The structural and binding characteristics of sHMA proteins

sHMA proteins are found in rice and are thought to function as negative regulators of plant immunity. They are the putative targets of the AVR-Pik effectors produced by the rice blast pathogen *Magnaporthe oryzae*. The interaction between the rice sHMA, sHMA1, and the AVR-PikD effector appears to stabilise the sHMA1 protein, which could lead to a less severe pattern-triggered immunity response and so increased survival of *Magnaporthe oryzae*. Over 8 weeks during this summer, I worked in Mark Banfield's lab at the John Innes Centre to investigate the structural and biochemical characteristics of sHMA proteins. The aims of the project were to investigate which AVR-Pik effectors bind to an alternative rice sHMA, sHMA2, attempt to crystallise an AVR-Pik/sHMA2 complex, and characterise the metal binding capabilities of sHMA proteins. In completing these objectives, I hoped to provide insight into the mechanism of suppressing host immunity by the rice blast pathogen.

The initial aims were to express and purify the sHMA2 protein, which is similar to sHMA1 with 74 % sequence identity, and then undertake analytical gel filtration of the sHMA2 protein mixed with AVR-Pik effectors to qualitatively investigate binding. This showed that different alleles of the AVR-Pik effector could form complexes with sHMA2. These were promising results, but further repeats remain to be conducted.

I had the time to show that one of the AVR-Pik alleles can form a complex with sHMA2 by co-expression in *E. coli*, and be purified at a sufficient yield to allow crystallisation experiments. These trials are ongoing, with the proportion of precipitation indicating the concentration range is conducive to crystallisation, which will hopefully occur after a more significant time period has passed. Any structural information gained from these trials could be used in further studies designed to disable effector binding to sHMA2, which ultimately has potential to reduce the virulence of the pathogen on rice.

I then tested the hypothesis, derived by collaborators, that sHMA proteins bind specific metal ions, by designing an *in vitro* metal binding assay. As part of this assay I tested a number of different divalent metal ions for sHMA1 binding. Interestingly, the results suggest sHMA1 binds strongly and specifically to certain divalent metal ions, whereas only non-specific, limited binding to other metal ions was observed. In the future, the assays I developed will hopefully help test related proteins such as sHMA2 and the integrated HMA domains present in NLR protein of rice that bind AVR-PikD.

During the 8 weeks, I have increased my knowledge of techniques such as crystallisation and developed a greater understanding of the molecular interactions between plants and pathogens, which I hope to take forward into future studies. I therefore thank the BSPP for the generous funding to provide me with this opportunity, and the members of the Banfield lab for an excellent summer.

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