# Comparative functional proteomics of MAP Kinase signalling in *Magnaporthe oryzae*

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I certify that all the material in this thesis which is not my own work has been identified and that no material has been previously submitted and approved for the award of a degree by this or any other University.

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# Abstract

Rice blast disease is caused by the heterothallic ascomycete fungus *Magnaporthe oryzae* and is one of the most severe diseases of cultivated rice throughout the world. The *PMK1* (Pathogenicity Mitogen-activated protein Kinase) gene in *M. oryzae* has been identified to play diverse roles during pathogenesis-related development. *PMK1* regulates appressorium formation and infectious hyphal growth. *PMK1* is functionally related to *Saccharomyces cerevisiae FUS3* and *KSS1* MAPK genes which regulate the transcription factor Ste12. The *STE12* homologue in *M. oryzae*, *MST12*, has also been identified and is known to be essential for appressorium mediated penetration and infectious growth. These observations imply that Pmk1 regulates a diverse set of targets important in both the initiation of appressorium development and the subsequent stages of invasive growth. In addition, the Mst12 transcription factor may function downstream of Pmk1 to regulate genes involved in appressorial penetration and infectious hyphal growth. We have used a comparative proteomic study between Guy11 (isogenic wild type), Δ*mst12* and Δ*pmk1* mutants to understand which genes are induced during appressorium formation and may be regulated by Mst12 and Pmk1. We carried out two-dimensional gel electrophoresis with proteins extracted from conidia that had germinated on a hydrophobic surface after 6h, 12h, 16h and 24h of incubation. *M. oryzae* underwent major changes in protein abundance and expression during the first 6 hours of spore germination in Guy11 which suggested that much of protein synthesis associated with appressorium morphogenesis and virulence occurs precociously during conidium germination on rice leaf surface. More than 394 differentially expressed proteins during conidium germination and appressorium formation have been identified by mass spectrometry. We confirmed many proteins already known as determinants of pathogenicity in *M. oryzae* such as enzymes involved in melanin biosynthesis or fatty

acid β-oxidation. However, we also identified proteins absent or lowly induced in the *Δpmk1* and *Δmst12* mutants involved in cell wall reorganisation, secondary metabolism, lipid metabolism, photomorphogenesis or found as proteins of unknown functions. We generated 28 targeted gene deletion mutants to test the putative function of proteins predicted by proteomics analysis to be associated with appressorium development. We obtained 7 mutants showing a significant reduction in virulence and confirmed importance of regulated proteolysis during appressorium development but also elucidated new processes involved in infection such as the phosphoinositide pathway and three proteins of unknown function.

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