Recognition of structurally related but sequence divergent pathogen effector proteins in *Magnaporthe oryzae* by distinct host NLR receptors

The rice blast fungus *Magnaporthe oryzae* causes devastating losses in one of our most important global crops. Some rice plants confer resistance by binding pathogenic effector proteins with NLR immune receptors. The exact molecular mechanisms of interaction by the wide range of effectors and their associated NLR receptors remains unclear, and understanding these specific processes could lead to methods for increased pathogen resistance in rice crops. I spent this summer working in Mark Banfield's lab at the John Innes Centre, which investigates the structural and biochemical properties of the proteins involved.

Recently, members of the Banfield lab found the crystal structure for the effector AVR-PikD bound to an integrated heavy metal associated (HMA) domain from the rice NLR Pik-1. Another HMA-domain containing NLR, RGA5, binds to two effectors – AVR-Pia and AVR1-CO39. These three effector proteins are sequence divergent but structurally similar, and therefore it would be valuable to elucidate the differences in the specific molecular interactions between these effectors and HMA domains.

To this end, a chimeric protein of AVR1-CO39 and AVR-PikD was generated. Curiously, analytical gel filtration showed that both AVR1-CO39 and the chimera bound to Pik-HMA, but *in planta* assays in *Nicotiana benthamiana* showed no immune response. The aim of my project was to confirm these results and purify potential complexes of AVR1-CO39/chimera with Pik-HMA using heterologous expression in *E.coli* and affinity chromatography with gel filtration. This could then be used in crystallisation to understand the structural binding properties.

Interestingly, preliminary analytical gel filtration results showed no binding of AVR1-CO39/chimera with Pik-HMA, and *in planta* assays by agroinfiltration in *N. benthamiana* confirmed no immune response. Repeat analytical gel filtration was done for AVR1-CO39 with Pik-HMA, which likely showed a complex the second time. Unfortunately there was no time for repeat analytical gel filtration of the chimera with Pik-HMA, and future investigations will have to be done to elucidate these qualitative binding properties. Circular dichroism was also performed for AVR1-CO39, with the results still to be analysed. This will provide additional understanding of the structure of this protein compared to AVR-PikD.

Co-expression of AVR1-CO39/chimera and Pik-HMA was attempted but purification of complexes was unsuccessful. Co-expression will have to be repeated to confirm insufficiency of this method. A third method of purification was also attempted, in which AVR1-CO39 and Pik-HMA were mixed together after tag cleavage and run on a gel filtration column. This method was more promising, but no complex was purified. In the future, it would be good to optimise these methods, as purified complexes would enable structural studies using crystallisation, paving the way for engineering NLRs able to recognise a wider range of effectors.

During my 8 weeks here, I have learned a lot about different biochemical methods and gained appreciation for how they can be used to solve practical problems in plant-pathogen interactions. It has been an inspiring summer, and I would like to thank Freya Varden my supervisor, all of the Banfield lab, and the BSPP for providing me with this excellent opportunity.

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