

**Investigating the role of lipid mobilisation and metabolism in the rice
blast fungus *Magnaporthe oryzae***

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Mohd. Termizi bin Yusof

Abstract

The rice blast fungus *Magnaporthe oryzae* infects plants by developing a specialised infection structure known as an appressorium. In *M. oryzae* the appressorium is a melanin-pigmented cell with a reinforced cell wall, allowing the cell to generate enormous internal turgor to enable penetration of the plant tissue by a narrow penetration hypha. Previously it has been shown that mobilisation of lipid droplets to the nascent appressorium is essential for successful plant infection. In this thesis, I describe a series of studies that have identified and characterised genes associated with infection-associated lipid metabolism in *M. oryzae*, including the role of fatty acid β -oxidation, acetyl-CoA transport and metabolism and regulation of lipid body breakdown. First, I report identification of *FAR1* and *FAR2*, which encode putative Zn²⁺-Cys⁶ binuclear proteins that appear to act as transcriptional regulators of lipid metabolism. Deletion mutants of *M. oryzae* *FAR1* and *FAR2* were deficient in growth on long chain fatty acids. In addition $\Delta far1$ mutants were unable to grow on acetate as a sole carbon source. *FAR1* and *FAR2* affect the expression of genes involved in fatty acid β -oxidation, acetyl-CoA translocation, peroxisomal biogenesis, the glyoxylate cycle and acetyl-CoA synthesis. Next, I functionally characterized the *CAR1*, *CAR2*, *CAR3* and *CAR4* genes, which encode enzymes involved in carnitine biosynthesis, which is required for translocation of acetyl-CoA between mitochondria, peroxisomes and the cytoplasm. Only a sub-set of carnitine biosynthetic enzymes was necessary for growth on fatty acids and lipids by *M. oryzae*, but redundancy was also apparent in carnitine biosynthesis, because *CAR1*, *CAR2*, *CAR3* and *CAR4* were dispensable for pathogenicity, while the carnitine acetyltransferase, *PTH2*, is essential for rice blast disease. To investigate the role of the appressorium acetyl-CoA pool in more detail, I functionally characterized the acetyl-CoA synthetase gene, *ACS2* and *ACS3*, and *CRC1*, which encodes the mitochondrial carnitine carrier, both of which are highly expressed during appressorium development and appear to play a role in appressorium physiology. Finally, to understand the onset of lipid droplet degradation in more detail, I characterised a putative perilipin, encoded by *CAP20*, which localizes specifically to the periphery of lipid droplets. Perilipins are known to play roles in lipid droplet mobilisation and lipase accessibility. Consistent with this idea, *M. oryzae* mutants lacking *CAP20*, were severely affected in fungal virulence due to impaired appressorium function. When considered together, the results presented in this thesis suggest that lipid body mobilisation and acetyl-CoA metabolism are fundamental processes required for appressoria to function correctly and cause rice blast disease.

Table of contents

	Page
Abstract	2
List of Figures	7
List of Tables	11
List of <i>Magnaporthe oryzae</i> strains utilized in this study	12
Acknowledgements	13
Abbreviations	14
1 Introduction	
1.1 Challenges in global food security	16
1.2 Phytopathogens and food security	17
1.3 Rice blast disease	18
1.4 The life cycle of <i>Magnaporthe oryzae</i>	20
1.5 Cell signalling in <i>Magnaporthe oryzae</i>	23
1.5.1 Cyclic AMP signalling	23
1.5.2 Mitogen-activated protein kinase (MAPK) pathways in <i>M. oryzae</i> and pathogenesis	25
1.6 Autophagy in <i>Magnaporthe oryzae</i>	29
1.7 Turgor and metabolism in <i>M. oryzae</i>	32
1.8 Major metabolic changes during appressorium-mediated plant infection by <i>Magnaporthe oryzae</i>	33
1.9 Lipid metabolism	37
1.9.1 Hydrolysis of triglycerides by triacylglycerol lipases (lipolysis)	37
1.9.2 Fatty acid β -oxidation	39
1.9.3 The glyoxylate cycle	41
1.10 Aim of this study	44
2 Materials & Methods	
2.1 Growth and maintenance of fungus stocks	46
2.2 Fungal genomic DNA extraction	47
2.3 Digestion of genomic or plasmid DNA with restriction enzymes	48
2.4 DNA gel electrophoresis	48
2.5 The polymerase chain reaction (PCR)	48
2.6 Gel purification of DNA fragments	49
2.7 Bacterial plasmid DNA preparations	50
2.7.1 Alkaline lysis plasmid mini preparations	50

2.7.2	High quality plasmid DNA preparations	51
2.8	DNA ligation and selection of recombinant clones	52
2.9	Preparation of competent cells	53
2.10	Transformation of bacterial hosts	54
2.11	Targeted gene deletion using split marker strategy	55
2.12	Fungal transformation	57
2.13	Southern blot analysis	58
2.14	Radio-labelled DNA probe construction	59
2.15	DNA gel blot hybridisations	59
2.16	Extraction of total <i>M. oryzae</i> RNA	60
2.17	Plant infection assays	61
2.18	Microscopy and live cell imaging	62
2.18.1	Microscopy using the Zeiss Axioskop 2 epifluorescence microscope	62
2.18.2	Microscopy analysis using the Olympus IX81 microscope	62
3	Regulation of lipid metabolism in <i>Magnaporthe oryzae</i> by <i>FAR1</i> and <i>FAR2</i>	
3.1	Introduction	63
3.2	Material and methods	66
3.2.1	Targeted deletion of genes that encodes <i>MoFAR1</i> , <i>MoFAR2</i> and <i>MoFAR1/MoFAR2</i>	66
3.2.2	Lipid staining	67
3.2.3	Construction of <i>FAR1:GFP:trpC</i> and <i>FAR2:GFP:trpC</i>	68
3.2.4	Quantitative Real Time PCR (QPCR) analysis	69
3.3	Results	72
3.3.1	Identification of gene that codes for <i>FAR1</i> and <i>FAR2</i> in <i>M. oryzae</i>	72
3.3.2	Targeted gene deletion of <i>FAR1</i> and <i>FAR2</i>	78
3.3.3	Expression profile of <i>FAR1</i> and <i>FAR2</i> and lipid utilisation of $\Delta far1$, $\Delta far2$ and $\Delta far1\Delta far2$ mutants	82
3.3.4	Expression profiling of genes involved in lipid metabolism in $\Delta far1$, $\Delta far2$ and $\Delta far1\Delta far2$ mutants	85
3.3.5	Acetate utilisation in $\Delta far1$, $\Delta far2$ and $\Delta far1\Delta far2$ mutants	89
3.3.6	Localisation of <i>FAR1</i> and <i>FAR2</i>	90
3.3.7	Appressorium development and lipid mobilisation in $\Delta far1$, $\Delta far2$ and $\Delta far1\Delta far2$ mutants	93
3.3.8	Plant infection analysis of $\Delta far1$, $\Delta far2$ and $\Delta far1\Delta far2$ mutants	99
3.4	Discussion	101

4	<i>Magnaporthe oryzae</i> perilipin homolog <i>CAP20</i> and its role in appressorial development and plant virulence	
4.1	Introduction	104
4.2	Material and methods	107
4.2.1	Targeted deletion of gene that encodes perilipin (<i>CAP20</i>) in <i>M. oryzae</i>	107
4.2.2	Lipid staining	108
4.2.3	Construction of RFP-tagged <i>CAP20</i> for localisation analysis	108
4.2.4	Yeast transformation	109
4.2.5	Yeast plasmid extraction	110
4.3	Results	112
4.3.1	Identification of gene that codes for perilipin in <i>M. oryzae</i>	112
4.3.2	Targeted gene deletion of <i>CAP20</i>	114
4.3.3	Localisation of perilipin	117
4.3.4	Appressorium development and lipid mobilisation in $\Delta cap20$ mutant	117
4.3.5	The effect of <i>CAP20</i> deletion on nuclear division in <i>M. oryzae</i>	121
4.3.6	Carbon utilisation and plant pathogenicity in $\Delta cap20$ mutants	121
4.4	Discussion	126
5	Acetyl-CoA metabolism and translocation in <i>M. oryzae</i>	
5.1	Introduction	130
5.1.1	Carnitine acetyltransferase (CAT)	131
5.1.2	Carnitine biosynthesis	134
5.1.3	Carnitine carrier	135
5.2	Material and methods	138
5.2.1	Targeted deletion of genes involved in carnitine biosynthesis (<i>CAR1</i> , <i>CAR2</i> , <i>CAR3</i> , <i>CAR4</i>) and cytoplasmic acetyl-CoA translocation (<i>ACS1</i> , <i>ACS2</i> , <i>ACS3</i> , <i>CRC1</i>) in <i>M. oryzae</i>	138
5.2.2	Lipid staining	139
5.3	Results	142
5.3.1	Importance of carnitine acetyl transferases (CATs) in plant infection development	142
5.3.2	Carnitine biosynthesis and its importance in plant infection development	147
5.3.2.1	Identification of genes involved in carnitine biosynthesis in <i>M. oryzae</i>	147

5.3.2.2	Targeted gene deletion of genes encoding carnitine biosynthesis enzymes	152
5.3.2.3	Expression profile, carbon utilisation and pathogenicity of carnitine biosynthesis mutants	158
5.3.3	Translocation of cytoplasmic acetyl-CoA	163
5.3.3.1	Identification of <i>ACS2</i> , <i>ACS3</i> and <i>CRC1</i>	163
5.3.3.2	Targeted deletion of <i>ACS1</i> , <i>ACS2</i> , <i>ACS3</i> and <i>CRC1</i> genes in <i>M. oryzae</i>	172
5.3.3.3	Carbon source utilisation and pathogenicity of $\Deltaacs2$, $\Deltaacs3$ and $\Deltacrc1$ mutants	177
5.3.3.4	Appressorium development and lipid mobilisation in $\Deltaacs2$, $\Deltaacs3$ and $\Deltacrc1$	180
5.4	Discussion	186
6	General discussion	194
	Bibliography	205