

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

Submitted by Romain Huguet

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Romain Huguet

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), $\Delta mst12$ and $\Delta pmk1$ 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.

Table of contents

	List of Figures	11
	List of Tables	17
	Acknowledgements	19
	Abbreviations	21
1.	General Introduction	24
1.1	Why investigate the molecular basis of fungal pathogenicity	24
1.2	Role of cell signalling in the pathfinder fungus	
	<i>Saccharomyces cerevisiae</i>	28
1.2.1	Cyclic AMP signalling in <i>S. cerevisiae</i>	28
1.2.2	MAPK signalling in <i>S. cerevisiae</i>	33
1.2.2.1	MAPK module	33
1.2.2.2	Pheromone response pathway in <i>S. cerevisiae</i>	35
1.2.2.3	Filamentous growth pathway in <i>S. cerevisiae</i>	38
1.2.2.4	High osmolarity/glycerol pathway in <i>S. cerevisiae</i>	49
1.2.2.5	Cell wall integrity pathway in <i>S. cerevisiae</i>	42
1.2.2.6	Spore wall assembly pathway in <i>S. cerevisiae</i>	44
1.3	The economic significance of rice blast disease	45
1.4	The life-cycle of <i>Magnaporthe oryzae</i>	46
1.5	Role of cell signalling in pathogenic development by <i>M. oryzae</i>	49
1.5.1	Cyclic AMP Signalling in <i>M. oryzae</i>	49
1.5.2	MAPK signalling in <i>M.oryzae</i>	53
1.6	General introduction to the research aims of this thesis	56

2.	Materials and Methods	57
2.1	Growth and maintenance of fungus stocks	57
2.2	Pathogenicity and infection-related development assays	57
2.2.1	Plant infection assays	57
2.2.2	Penetration assay	58
2.2.3	Assays for measuring rates of conidial germination and appressorium formation	58
2.3	Nucleic acid analysis	59
2.3.1	Fungal DNA extraction	59
2.3.1.1	Large-scale extraction of fungal genomic DNA	59
2.3.1.2	Small-scale extraction of fungal genomic DNA	60
2.3.2	DNA manipulation	61
2.3.2.1	Digestion of genomic DNA with restriction enzymes	61
2.3.2.2	DNA gel electrophoresis	61
2.3.2.3	The polymerase chain reaction (PCR)	62
2.3.2.4	Gel purification of DNA fragments	62
2.3.2.5	Southern blotting	62
2.3.2.6	Radio-labelled DNA probe construction	62
2.3.2.7	Hybridisation conditions	64
2.3.3	Fungal transformations	65
2.4	Microscopy	66
2.4.1	Microscopic analysis using the Confocal-Laser Scanning Microscope (CLSM)	66
2.4.2	Microscopic analysis using the Zeiss Axioskop 2 microscope	67
2.4.3	Microscopic analysis using the Olympus IX81 microscope	67
2.5	Proteomic methods	67

2.5.1	Protein extraction	67
2.5.2	Protein quantification	69
2.5.3	2-Dimensional gel electrophoresis: IEF and SDS PAGE	69
2.5.3.1	Isoelectric focusing (1st dimension)	71
2.5.3.1.1	Precautionary steps	71
2.5.3.1.2	pH gradient choice	71
2.5.3.1.3	Equilibration of IEF gels	72
2.5.3.1.4	IEF migration	72
2.5.3.1.5	Equilibration of IEF gels	73
2.5.3.2	Protein separation in SDS-PAGE gel (2nd dimension)	73
2.5.3.3	Gel staining methods	74
2.5.3.4	Gel drying	75
2.5.4	Spot analysis and protein identification	75
2.5.4.1	Computational analysis of 2D-gels	75
2.5.4.2	Spot identification using mass spectrometry	75
2.5.4.3	Spot sampling	76
2.5.4.4	MALDI-ToF mass spectrometry	78
2.5.4.5	Tandem MS	80
2.5.4.6	Analysers used in proteomic studies	83
2.5.4.7	Analysis of MALDI-ToF data	86
2.5.4.7.1	Protocol for MALDI-ToF Mass Spectrometry	86
2.5.4.7.2	Data analysis	85
2.5.4.8	Analysis of LC-MS/MS data	85
2.5.4.9	Bioinformatic analysis	85

3.	Comparative phenotypic analysis of cAMP and MAPK signalling mutants of <i>Magnaporthe oryzae</i>	88
3.1	Introduction	88
3.2	Materials and methods	95
3.2.1.	Generation of GFP fusion protein recombinants and <i>Magnaporthe oryzae</i> strains	97
3.2.2.	Conidial suspension preparation and visualisation of GFP expression by laser excitation epifluorescence microscopy	97
3.3	Results	97
3.3.1	Conidium germination and appressorium differentiation in developmental mutants of <i>M.oryzae</i>	97
3.3.2	Microtubule dynamics during germination, appressorium differentiation and maturation	101
3.3.3	Actin organisation during germination, appressorium differentiation and maturation	108
3.3.4	Localisation and quantification of autophagosomes during germination, appressorium differentiation and maturation	124
3.4	Discussion	132
4.	Comparative proteomic analysis of MAPK signalling mutants of <i>Magnaporthe oryzae</i>	135
4.1	Introduction	135
4.2	Materials and methods	136
4.2.1	Protein preparation from <i>M. oryzae</i> during appressorium development	136
4.2.2	Protein identification	138
4.3	Results	139

4.3.1	Pilot experiment for comparative proteomic analysis of appressorium development	140
4.3.2	Large scale sample preparation for comparative proteomic analysis	142
4.3.3	Protein spot quantification	144
4.3.4	Differential proteomic analysis	144
4.3.5	Protein identification of statistics-based selected spots	155
4.3.5.1	Physico-chemical properties of proteins in 2D-gels	159
4.3.5.2	Functional classification of proteins identified by differential proteomics	159
4.3.6	Subsets of proteins identified during germination and appressorium morphogenesis in <i>M. oryzae</i>	162
4.3.6.1	Group 0: Protein spots constitutively expressed during appressorium morphogenesis in <i>M. oryzae</i>	162
4.3.6.2	Group 1: Proteins predicted to play a function in conidial germination of <i>M. oryzae</i>	162
4.3.6.3	Group 2: Proteins predicted to play a function in appressorium morphogenesis	164
4.3.6.4	Group 3: Proteins predicted to play a function in appressorium maturation	166
4.3.7	Significance of identified proteome variation during appressorium development	167
4.3.7.1	Amino-acid and protein metabolism	167
4.3.7.2	Carbohydrate metabolism, cell wall biogenesis and carbon source scavenging	171
4.3.7.3	Lipid metabolism and the peroxisome	180

4.3.7.4	Lipid signalling	184
4.3.7.5	Secondary metabolism	189
4.3.7.6	Other significant changes in protein expression	191
4.4	Discussion	199
5.	Generation and characterization of mutants based on proteomics-analysis	203
5.1	Introduction	203
5.2	Materials and methods	204
5.2.1	Experimental design	204
5.2.2	Split-marker deletion method	206
5.2.3	Fungal transformation	208
5.2.4	PCR colony check	208
5.2.5	Pathogenicity and infection-related development assays	209
5.2.5.1	Plant infection assay	209
5.2.5.2	Assays for measuring rates of conidial germination and appressorium formation	209
5.2.5.3	Penetration assay on onion epidermis	210
5.2.5.4	Leaf sheath inoculation on intact plants	210
5.3	Results	210
5.3.1	Proteomics based selection of 30 genes for targeted-gene deletion	210
5.3.2	Twenty eight targeted gene deletion mutants were obtained with success	213
5.3.3	Plant infection assay of the 28 gene deletion mutants	215
5.3.4	Characterization of seven gene deletion mutants showing	

	a significant reduction in virulence	218
5.3.4.1	Gene deletion mutant $\Delta psg1$	218
5.3.4.2	Gene deletion mutant $\Delta psg2$	225
5.3.4.3	Gene deletion mutant $\Delta psg3$	225
5.3.4.4	Gene deletion mutant $\Delta psg4$	229
5.3.4.5	Gene deletion mutant $\Delta psg5$	237
5.3.4.6	Gene deletion mutant $\Delta psg6$	241
5.3.4.7	Gene deletion mutant $\Delta psg7$	245
5.4	Discussion	249
6.	Summary	265
	Marie Curie research training network	269
	References	270
	Appendices 1-9 Proteomics supplementary Tables	CD
	Appendix 10 Proteomic 2D-gel maps	CD
	Appendix 11 SIGNALPATH publication 2009	CD