synthesising the enzyme in *E. coli* and identifying its substrate. From this we can try to work out its direct effect on bacterial growth and infection. It will also be important to repeat the infection experiments described above and track bacterial growth at later time points to further understand how the enzymes effects bacterial growth *in planta*.

I thoroughly enjoyed my summer project and now feel confident heading into my third year project with an enhanced laboratory technique and an interest for plant pathology. I'd like to thank the BSPP for my funding, as without it my project would not have been possible, Professor Julie Gray and my supervisor Christian Dutton for guiding me throughout my time in the lab.

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