Polyketide synthesis

in

Stagonospora nodorum

Dipl. Biol. Christian Krill

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There is a theory which states that if anybody ever discovers exactly what the Universe is and why it is here, it will instantly disappear and be replaced by something even more bizarre and inexplicable.

There is another theory which states that this has already happened.

- Douglas Adams
DECLARATION

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

______________________________
Christian Krill
ACKNOWLEDGEMENTS

nani gigantum humeris insidentes

-Bernard of Chartres

I would like to express my sincere gratitude and appreciation towards everybody who helped me along this epic journey, the destination of which not only being this thesis, but what still lies ahead. As a scientist, I am standing on the shoulders of giants, and you have helped me to widen my horizon far beyond what I ever thought possible.

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Then, there are three particularly colourful characters that came along the way with me: KC, Master of Rackets, Proteins and Photography; Joel, the externalised extension to my own Id; and Garth, the Obi Wan Kenobi of MS, Music and all things SciFi. I can only think of one word that describes the importance of every single one of you to me - Friend!
Finally, to my parents and to Eva, you deserve special thanks. You are the constants in my life, my resting place. Without your support in all these years, your love and understanding, I would not have accomplished anything. You brought me this far, together we will go much, much further still.
ABSTRACT

*Stagonospora nodorum* is a necrotrophic fungal pathogen of wheat and related grasses. It is the causal agent of *Stagonospora nodorum* blotch of wheat, a major disease causing upwards of 100 million dollars (AU$) damage in yield loss per annum.

*Stagonospora nodorum* is a member of the Dothideomycete class of filamentous ascomycetes. Within this class are numerous plant pathogens that rely on secondary metabolite (SM) phytotoxins of the polyketide class as pathogenicity and/or virulence factors. While the production of proteinaceous host specific toxins has been studied extensively in *S. nodorum*, the role of secondary metabolites in the pathogenic lifecycle of this fungus is completely unexplored.

In this study a combination of bioinformatics, molecular biology and analytical chemistry techniques are used to investigate polyketide synthesis in *S. nodorum*. *In silico* analysis of polyketide synthase (PKS) gene and protein sequences was used to catalogue and classify the PKS repertoire of *S. nodorum*, assign putative functions to PKS genes based on homology, identify PKS gene clusters and elucidate the phylogenetic history of PKSs in *S. nodorum*. Transcriptomics techniques were used to identify genes active during important stages of the pathogenic lifecycle as candidates for targeted gene deletion experiments. The role these genes play in host colonisation and disease progression was analysed using knockout mutagenesis and *in vitro* and *in planta* characterisation of mutant strains. Secondary metabolite extraction and LC-MS analysis techniques were evaluated to identify key compounds produced by the wild type fungus that were differentially abundant in the knockout mutants.
With this approach, a highly conserved alternative melanisation pathway gene cluster involving the putative DHN-melanin synthase *MEL1*, a putative oxidoreductase and a putative transcription factor has been identified. Further findings highlighted a rapid evolution and plasticity of PKS genes in *S. nodorum*. Knockout mutants for the *SMS1*, *PKS1* and *PKS3* polyketide synthase genes have been generated and were tested for defects in pathogenicity, metabolism and sporulation. No significant differences to the wild type were detected, indicating a menial role for these genes during pathogenesis. An SPE based method for isolating SM from culture filtrate was developed and used to identify compounds produced by *S. nodorum* in liquid culture, as well as two putative polyketide products absent in cultures of the *SMS1* knockout mutant putatively linked to a cryptic mycotoxin pathway.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>US/AU$</td>
<td>US/Australian Dollar</td>
</tr>
<tr>
<td>(g/c)DNA</td>
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</tr>
<tr>
<td>(m)RNA</td>
<td>(messenger) Ribonucleic acid</td>
</tr>
<tr>
<td>(q)PCR</td>
<td>(quantitative) Polymerase Chain Reaction</td>
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<tr>
<td>[M+H/Na]$^+$</td>
<td>Proton/Sodium adduct ion</td>
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<td>Acyl Carrier Protein (domain)</td>
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<td>Alternariol Monemethylether</td>
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<td>AMU</td>
<td>Atomic Mass Unit</td>
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<td>Complete Supplement</td>
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<td>cv</td>
<td>cultivar</td>
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</tr>
<tr>
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<td>Formic Acid</td>
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<tr>
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<td>Fumonisin B1</td>
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<td>f.sp.</td>
<td>forma specialis</td>
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<td>gas chromatography</td>
</tr>
<tr>
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<td>liquid chromatography</td>
</tr>
<tr>
<td>(l)N₂</td>
<td>(liquid) Nitrogen</td>
</tr>
<tr>
<td>LPA</td>
<td>Latent Period Assay</td>
</tr>
<tr>
<td>m</td>
<td>Milli-</td>
</tr>
<tr>
<td>M</td>
<td>molar, also: Mega-, Molecular Ion</td>
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<td>m/z</td>
<td>mass per charge ratio</td>
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<tr>
<td>MAPK</td>
<td>Mitogen Activated Protein Kinase</td>
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<td>min</td>
<td>minute(s)</td>
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<tr>
<td>mQ H₂O</td>
<td>double distilled (MilliQ®) Water</td>
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<td>6-methylsalicylic acid</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
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<tr>
<td>n</td>
<td>Nano-</td>
</tr>
<tr>
<td>NRPS</td>
<td>Non-ribosomal peptide synthase</td>
</tr>
<tr>
<td>p.a.</td>
<td>per year (per annum)</td>
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<td>pathogen associated molecular pattern</td>
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<tr>
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<td>Polyketide Synthase</td>
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phleo  phleomycin
psi  pounds per square inch
Rif  Rifampicin
RBH  reciprocal best hit
RP(18)  Reverse Phase (octadecyl silica)
rpm  rounds per minute
RT  retention time, also: Reverse Transcriptase/Transcription, Room Temperature
s  second(s)
SM  secondary metabolite
SPE  Solid Phase extraction
stdev  standard deviation
TE  Thioesterase (domain)
(q)TOF  (quadrupole) Time-of-flight
V  Volt
v/v  volume per volume
V8  Campbell’s V8 Juice
WPS  Whole Plant Spray
YMG  Yeast Extract, Malt Extract, Glucose Medium