

## 10-week Summer Studentship opportunity available at the BBSRC funded Institute Rothamsted Research, Herts, UK

### Exploring the DNA sequence diversity of fungal genes predicted to contribute to the virulence of plant pathogens

**Supervisors:** Jason Rudd, Alex Amaral and Kim Hammond-Kosack (Wheat Pathogenomics team)

Two globally important Ascomycete pathogens of bread wheat (*Triticum aestivum*), namely *Fusarium graminearum* (*Fg*) and *Mycosphaerella graminicola* (*Mg*), cause disease by initially invading the intercellular spaces of plant tissue. For both species, the fungal 'within season' life cycle is subsequently completed via asexual sporulation in association with dead host tissue, i.e. by a non-biotrophic lifestyle (1-4).

Full genome sequence information is available for both species (5, 6). At Rothamsted, the wheat pathogenomics team have been recently using various bioinformatics analyses to define the *Fg* and *Mg* genes that code for small secreted proteins. Then by using a next generation sequencing approach, the fungal transcriptome expressed within a few days of wheat infection has been determined for both species. Using these data sets we have been able to predict the set of early *in planta* induced small secreted fungal proteins (i.e the early secretome of each species). Some of these small secreted proteins are anticipated to be the effectors contributing to fungal virulence (7).

In this summer project, the appointed student will explore the sequence diversity of many of the genes coding for these early *in planta* expressed small fungal genes. Some of these sequences are shared by *Fg* and *Mg*, whilst others are not. This analysis will be done by using nine differential isolates available for *Mg* and a range of *Fg* isolates from various geographical locations and mycotoxin chemotypes (8, 9). The initial experiment will involve PCR amplification of the gene sequence from each isolate using a DNA template to identify qualitative genetic differences among isolates (10). Then the PCR products amplified will be analysed using the high resolution melt (HRM) technique to identify in a high throughput manner the subset of gene sequences exhibiting significant polymorphism (11). Direct sequencing of the PCR products will be used to define the exact DNA sequence changes and to predict the amino acid. These sequencing results will then be analysed using various software algorithms to predict the evolutionary path(s) which lead to the sequence diversity observed. The number of synonymous to non-synonymous substitutions in each gene sequences will also be determined to identify the domains undergoing positive selection. For a few sequences where either extreme polymorphism or no polymorphism is observed, linearly amplified template DNA already prepared from numerous samples collected from the Broadbalk classical wheat experiment over the past 150 years, and which are known to be positive for *Fg* or *Mg* (12), will be used to explore gene sequence diversity at the fungal population level.

During this project, the appointed student will learn a range of molecular biology techniques as well as how to use various software algorithms to explore gene evolution. Towards the end of the student's stay at Rothamsted, he or she will be given the opportunity, if they so wish, to give an oral presentation on their findings to the wheat pathogenomics team at their weekly laboratory meeting. The results from this project will form the basis of a future publication and will also help the two supervisors to select candidate genes for functional analyses using a reverse genetics approach.

#### References:

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2. Beacham AM, Antoniw J and Hammond-Kosack KE (2009) A genomic fungal foray. The Biologist 56, 98-105.
3. Keon J, Antoniw J, Carzaniga R, Deller D, Ward JL, Baker JM, Beale MH, Hammond-Kosack KE and Rudd JR (2007) Transcriptional adaptation of *Mycosphaerella graminicola* to programmed cell death (PCD) of its susceptible wheat host. Mol. Plant-Microbe Interact 20, 178-193.
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5. Cuomo et al., (2007) The genome sequence of *F. graminearum* reveals localized diversity and pathogen specialization. Science 317, 1400-1402.  
[http://www.broadinstitute.org/annotation/genome/fusarium\\_verticillioides/MultiHome.htm](http://www.broadinstitute.org/annotation/genome/fusarium_verticillioides/MultiHome.htm) and <http://mips.helmholtz-muenchen.de/genre/proj/fusarium>
6. *Mycosphaerella graminicola* genome portal <http://genome.jgi-psf.org/Mycgr3/Mycgr3.home.html>
7. Walton JD, Avis TJ, Alfano JR, Gijzen M, Spanu P, Hammond-Kosack K and Sanchez F (2009) Effectors, Effectors et encore des Effectors: The XIV International Congress on Molecular-Plant Microbe Interactions, Quebec. Molecular Plant Microbe Interactions 22, 1479-1483
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9. Lowe RGT, Allwood JW, Galster A, Urban M, Daudi A, Canning C, Ward JL, Beale MH and Hammond-Kosack KE (2010) A combined <sup>1</sup>H NMR and ESI-MS analysis to understand the basal metabolism of plant pathogenic *Fusarium* species. Mol. Plant-Microbe Interact 23, 1605-1618.
10. Rudd JJ, Antoniw J, Marshall R, Motteram J, Fraaije B and Hammond-Kosack KE (2009) Identification and characterisation of *Mycosphaerella graminicola* secreted or surface-associated proteins with variable intragenic coding repeats. Fungal Genetics and Biology 47, 19-32.
11. Hofinger BJ, Jing HC, Hammond-Kosack KE and Kanyuka K (2009) High-resolution melting analysis of cDNA-derived PCR amplicons for rapid and cost-effective identification of novel alleles in barley. Theoretical and Applied Genetics 119, 851-865.
12. Beachell SJ, Fraaije BA, Shaw MW and Fitt BDL (2005) Wheat archive links long-term fungal pathogen population dynamics to air pollution. Proc Natl Acad Sciences USA 102, 5438-5442.

### How to apply?

Interested undergraduate students, in the middle year(s) of their degree course, should contact in the 1<sup>st</sup> instance either Dr Jason Rudd ([jason.rudd@bbsrc.ac.uk](mailto:jason.rudd@bbsrc.ac.uk)) or Prof Kim Hammond-Kosack ([kim.hammond-kosack@bbsrc.ac.uk](mailto:kim.hammond-kosack@bbsrc.ac.uk)).

The intention is that the student selected by **early March**, will then apply for funding from The Genetic Society. The application form must be submitted to the Genetics Society by **31<sup>st</sup> March 2011**.