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Complexity of roles and regulation of the *PMK1*-MAPK pathway in mycelium development, conidiation and appressorium formation in *Magnaporthe oryzae*

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ABSTRACT

MST50, *MST11*, *MST7*, *PMK1* and *GAS1*/*GAS2* genes are the important components in the *PMK1*-MAPK signal transduction pathway in fungi. Mutants with deletion of these five genes of *Magnaporthe oryzae*, a pathogen of the rice blast, were constructed. A cDNA array containing 4108 unique genes of *M. oryzae* was developed and used to analyze the gene expression profiles of these mutants against the wild type to dissect the gene expression regulation networks responsible for conidiation and appressorium formation. With this approach, differentially regulated genes by these five components were identified. The vast majority of the regulated genes were mutant-specific, while only a small proportion were in common for all of the mutants, suggesting that each of these genes has its own regulon. Functional groups and expression patterns of the regulated genes showed that (1) gene members in the *PMK1*-MAPK pathway are associated with multiple signaling pathways; (2) the regulation of *PMK1*-mediated signaling pathways is very complex and likely involved in other signaling networks; (3) glucose metabolism and signals are required in mycelium development; and (4) appressorium formation likely shares the mechanisms responsible for sexual conjugation and meiosis, which is affected by carbohydrate metabolism.

Mitogen-activated protein kinase (MAPK) signal transduction pathways are vital in regulation of many biological processes in eukaryotes, and thus have been considered as potential drug target pathways for treatment and prevention in many human diseases (Bardwell, 2006; Boldt and Kolch, 2004; Lengeler et al., 2000). MAPK pathways can be triggered by exogenous factors, and usually lead to signaling cascade through multiple-step phosphorylation to activate transcription factors. In fungi, MAPK-mediated signaling transduction is associated with sexual reproduction, mycelium growth, osmotic stress response, cell integrity, and asexual spore formation (Bardwell, 2006).

Magnaporthe oryzae is a pathogen of the rice blast, and its infection process is associated with three pathways mediated, respectively, by MAPK kinase kinase (MAPKKK, encoded by *MST11*), MAPK kinase (MAPKK, encoded by *MST7*), and MAPK (encoded by *PMK1*) (Lengeler et al., 2000; Xu, 2000). During infection process, *MPS1* plays a role in regulation of the integrity of hyphal cells (Xu et al., 1998), *OSM1* is responsible to osmotic stress of hyphal cells (Dixon et al., 1999), and *PMK1* is required for pathogenicity (Xu and Hamer, 1996). Constitutive expression of *PMK1* in *M. oryzae* can make a notable impact on both growth and pathogenicity of this pathogen (Bruno et al., 2004; Xu and Hamer, 1996; Zhao et al., 2005). Adaptor protein Mst50 has been shown to be the regulator lying upstream of the *PMK1*-dependent MAPK pathway (*PMK1*-MAPK pathway), where it binds to Mst11 and Mst7 to maintain the stability of the Mst11–Mst7 complex for the phosphorylation of *PMK1*-encoded protein (Park et al., 2006; Zhao et al., 2005). Genes downstream the *PMK1* in the *PMK1*-MAPK pathway include transcription factor *MST12* and pathogenicity-related genes *GAS1* and *GAS2* (Park et al., 2002), both of which were found to express during appressorium morphogenesis (Xue et al., 2002). However, expression of *GAS1*/*GAS2* is controlled by other unknown transcription factors instead of *MST12* (Xue et al., 2002). In addition, other signaling related genes such as *MGB1*, *MST20* and *CHM1* are also involved in *PMK1*-MAPK pathway in *M. oryzae* (Li et al., 2004; Nishimura et al., 2003). Up to date, only affect of *PMK1* involved in the MAPK pathway on gene regulation was detected during conidial germination of *Δpmk1* mutant with RNA-Seq and HT-SuperSAGE techniques (Soanes et al., 2012). A systematic transcriptome analysis of *PMK1*-MAPK pathway will be necessary for overall understanding on the development and pathogenicity of the fungus.

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In this study, gene expression patterns of null mutants of each of the five *PMK1*-MAPK pathway components in *M. oryzae* were monitored and compared to that of the wild-type strain and to each other using a set of the cDNA array spotted with 4108 unique genes of *M. oryzae*. The results showed that each of these genes regulates different set of genes that belong to different signaling pathways and function in different biological processes, demonstrating the complexity of roles and functions of the *PMK1*-MAPK pathway.

1. Results

1.1. Characterization of MAPK pathway null mutants

The characteristics of the mutants of the MAPK pathway component null mutants were summarized in Table 1. The mutants and showed no significant differences to the wild type strains in term of growth rate. However, mutants *Δmst50*, *Δmst11* and *Δmst7*, showed a very low level of conidiation and hardly produced appressoria when compared. Moreover, significant difference in conidiation level was found between mutant *Δpmk1* and *Δgas1/gas2*, the latter could produce appressoria.

Microscopic analysis revealed that there was no significant difference between the mutants and the wild-types in hypha growth, agreeing with previous reports (Park et al., 2006; Xu and Hamer, 1996; Xue et al., 2002; Zhao et al., 2005).

1.2. Quality evaluation of the cDNA array data

A cDNA array covering 4108 unique genes from *M. oryzae* strain Y34 was employed to investigate the alteration of gene expression in mutant *Δmst50*, *Δmst11*, *Δmst7*, *Δpmk1*, and *Δgas1/gas2*. Materials only from mycelia without production of conidia were used for cDNA array analysis. The coefficient (r^2) of results from two hybridization repeats was 0.94 for the wild type 70-15, 0.98 for the wild type Guy11, 0.96 for *Δmst50*, 0.95 for *Δmst11*, 0.95 for *Δmst7*, 0.96 for *Δpmk1*, and was 0.98 for *Δgas1/gas2*, indicating the high repeatability of hybridization. Expression of 14 cDNA array hybridization spots from 14 genes randomly selected was further assayed by qRT-PCR. On the whole, they were consistent with the results from the cDNA array analysis (Supplementary Fig. 1) and the correlation coefficient (r^2) of most genes was larger than 0.60 (Table 2), confirming that data from the cDNA array were reliable.

1.3. Gene expression profiles in the mutants

It was known that the mutant *Δmst50*, *Δmst11*, *Δmst7* was originated from wild type 70-15 and *Δpmk1*, *Δgas1/gas2* from wild type Guy11. The expression profile between 70-15 and Guy11 was investigated, and there were 621 differentially expressed genes (Supplementary Table 1). But these genes did not exhibited differential expression within each mutant/wild type pair, indicating expression profile difference between two wild type strains was not reflected significantly in the respective mutants. So only differentially expressed genes in each mutant compared to the respective wild type was identified and discussed in the following contents.

Of 4108 genes arrayed, a total of 938 (23%) were significantly regulated, up or down (Fig. 1). Eighty-six genes in *Δmst50*, 60 genes in *Δmst11*, 151 genes in *Δmst7*, 264 genes in *Δpmk1*, and 631 genes in *Δgas1/gas2* with deletion of *GAS1/GAS2* (Fig. 2).

Table 1
Growth rate, conidiation and appressorium formation of *M. oryzae* strains.

Phenotype[a]	Strains						
	Wild types[c]		Mutants				
	70-15	Guy11	*Δmst50*	*Δmst11*	*Δmst7*	*Δpmk1*	*Δgas1/gas2*
Growth rate (mm/d)	6.0 ± 0.1	5.7 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.4 ± 0.2	5.4 ± 0.1	5.1 ± 0.5
Conidiation ($\times 10^6$/ml)	8.1 ± 0.7	5.1 ± 0.3	0.05 ± 0.01	0.04 ± 0.008	0.03 ± 0.007	4.4 ± 0.2	4.9 ± 0.6
Appressorium formation (%)[b]	94.1 ± 2.7	92.1 ± 2.1	0	0	0	0	90.1 ± 1.3

[a] Each datum is mean ± standard errors, which were from three independent replicates.
[b] Percentage of appressoria formed on GelBond film by 20 h.
[c] *Δmst50*, *Δmst11* and *Δmst7* were all mutants of the wild type 70-15, and *Δpmk1* and *Δgas1/gas2* were that of mutants of the wild type Guy11.

Table 2
Correlation analysis of 14 gene expression data obtained by cDNA array compared with qRT-PCR.

Name	IDI4	MAGB	MPG1	Grg1	CPC1	SFP1	Seb1	Sec4	TP	GCD1	OSM1	Wis4	Mkh1	MAC1
r^2 Value	0.80	0.63	0.95	0.60	0.90	0.76	0.04	0.27	0.73	0.65	0.55	0.69	0.88	0.46

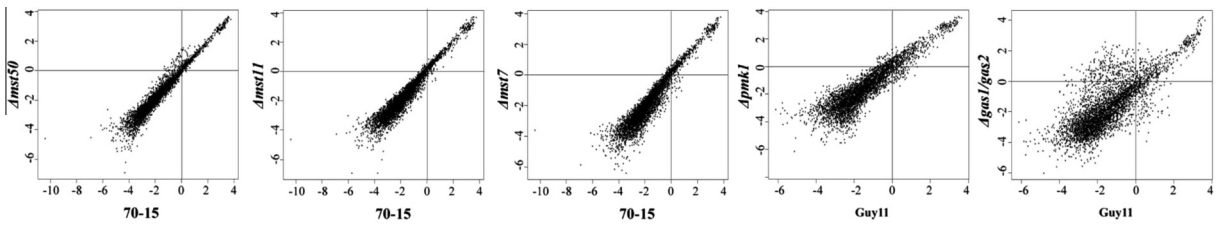

Fig. 1. Distribution of the signal intensity of the cDNA array hybridization among MAPK signal transduction pathway defective mutants compared with wild-types of *M. oryzae*. Each data point represents the mean of ln nARVOL values of four-spot data sets of each gene.