Functional genomics and mycotoxin discovery in the wheat glume blotch pathogen *Stagonospora nodorum*

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***Stagonospora nodorum* is a fungal pathogen of wheat and is responsible for over $100 million in yield losses in Australia each year[1,2]. Significant progress has recently been made in understanding how *S. nodorum* causes disease on wheat. These pathogens, known as a necrotrophs, were thought to secrete a battery of lytic and degradative enzymes during infection. These enzymes would simply degrade host tissue, allowing the infecting pathogen to feed off the lysed cellular contents. Recent studies have shown that this is not so, and that these fungi secrete unique effector proteins during the early stages of infection, which appear to be translocated into wheat cells. Once inside, these proteins interact (either directly or indirectly) with the products of dominant susceptibility loci leading to a localised programmed cell death response. Consequently, it is through an intricate gene-for-gene mechanism involving the interaction of pathogen effector proteins and host dominant susceptibility genes that *S. nodorum* infects its wheat host, not through a crude secretion of cell lysis enzymes. These findings have been recently reviewed by Oliver and Solomon[3]. This short article focuses on how modern functional genomics techniques have been exploited to reveal a new dimension to the wheat pathogen *S. nodorum*.**

Along with the study of these effector proteins, my laboratory, together with Professor Richard Oliver at Curtin University, has a strong interest in understanding how the pathogen sporulates. *Stagonospora nodorum* is a polycyclic pathogen, meaning that it must undergo multiple rounds of asexual sporulation in the field for the infection to affect yield. As part of the dissection of this developmental process, we identified that cAMP-dependent signalling was required for sporulation[1]. To identify exactly what the cAMP-signalling pathway was controlling, we compared the proteomes of wild-type and mutant strains of the fungus lacking an intact cAMP-signalling pathway. Multiple proteins were identified that were either increased or decreased in abundance in the cAMP-signalling mutant strains[4]. One such protein was *Sch1*, an unknown short-chain dehydrogenase that was only present in the wild-type. The role of the *Sch1* was interrogated by disrupting the endogenous copy through homologous recombination and characterising the resulting mutants. The *Sch1* mutants displayed altered vegetative growth when grown *in vitro* and appeared unable to differentiate mature pycnidia (macro bodies containing the asexual pycnidiospores). This was confirmed by subsequent histology was showed that the *Sch1* mutant pycnidia appeared immature and resulted in only a very poor yield of viable spores.

Consequently this strategy identified that *Sch1* was required for sporulation and was also positively regulated by the cAMP-signal transduction pathway. However, we still had little idea of the role of *Sch1* during sporulation. To address this, and given that *Sch1* encoded a protein possibly involved in metabolism (a short-chain dehydrogenase), we compared the metabolome of the *Sch1* mutant to that of the wild-type strain. Metabolomics is the latest in the omics suite of tools to be embraced by the functional genomics revolution. Metabolomics is the measure of the metabolome, which in turn is defined as the complement of metabolites present in a system at a specific point of time under a given set of conditions. For the comparison of the *Sch1* mutants to that of the wild-type, a gas chromatography-mass spectrometry (GC-MS) technique was used. GC-MS is a popular method used to analyse small polar metabolites (for example, the primary metabolome). In our study, only one metabolite was found to be significantly different when comparing the *Sch1* mutants and the wild-type (Figure 1). A compound that eluted at 45.57 minutes was approximately two orders of magnitude more abundant in the *Sch1* mutant than in the wild-type. A comparison of the fragmentation profile of this compound to publicly available databases tentatively identified it as alternariol[5]. This tentative identification was confirmed by further mass spectral analysis (MS/MS) of the putative alternariol to a purchased standard.

So what is alternariol? Alternariol is a mycotoxin produced predominantly by *Alternaria alternata*, a post-harvest pathogen of cereals and fruits. Alternariol has demonstrated carcinogenic and toxigenic properties and is considered harmful to human health[6]. This is a particularly significant finding as alternariol is the first mycotoxin identified in *S. nodorum*. *Stagonospora nodorum* has long been known as a cause of significant yield losses, but never as a potential health hazard. This finding has now shown that *S. nodorum* harbours the metabolic potential to be able to synthesise at least a mycotoxin and possibly others. Studies are now ongoing to determine the prevalence of alternariol in Australian wheat fields, its mechanism of synthesis in *S. nodorum* and whether or not *S. nodorum* and other related fungal pathogens synthesise other harmful mycotoxins.

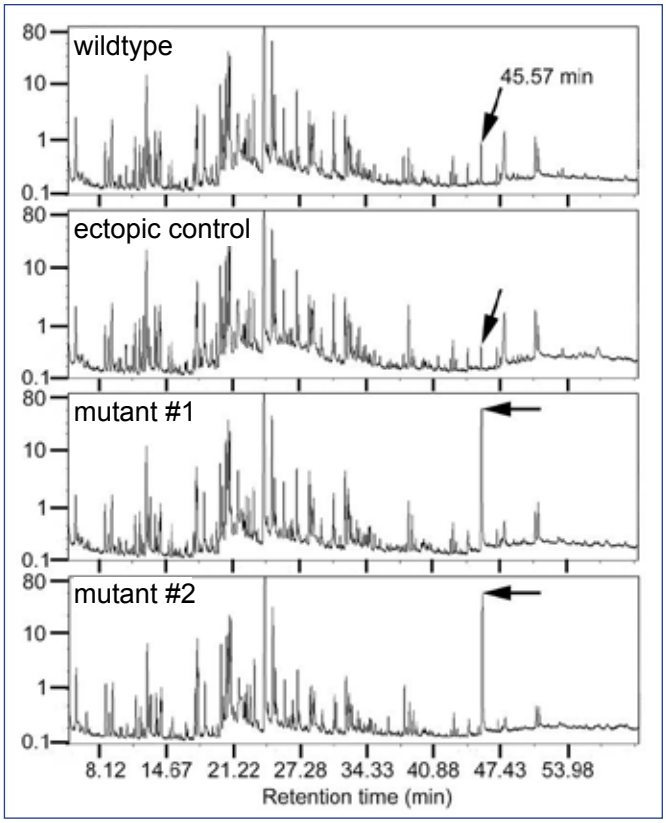

Figure 1. GC-MS total ion chromatograms (TIC) of polar extracts from *S. nodorum* strains harbouring Sch1 (wild-type and ectopic control) and also of mutant isolates (mutants #1 and #2).The y-axes are in a log-scale. The elution of RT4557 (alternariol) is marked with an arrow.

References

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Biography

Dr Peter Solomon completed his undergraduate degree and subsequent PhD studies at The University of Queensland investigating the role of molybdenum-containing enzymes in the photosynthetic bacterium *Rhodobacter capsulatus*. In 1998, he undertook a postdoctoral position at the Carlsberg Laboratory in Denmark investigating the nutritional basis of the tomato-*Cladosporium fulvum* interaction. In 2000, he moved to the Australian Centre for Necrotrophic Fungal Pathogens located at Murdoch University in Perth to further investigate fungal-plant interactions using the *Stagonospora nodorum*-wheat interaction. In 2008, Dr Solomon accepted a laboratory leader position in the Research School of Biology at The Australian National University, where he continues to investigate the molecular basis of necrotrophic fungal wheat diseases.

Microbial communities in Antarctic lakes: Entirely new perspectives from metagenomics and metaproteomics

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Driven by advances in DNA sequencing technologies, an astounding amount of data is being generated from genetic material sourced directly from the environment, and this exponential growth of data is set to continue. By surmounting the challenges of working with such vast datasets, a whole new level of understanding is being gained about microbial diversity, microbial evolution and whole ecosystem function. For precious, pristine and logistically difficult to obtain Antarctic samples, metagenomic and metaproteomic approaches are providing the basis for fundamental new discoveries about how Antarctic systems function.

The application of new technologies capable of large-scale