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INVESTIGATION OF METABOLITES PRODUCED BY RICE BLAST FUNGUS, Magnaporthe oryzae DURING APPRESSORIUM DEVELOPMENT

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By

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Chairman : Mohd Termizi bin Yusof, PhD
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Magnaporthe oryzae is important fungal pathogen that caused serious rice blast disease worldwide. The disease cycle of *M. oryzae* is unique, required appressorium in order to penetrate the host cells. Appressorium is critical structure to cause disease but metabolites produced during appressorium development is poorly understood. To date, there is limited information on fungal metabolites and their functions produced by *M. oryzae* during appressoria development. Therefore, the aim of this study was to determine metabolites produced by *M. oryzae* during appressorium development *in vitro* and revealed important metabolic pathways involved. Untargeted metabolomics of Proton Nuclear Magnetic Resonance (^{1}H NMR) was used to determine metabolites from *M. oryzae* extracts and any metabolite changes was observed during 0 h, 8 h and 24 h development stage. Spectra of ^{1}H NMR were analyzed using multivariate data analysis (MVDA) and model validation was studied. Rich numbers of primary metabolites were detected from ^{1}H NMR spectra and there were 43 metabolites identified putatively based on metabolomics library and previous reports. Partial least square discriminant analysis (PLS-DA) disclosed metabolites pattern among 0 h, 8 h, 24 h and mycelia. Metabolites that showed significant changes ($p < 0.05$) among groups of 0 h, 8 h, 24 h and mycelia including butyrate, leucine, isoleucine, valine, isobutyrate, ethanol, methylmalonate, threonine, lactate, alanine, lysine, arginine, 4-aminobutyrate, glutamate, homoserine, isocitrate, glutamine, choline, glucose, xylose, mannose, glycerol, mannitol, glucitol, tyrosine, sucrose and tryptophan. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) revealed metabolites produced during each time-point of 0 h, 8 h, 24 h. Number of metabolites at 8 h and 24 h were produced the highest compared to 0 h. Then, metabolomics pathway analysis (MetPa 4.0) from Metaboanalyst.ca was used to illustrate metabolic pathways involved during appressorium development. There were eight key metabolic pathways that highly involved during appressorium development including amino acids, carbohydrates and lipid metabolisms. Fungal metabolites produced by *M. oryzae* have potential for

targeted metabolomics to target specific metabolite or pathways required for pathogenicity thus provide opportunity in developing inhibitors for rice blast disease.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KAJIAN PENGHASILAN METABOLIT OLEH KULAT KARAH PADI, *Magnaporthe oryzae* SEMASA PERKEMBANGAN "APPRESSORIUM"

Oleh

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Magnaporthe oryzae ialah sejenis kulat patogen penyebab penyakit karah padi yang menjangkiti padi di seluruh dunia. Kitaran penyakit karah yang disebabkan oleh *Magnaporthe oryzae* ini unik, di mana ia memerlukan appressorium untuk menembusi sel perumah. Appressorium ialah struktur kritikal yang diperlukan untuk terjadinya penyakit, namun dari sudut penghasilan metabolit semasa perkembangan appressorium, ianya sangat kurang diketahui. Sehingga kini, maklumat mengenai metabolit kulat *M. oryzae* serta fungsi-fungsi penghasilannya semasa perkembangan appressoria amat terhad. Oleh itu, tujuan penyelidikan ini adalah untuk menentukan metabolit yang dihasilkan oleh *M. oryzae* semasa perkembangan appressoria secara *in vitro* dan menerokai laluan metabolik yang terlibat. Kaedah metabolomik tidak sasar daripada Proton Nuklear Magnetik Resonan (^{1}H NMR) telah digunakan untuk menentukan metabolit yang dihasilkan dari ekstrak *M. oryzae* dan sebarang perubahan metabolit telah dikenalpasti pada setiap peringkat perkembangan iaitu 0 jam, 8 jam dan 24 jam. Spektra ^{1}H NMR telah dianalisis menggunakan analisa multivariat (MVDA) dan model validasi telah dikaji. Terdapat banyak metabolit "primary" berdasarkan dari spektra ^{1}H NMR dan sebanyak 43 metabolit telah dikenaplasti secara "putative" hasil carian rujukan metabolomik dan kajian terdahulu. Analisa separa persegi diskriminan (PLS-DA) menunjukkan pola metabolit pada 0 jam, 8 jam, 24 jam dan miselia. Di antara metabolit yang menunjukkan perubahan yang signifikan ($p < 0.05$) antara kumpulan 0 jam, 8 jam, 24 jam dan miselia ialah butirat, leusina, isoleusina, valina, isobutirat, etanol, metilmalonat, treonina, laktat, alanina, lisina, arginina, 4-aminobutirat, glutamat, homoserina, isositrat, glutamina, kolina, glukosa, xilosa, manosa, gliserol, manitol, glusitol, tirosina, sukrosa dan triptofan. Analisa unjuran orthogonal kepada struktur laten diskriminan (OPLS-DA) memberikan gambaran metabolit yang dihasilkan pada setiap masa perkembangan iaitu 0 jam, 8 jam dan 24 jam. Banyak penghasilan metabolit dilihat berlaku pada 8 jam dan 24 jam berbanding 0 jam. Analisa laluan metabolomik (MetPa 4.0) daripada Metaboanalyst.ca digunakan untuk menerangkan laluan metabolik yang terlibat

semasa perkembangan appressoria. Terdapat lapan laluan metabolik penting dikenalpasti terlibat semasa perkembangan appressoria termasuk metabolisme asid amino, metabolisme karbohidrat dan metabolisme lipid. Metabolit-metabolit yang dihasilkan oleh kulat *M. oryzae* ini berpotensi untuk kajian sasaran metabolomik dalam menyasarkan metabolit atau laluan metabolik tertentu yang penting terlibat dalam perkembangan penyakit karah padi sekaligus memberi peluang ke arah perkembangan menghasilkan perencat kepada penyakit karah padi.

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highlighted in 3 colours; red- 0 h, green- 8 h and 24 and purple- 24 h. Multiple arrows in pathways represent series of reactions that were not shown. Metabolic pathways were analyzed using MetPa (Metaboanalyst.ca) and KEGG (Kyoto Encyclopedia of Genes and Genomes) database.

LIST OF ABBREVIATIONS

M. oryzae	*Magnaporthe oryzae*
h	Hour
m	Mycelia
MPa	Megapascal
µm	Micrometre
ºC	Degree Celcius
min	minute
^{1}H NMR	Proton Nuclear Magnetic Resonance
MVDA	Multivariate Data Analysis
PCA	Principal Component Analysis
PLS-DA	Partial Least Squares Discriminant Analysis
OPLS-DA	Orthogonal Partial Least Square Discriminant Analysis
TSP	Trimethylsilyl-2,2,3,3-tetradeuteropropionic acid
ppm	chemical shift
VIP	Variable influence on projection
TCA	Tricarboxylic acid cycle/citric acid cycle
s	singlet
d	doublet
dd	double doublet
t	triplet
m	multiplet
q	quartet
J	coupling constant
GABA	gamma-aminobutyrate

CHAPTER 1

INTRODUCTION

Rice blast disease is one of the devastating diseases in agriculture caused by fungal pathogen, *Magnaporthe oryzae*. The rice yield loss about 10-30% worldwide because of rice blast every year with estimation about US$6 billion sufficient to feed another 60 million people (Pennisi, 2010; Talbot, 2003). As rice is a staple food, the impact of rice blast to human signify a threat to food security considering increase of rice production demands consumed by approximately 23% human population (Wilson and Talbot, 2009). In Malaysia, 10% of Muda Agricultural Development Authority (MADA) crop area approximately more than 60,000 tons of rice was attacked by rice blast in 2016-2017 season and rice price was cut-down by 50% which brought huge losses to the rice production. Susceptible rice crop now at risk as it could be affected easily by this disease (Berita Harian, 2017). Interestingly, the fungus has become intense research subject in years to obtain in-depth understanding of *Magnaporthe oryzae* infection stage, from early infection until it took over the host entirely. Knowledge of how this pathogen could survive in harsh environment and successfully invade host can help in developing reliable disease countermeasure or finding inhibitors to combat fungal disease (Skamnioti and Gurr, 2009; Dunn *et al*., 2009).

Magnaporthe oryzae formed essential infection structure called appressorium. The appressorium can be induced away from rice leaf (*in vitro)* (Soanes *et al*., 2012) by providing hydrophobic, hard surface and nutrient-free condition. Conidium undergoes germination to form germ tube and once the germ tube has met its required physical signals, it becomes swollen and differentiates to become appressorium (Wilson and Talbot, 2009). The appressorium accumulates high glycerol level so that enormous turgor pressure can be driven by osmosis (water moves into the appressorium) and presses down the base of appressorium by forming small penetration peg hence develops invasive hyphae from inside host (Fernandez and Orth, 2018). Complex infection mechanisms of *M. oryzae* were controlled by various specific genes, proteins, metabolites, regulators and enzymes activities (Fernandez and Orth, 2018; Jones *et al*., 2011; Aliferis and Jabaji, 2010; Foster *et al*., 2003).

Integrated control measures have been implemented to decrease the severity of rice blast but complete eradication is still unachievable. Methods like developing resistant cultivar, chemical control, biological control, nutritional management have been introduced to control the disease (Miah *et al*., 2017) but comprehensive study is also highly needed for better understanding of *M. oryzae* physiological processes. In addition, metabolites produced by *M. oryzae* during appressorium development and their functions in pathogenicity are still unclear. So, this study focused on metabolites produced by *M. oryzae* to provide information on possible metabolites activated during each time of development stage hence could reveal underlying biochemical mechanisms of *M. oryzae* and allow development of effective control measures. Metabolites production levels

were influenced by time of appressoria development whereby early appressoria development stage produced low number of metabolites compared to appressoria maturation stage. Previous study of *M. oryzae* metabolites unveil several metabolites such as alanine, malate, glutamine and unknown sugar exhibited high levels after 24 h post inoculation (Jones *et al*., 2011). Targeted gene studies of *M. oryzae* indicated amino acids like arginine, isoleucine, valine and lysine (Zhang *et al*., 2015; Chen *et al*., 2014; Du *et al*., 2013) were essential for appressoria formation, conidia development and pathogenicity.

Fungal metabolites could be identified using a robust technique of NMR-based metabolomics. A combination of advanced instrumentation and analytical software have allowed metabolomics emerged as powerful tools and unbiased for determination of endogenous metabolites derived from living samples such as urine, serum, microbes or biological tissue extract in response to genetic modification or developmental stimuli (Clark and Haselden, 2008). In this study, *M. oryzae* was germinated on artificial surface to produce appressoria under 0 h, 8 h, 24 h time-point development to assess metabolites production and changes of metabolites within 0 h, 8 h and 24 h compared with mycelia. Our study provided new insight of metabolites analytical analysis and possible mechanisms involved during appressoria development in *Magnaporthe oryzae* in hope for better understanding regarding fungal pathogen metabolites.

Hence, the research objectives were to:

1. identify metabolites produced by *Magnaporthe oryzae* during appressoria development stage.

2. determine metabolites associated changes during each development time-point and propose metabolic pathways involved in *Magnaporthe oryzae* during appressoria development stage.

REFERENCES

Abdel-Farid, I. B., Jahangir, M., van den Hondel, C. A. M. J. J., Kim, H. K., Choi, Y. H. and Verpoorte, R. (2009). Fungal infection-induced metabolites in *Brassica rapa*. *Plant Science*, 176(5), 608-615.

Adachi, K. and Hamer, J. E. (1998). Divergent cAMP signaling pathways regulate growth and pathogenesis in the rice blast fungus *Magnaporthe grisea*. *The Plant Cell*, 10(8), 1361-1373.

Aliferis, K. A. and Jabaji, S. (2010). ^{1}H NMR and GC-MS metabolic fingerprinting of developmental stages of *Rhizoctonia solani* sclerotia. *Metabolomics*, 6(1), 96-108.

Ansell, R., Granath, K., Hohmann, S., Thevelein, J. M. and Adler, L. (1997). The two isoenzymes for yeast NAD+-dependent glycerol 3-phosphate dehydrogenase encoded by GPD1 and GPD2 have distinct roles in osmoadaptation and redox regulation. *The EMBO Journal*, 16(9), 2179-2187.

Azam, A. A., Pariyani, R., Ismail, I. S., Ismail, A., Khatib, A., Abas, F. and Shaari, K. (2017). Urinary metabolomics study on the protective role of *Orthosiphon stamineus* in Streptozotocin induced diabetes mellitus in rats via ^{1}H NMR spectroscopy. *BMC Complementary and Alternative Medicine*, 17(1), 278.

Berita Harian (2017). *60 000 tan Padi Terdedah Serangan Karah*. Retrieved 6 July 2020, from https://www.pressreader.com/malaysia/berita-harian/20170220/282527248198351

Bhambra, G. K., Wang, Z. Y., Soanes, D. M., Wakley, G. E. and Talbot, N. J. (2006). Peroxisomal carnitine acetyl transferase is required for elaboration of penetration hyphae during plant infection by *Magnaporthe grisea*. *Molecular Microbiology*, 61(1), 46-60.

Böhnert, H. U., Fudal, I., Dioh, W., Tharreau, D., Notteghem, J. L. and Lebrun, M. H. (2004). A putative polyketide synthase/peptide synthetase from *Magnaporthe grisea* signals pathogen attack to resistant rice. *The Plant Cell*, 16(9), 2499-2513.

CABI.org. (2018). *CABI Invasive Species Compendium: Magnaporthe oryzae (rice blast disease)*. Retrieved 28 November 2018, from https://www.cabi.org/isc/datasheet/46103/

Cappucino, J. G., and Sherman, N. (2011). *Microbiology A Laboratory Manual International Edition 9th*. Pearson Education, Inc.

Chen, Y., Zuo, R., Zhu, Q., Sun, Y., Li, M., Dong, Y. and Zhang, Z. et al. (2014). MoLys2 is necessary for growth, conidiogenesis, lysine biosynthesis, and

pathogenicity in *Magnaporthe oryzae*. *Fungal Genetics and Biology*, 67, 51-57.

Choi, W. and Dean, R. A. (1997). The adenylate cyclase gene MAC1 of *Magnaporthe grisea* controls appressorium formation and other aspects of growth and development. *The Plant Cell*, 9(11), 1973-1983.

Choi, Y. H., Kim, H. K., Hazekamp, A., Erkelens, C., Lefeber, A. W. and Verpoorte, R. (2004). Metabolomic Differentiation of *Cannabis sativa* Cultivars Using ^{1}H NMR Spectroscopy and Principal Component Analysis. *Journal of Natural Products*, 67(6), 953-957.

Chong, J., Wishart, D. S. and Xia, J. (2019). Using metaboanalyst 4.0 for comprehensive and integrative metabolomics data analysis. *Current Protocols in Bioinformatics*, 68(1), e86.

Clarke, C. J. and Haselden, J. N. (2008). Metabolic profiling as a tool for understanding mechanisms of toxicity. *Toxicologic Pathology*, 36(1), 140-147.

Collemare, J., Billard, A., Böhnert, H. U. and Lebrun, M. H. (2008). Biosynthesis of secondary metabolites in the rice blast fungus *Magnaporthe grisea*: the role of hybrid PKS-NRPS in pathogenicity. *Mycological Research*, 112(2), 207-215.

Couch, B. C. and Kohn, L. M. (2002). A multilocus gene genealogy concordant with host preference indicates segregation of a new species, *Magnaporthe oryzae*, from *M. grisea*. *Mycologia*, 94(4), 683-693.

Couch, B. C., Fudal, I., Lebrun, M. H., Tharreau, D., Valent, B., Van Kim, P. and Kohn, L. M. et al. (2005). Origins of host-specific populations of the blast pathogen *Magnaporthe oryzae* in crop domestication with subsequent expansion of pandemic clones on rice and weeds of rice. *Genetics*, 170(2), 613-630.

Davis, D. J., Burlak, C. and Money, N. P. (2000). Biochemical and biomechanical aspects of appressorial development in *Magnaporthe grisea*. In *Advances in Rice Blast Research* (pp. 248-256). Springer, Dordrecht.

de Jong, J. C., McCormack, B. J., Smirnoff, N. and Talbot, N. J. (1997). Glycerol generates turgor in rice blast. *Nature*, 389(6648), 244.

Dean, R. A., Talbot, N. J., Ebbole, D. J., Farman, M. L., Mitchell, T. K., Orbach, M. J. and Read, N. D. et al. (2005). The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature*, 434(7036), 980.

DeZwaan, T. M., Carroll, A. M., Valent, B. and Sweigard, J. A. (1999). *Magnaporthe grisea* pth11p is a novel plasma membrane protein that mediates appressorium differentiation in response to inductive substrate cues. *The Plant Cell*, 11(10), 2013-2030.

Diehl, B. (2008). Principles in NMR spectroscopy. *NMR Spectroscopy in Pharmaceutical Analysis* (pp. 1-41). Elsevier.

Divon, H. H. and Fluhr, R. (2007). Nutrition acquisition strategies during fungal infection of plants. *FEMS Microbiology Letters*, 266(1), 65-74.

Dona, A. C., Kyriakides, M., Scott, F., Shephard, E. A., Varshavi, D., Veselkov, K. and Everett, J. R. (2016). A guide to the identification of metabolites in NMR-based metabonomics/metabolomics experiments. *Computational and Structural Biotechnology Journal*, 14, 135-153.

Du, Y., Hong, L., Tang, W., Li, L., Wang, X., Ma, H. and Zhang, Z. et al. (2014). Threonine deaminase MoIlv1 is important for conidiogenesis and pathogenesis in the rice blast fungus *Magnaporthe oryzae*. *Fungal Genetics and Biology*, 73, 53-60.

Du, Y., Zhang, H., Hong, L., Wang, J., Zheng, X. and Zhang, Z. (2013). Acetolactate synthases MoIlv2 and MoIlv6 are required for infection-related morphogenesis in *Magnaporthe oryzae*. *Molecular Plant Pathology*, 14(9), 870-884.

Dunn, M. F., Ramirez-Trujillo, J. A. and Hernández-Lucas, I. (2009). Major roles of isocitrate lyase and malate synthase in bacterial and fungal pathogenesis. *Microbiology*, 155(10), 3166-3175.

Dunn, W. B., Bailey, N. J. and Johnson, H. E. (2005). Measuring the metabolome: current analytical technologies. *Analyst*, 130(5), 606-625.

Ebbole, D.J. (2007). *Magnaporthe* as a model for understanding host-pathogen interactions. *Annual Review of Phytopathology*, 45, 437-456.

Egan, M. J., Wang, Z. Y., Jones, M. A., Smirnoff, N. and Talbot, N. J. (2007). Generation of reactive oxygen species by fungal NADPH oxidases is required for rice blast disease. *Proceedings of the National Academy of Sciences*, 104(28), 11772-11777.

Eriksson, L., Johansson, E., Kettaneh-World, N.n Tyrgg, J., Wikstorm, C. and Wold, S. (2006). *Multi- and Megavariate Data Analysis Part 1: Basic Principles and Applications*. Umeå, Sweden: Umetrics AB.

Fernandez, J. and Orth, K. (2018). Rise of a Cereal Killer: The Biology of *Magnaporthe oryzae* Biotrophic Growth. *Trends in Microbiology*, 26(7), 582-597.

Foster, A. J., Jenkinson, J. M. and Talbot, N. J. (2003). Trehalose synthesis and metabolism are required at different stages of plant infection by *Magnaporthe grisea*. *The EMBO Journal*, 22(2), 225-35.

Galhano, R. and Talbot, N. J. (2011). The biology of blast: Understanding how *Magnaporthe oryzae* invades rice plants. *Fungal Biology Reviews*, 25(1), 61-67.

Gilbert, R. D., Johnson, A. M. and Dean, R. A. (1996). Chemical signals responsible for appressorium formation in the rice blast fungus *Magnaporthe grisea*. *Physiological and Molecular Plant Pathology*, 48(5), 335-346.

Gokce, E., Franck, W. L., Oh, Y., Dean, R. A. and Muddiman, D. C. (2012). In-depth analysis of the *Magnaporthe oryzae* conidial proteome. *Journal of Proteome Research*, 11(12), 5827-5835.

Goodacre, R., Vaidyanathan, S., Dunn, W. B., Harrigan, G. G. and Kell, D. B. (2004). Metabolomics by numbers: acquiring and understanding global metabolite data. *TRENDS in Biotechnology*, 22(5), 245-252.

Habibuddin, H. (2012). Managing pests and diseases of rice using resistant varieties. *MARDI Press* (pp.45).

Howlett, B. J. (2006). Secondary metabolite toxins and nutrition of plant pathogenic fungi. *Current Opinion in Plant Biology*, 9(4), 371-375.

Hubert, J., Mabagala, R. B. and Mamiro, D. P. (2015). Efficacy of selected plant extracts against *Pyricularia grisea*, causal agent of rice blast disease. *American Journal of Plant Sciences*, 6, 602-611.

Jones, O. A., Maguire, M. L., Griffin, J. L., Jung, Y. H., Shibato, J., Rakwal, R. and Jwa, N. S. et al. (2011). Using metabolic profiling to assess plant-pathogen interactions: an example using rice (*Oryza sativa*) and the blast pathogen *Magnaporthe grisea*. *European Journal of Plant Pathology*, 129(4), 539-554.

Keller, N. P., Turner, G. and Bennett, J. W. (2005). Fungal secondary metabolism- from biochemistry to genomics. *Nature Reviews Microbiology*, 3(12), 937.

Kershaw, M. J. and Talbot, N. J. (2009). Genome-wide functional analysis reveals that infection-associated fungal autophagy is necessary for rice blast disease. *Proceedings of the National Academy of Sciences*. 106(37), 15967-15972.

Kim, H. K. and Verpoorte, R. (2010). Sample preparation for plant metabolomics. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, 21(1), 4-13.

Kim, H. K., Choi, Y. H. and Verpoorte, R. (2010). NMR-based metabolomic analysis of plants. *Nature Protocols*, 5(3), 536.

Kim, H. K., Choi, Y. H. and Verpoorte, R. (2011). NMR-based plant metabolomics: where do we stand, where do we go?. *Trends in Biotechnology*, 29(6), 267-275.

Klionsky, D. J. (2007). Autophagy: from phenomenology to molecular understanding in less than a decade. *Nature Reviews Molecular Cell Biology*, 8(11), 931.

Kohli, M. M., Mehta, Y. R., Guzman, E., De Viedma, L. and Cubilla, L. E. (2011). *Pyricularia* blast–a threat to wheat cultivation. *Czech Journal of Genetic and Plant Breeding*, 47, S130-S134.

Lang, T., Schaeffeler, E., Bernreuther, D., Bredschneider, M., Wolf, D. H. and Thumm, M. (1998). *Aut2p* and *Aut7p*, two novel microtubule-associated proteins are essential for delivery of autophagic vesicles to the vacuole. *The EMBO Journal*, 17(13), 3597-3607.

Lee, Y. H. and Dean, R. A. (1993). cAMP regulates infection structure formation in the plant pathogenic fungus *Magnaporthe grisea*. *The Plant Cell*, 5(6), 693-700.

Li, Y., Zhang, X., Hu, S., Liu, H. and Xu, J. R. (2017). PKA activity is essential for relieving the suppression of hyphal growth and appressorium formation by *MoSfl1* in *Magnaporthe oryzae*. *PLoS Genetics*, 13(8), e1006954.

Liu, X. H., Liang, S., Wei, Y. Y., Zhu, X. M., Li, L., Liu, P. P. and Fernandes, C. M. et al. (2019). Metabolomics analysis identifies sphingolipids as key signaling moieties in appressorium morphogenesis and function in *Magnaporthe oryzae*. *MBio*, 10(4), e01467-19.

Liu, H., Suresh, A., Willard, F. S., Siderovski, D. P., Lu, S. and Naqvi, N. I. (2007). Rgs1 regulates multiple Gα subunits in *Magnaporthe* pathogenesis, asexual growth and thigmotropism. *The EMBO Journal*, 26(3), 690-700.

Liu, X., Cai, Y., Zhang, X., Zhang, H., Zheng, X. and Zhang, Z. (2016). Carbamoyl phosphate synthetase subunit *MoCpa2* affects development and pathogenicity by modulating arginine biosynthesis in *Magnaporthe oryzae*. *Frontiers in Microbiology*, *7*, 2023.

Lowe, R. G., Lord, M., Rybak, K., Trengove, R. D., Oliver, R. P. and Solomon, P. S. (2008). A metabolomic approach to dissecting osmotic stress in the wheat pathogen *Stagonospora nodorum*. *Fungal Genetics and Biology*, 45(11), 1479-1486.

Manandhar, H. K., Jørgensen, H. L., Mathur, S. B. and Smedegaard-Petersen, V. (1998). Resistance to rice blast induced by ferric chloride, di-potassium hydrogen phosphate and salicylic acid. *Crop Protection*, 17(4), 323-329.

Marroquin-Guzman, M. and Wilson, R. A. (2015). GATA-dependent glutaminolysis drives appressorium formation in *Magnaporthe oryzae* by suppressing TOR inhibition of cAMP/PKA signaling. *PLoS Pathogens*, 11(4), e1004851.

Miah, G., Rafii, M. Y., Ismail, M. R., Sahebi, M., Hashemi, F. S. G., Yusuff, O. and Usman, M. G. (2017). Blast disease intimidation towards rice

cultivation: a review of pathogen and strategies to control. *The Journal of Animal and Plant Sciences*, 27(4), 1058-1066.

NCBI taxonomy database. (2018). *Magnaporthe oryzae*. Retrieved 14 December 2018, from https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi

NurulNahar, E., Adam, P., Mazidah, M., Roslan, I. and Rafii, Y. M. (2020). Rice blast disease in Malaysia: Options for its control. *Journal of Tropical Agriculture and Food Science*, 48, 1.

Pennisi, E. (2010). Armed and Dangerous. *Science*, 327(5967), 804-805.

Pusztahelyi, T., Holb, I. J. and Pócsi, I. (2015). Secondary metabolites in fungus-plant interactions. *Frontiers in Plant Science*, 6, 573.

Redkar, R. J., Locy, R. D. and Singh, N. K. (1995). Biosynthetic pathways of glycerol accumulation under salt stress in *Aspergillus nidulans*. *Experimental Mycology*, 19(4), 241-246.

Rönnow, B. and Kielland-Brandt, M. C. (1993). *GUT2*, a gene for mitochondrial glycerol 3-phosphate dehydrogenase of *Saccharomyces cerevisiae*. *Yeast*, 9(10), 1121-1130.

Saunders, D. G., Aves, S. J. and Talbot, N. J. (2010). Cell cycle–mediated regulation of plant infection by the rice blast fungus. *The Plant Cell*, 22(2), 497-507.

Scharf, D. H., Heinekamp, T. and Brakhage, A. A. (2014). Human and plant fungal pathogens: the role of secondary metabolites. *PLoS Pathogens*, 10(1), e1003859.

Shen, B., Hohmann, S., Jensen, R. G. and Bohnert, H. J. (1999). Roles of sugar alcohols in osmotic stress adaptation. Replacement of glycerol by mannitol and sorbitol in yeast. *Plant Physiology*, 121(1), 45-52.

Skamnioti, P. and Gurr, S.J. (2009). Against the grain: safeguarding rice from rice blast disease. *Trends in Biotechnology*, 27(3), 141-150.

Soanes, D. M., Chakrabarti, A., Paszkiewicz, K. H., Dawe, A. L. and Talbot, N. J. (2012). Genome-wide transcriptional profiling of appressorium development by the rice blast fungus *Magnaporthe oryzae*. *PLoS Pathogens*, 8(2), e1002514.

Talbot, N. J. (1995). Having a blast: exploring the pathogenicity of *Magnaporthe grisea*. *Trends in Microbiology*, 3(1), 9-16.

Talbot, N. J., McCafferty, H. R. K., Ma, M., Moore, K. and Hamer, J. E. (1997). Nitrogen starvation of the rice blast fungus *Magnaporthe grisea* may act as an environmental cue for disease symptom expression. *Physiological and Molecular Plant Pathology*, 50(3), 179-195.

Talbot, N.J. (2003). On the trail of a cereal killer: exploring the biology of *Magnaporthe grisea*. *Annual Reviews in Microbiology*, 57(1), 177-202.

Thines, E., Aguirre, J., Foster, A. J. and Deising, H. B. (2006). Genetics of phytopathology: secondary metabolites as virulence determinants of fungal plant pathogens. *Progress in Botany* (pp. 134-161). Springer, Berlin, Heidelberg.

Thines, E., Heidrun, A. N. K. E. and Weber, R. W. (2004). Fungal secondary metabolites as inhibitors of infection-related morphogenesis in phytopathogenic fungi. *Mycological Research*, 108(1), 14-25.

Thines, E., Weber, R. W. and Talbot, N. J. (2000). MAP kinase and protein kinase A–dependent mobilization of triacylglycerol and glycogen during appressorium turgor generation by *Magnaporthe grisea*. *The Plant Cell*, 12(9), 1703-1718.

Tucker, S. L., Thornton, C. R., Tasker, K., Jacob, C., Giles, G., Egan, M. and Talbot, N. J. (2004). A fungal metallothionein is required for pathogenicity of *Magnaporthe grisea*. *The Plant Cell*, 16(6), 1575-1588.

UniProt.org. (2018). *Taxonomy - Magnaporthe oryzae (Rice blast fungus) (Pyricularia oryzae).* Retrieved 14 December 2018, from https://www.uniprot.org/taxonomy/318829/

Veneault-Fourrey, C., Barooah, M., Egan, M., Wakley, G. and Talbot, N. J. (2006). Autophagic fungal cell death is necessary for infection by the rice blast fungus. *Science*, 312(5773), 580-583.

Wang, F., Han, J., He, Q., Geng, Z., Deng, Z. and Qiao, D. (2015). Applying ^{1}H NMR spectroscopy to detect changes in the urinary metabolite levels of Chinese half-pipe snowboarders after different exercises. *Journal of Analytical Methods in Chemistry*, 2015.

Wang, H., Chen, J., Feng, Y., Zhou, W., Zhang, J., Yu, Y. U. and Zhang, P. et al. (2015). ^{1}H nuclear magnetic resonance-based extracellular metabolomic analysis of multidrug resistant Tca8113 oral squamous carcinoma cells. *Oncology Letters*, 9(6), 2551-2559.

Wang, Z. Y., Soanes, D. M., Kershaw, M. J. and Talbot, N. J. (2007). Functional analysis of lipid metabolism in *Magnaporthe grisea* reveals a requirement for peroxisomal fatty acid β-oxidation during appressorium-mediated plant infection. *Molecular Plant-Microbe Interactions*, 20(5), 475-491.

Wang, Z. Y., Thornton, C. R., Kershaw, M. J., Debao, L. and Talbot, N. J. (2003). The glyoxylate cycle is required for temporal regulation of virulence by the plant pathogenic fungus *Magnaporthe grisea*. *Molecular Microbiology*, 47(6), 1601-1612.

Wiklund, S. (2008). Multivariate data analysis for Omics. *Umeå: Umetrics AB*.

Wilson, R.A and Talbot, N.J. (2009). Under pressure: investigating the biology of plant infection by *Magnaporthe oryzae*. *Nature Reviews Microbiology*, 7(3), 185–195.

Wishart, D. S. (2008). Quantitative metabolomics using NMR. *TrAC Trends in Analytical Chemistry*, 27(3), 228-237.

Worley, B. and Powers, R. (2013). Multivariate analysis in metabolomics. *Current Metabolomics*, 1(1), 92-107.

Xu, J. R. and Hamer, J. E. (1996). MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus *Magnaporthe grisea*. *Genes and Development*, 10(21), 2696-2706.

Zabek, A., Klimek-Ochab, M., Jawień, E. and Młynarz, P. (2017). Biodiversity in targeted metabolomics analysis of filamentous fungal pathogens by ^{1}H NMR-based studies. *World Journal of Microbiology and Biotechnology*, 33(7), 132.

Zabriskie, T. M. and Jackson, M. D. (2000). Lysine biosynthesis and metabolism in fungi. *Natural Product Reports*, 17(1), 85-97.

Zhang, Y., Shi, H., Liang, S., Ning, G., Xu, N., Lu, J. and Lin, F. et al. (2015). *MoARG1*, *MoARG5*, 6 and *MoARG7* involved in arginine biosynthesis are essential for growth, conidiogenesis, sexual reproduction, and pathogenicity in *Magnaporthe oryzae*. *Microbiological Research*, 180, 11-22.

Zhang, H., Ma, H., Xie, X., Ji, J., Dong, Y., Du, Y. and Zhang, Z. et al. (2014). Comparative proteomic analyses reveal that the regulators of G-protein signaling proteins regulate amino acid metabolism of the rice blast fungus *Magnaporthe oryzae*. *Proteomics*, 14(21-22), 2508-2522.

Zhao, X., Kim, Y., Park, G. and Xu, J. R. (2005). A mitogen-activated protein kinase cascade regulating infection-related morphogenesis in *Magnaporthe grisea*. *The Plant Cell*, 17(4), 1317-1329.

Zhao, X., Mehrabi, R. and Xu, J. R. (2007). Mitogen-activated protein kinase pathways and fungal pathogenesis. *Eukaryotic Cell*, 6(10), 1701-1714.