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Identification of Genes Encoding Heat Shock Protein 40 Family and the Functional Characterization of Two *Hsp40s*, *MHF16* and *MHF21*, in *Magnaporthe oryzae*

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Magnaporthe oryzae*, the causal agent of the rice blast disease, poses a worldwide threat to stable rice production. The large-scale functional characterization of genes controlling the pathogenicity of *M. oryzae* is currently under way, but little is known about heat shock protein 40 (Hsp40) function in the rice blast fungus or any other filamentous plant pathogen. We identified 25 genes encoding putative Hsp40s in the genome of *M. oryzae* using a bioinformatic approach, which we designated *M. oryzae* heat shock protein forty (*MHF1-25*). To elucidate the roles of these genes, we characterized the functions of *MHF16* and *MHF21*, which encode type III and type II Hsp40 proteins, respectively. *MHF16* and *MHF21* expression was not significantly induced by heat shock, but it was down-regulated by cold shock. Knockout mutants of these genes (Δ*mhf16* and Δ*mhf21*) were viable, but conidiation was severely reduced. Moreover, sectoring was observed in the Δ*mhf16* mutant when it was grown on oatmeal agar medium. Conidial germination, appressorium formation, and pathogenicity in rice were not significantly affected in the mutants. The defects in conidiation and colony morphology were fully complemented by reintroduction of wild type *MHF16* and *MHF21* alleles, respectively. These data indicate that *MHF16* and *MHF21* play important roles in conidiation in the rice blast fungus.

Keywords : conidiation, heat shock protein 40, *MHF*, sectoring

Heat shock proteins (Hsps) were initially described based on their induction by heat shock, but several Hsps are constitutively expressed or are induced by stimuli other than heat treatment (Burnie et al., 2006; Kaufmann, 1990). In addition, the differential expression of Hsps during embryogenesis and sexual reproduction in plants and animals suggests that they play specific roles during these developmental stages (Heikkila, 1993). Differential Hsp expression during development, including during conidiation, has also been reported in fungi (Bonato et al., 1987; Rensing et al., 1998; Werner-Washburne et al., 1989).

Hsps, which are grouped according to their molecular weight and level of sequence homology (Burnie et al., 2006), are currently considered to be one of the main groups of chaperones that assist in such cellular processes as protein folding and refolding, translocation, and proteolytic degradation (Gething and Sambrook, 1992). Hsp40 and Hsp70, the best-known components of the cellular chaperone network that mediateds the response to stress, exist alone or in combination in all major cellular compartments in eukaryotes (Gething, 1997). By itself, Hsp70 possesses very weak ATPase activity, but the co-chaperones Hsp40 and nucleotide exchange factor are able to increase the activity of Hsp70. Hsp40 modulates the binding and release of substrates by Hsp70 by altering the rate of ATP hydrolysis, recruits the substrates to Hsp70, or involves in the translocation of Hsp70 (Fan et al., 2004).

All Hsp40 family members possess a J-domain, which is comprised of about 70 amino acids linked together to create four helices and includes a conserved tripeptide, HPD (Kelly, 1998). The second helix and tripeptide at the turn between helices II and III are involved in the interaction of Hsp40 with the ATPase domain of Hsp70, which stimulates ATP hydrolysis (Greene et al., 1998). The Hsp40 family can be further categorized into 3 sub-groups based on the degree of conservation with the Hsp40 homologue in *E. coli*, DnaJ (Cheetham and Caplan, 1998). Type I Hsp40s are the true descendents of DnaJ, and they contain all of the typical structural domains: the J-domain, a G/F-rich region, a zinc finger (-like) domain, and a carboxy-terminal domain. In contrast, type II Hsp40s do not contain a zinc finger (-like) domain, and type III Hsp40s contain only the J-domain (Cheetham and Caplan, 1998).

In *Saccharomyces cerevisiae*, the null mutants of several *Hsp40*s, including *SIS1*, were found to be inviable (Luke et al., 1991) (http://www.yeastgenome.org/), and several mutants were sensitive to high temperatures (Caplan and Douglas,

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1991; Nishikawa and Endo, 1997; Rowley et al., 1994; Westermann and Neupert, 1997). Moreover, the *ydj1* mutant displayed pleiotrophic developmental phenotypes, including defects in mating, cell wall integrity, and mat formation (Martineau et al., 2007; Meacham et al., 1999; Wright et al., 2007). In filamentous fungi, no direct evidence exists that Hsps function as pathogenicity factors for plant pathogens; however, Hsps may play roles in the process of infection because the conditions *in planta* are considered to be very stressful for pathogens.

Magnaporthe oryzae is the causal agent of the rice blast, which is the greatest threat to stable rice production across the globe (Ou, 1985). The interactions between *M. oryzae* and rice have been used as a model system for the study of host plant-fungal pathogen interactions (Ebbole, 2007; Valent, 1990). Such research has been facilitated by genetic tractability and availability of genome sequence information for both the pathogen and host (Dean et al., 2005; Yu et al., 2002). A large-scale analysis of the functions of the genes in *M. oryzae* is currently under way (Jeon et al., 2007), but no information is available regarding the Hsp40s encoded by the *M. oryzae* genome. Moreover, no report has described the role of Hsp40s in plant pathogenic fungi at the molecular level in spite of their great conservation in the genome and predicted importance in various biological processes. In this study, we identified 25 genes encoding putative Hsp40s in *M. oryzae* by *in-silico* analysis and functionally characterized two of the genes, which were found to be involved in the conidiation of the fungus at the molecular level.

Materials and Methods

Strains and media. *M. oryzae* strain KJ201 was used as the wild type strain (Jeon et al., 2007). All strains were incubated on oatmeal agar medium (OMA, 5% oatmeal (w/v) and 2.5% agar powder (w/v)) at 25°C with constant fluorescent light to promote conidiation. To extract genomic DNA, strains were cultured in liquid complete medium (0.6% yeast extract (w/v), 0.6% casamino acid (w/v), and 1% sucrose (w/v)) at 25°C for 3 days with agitation (150 rpm). To extract total RNA, conidia suspensions of each strain were cultured in liquid complete medium for 3 days with agitation as previously described (Talbot et al., 1993), and treated with stresses as designated (cold shock at 4°C and heat shock at 42°C).

Nucleic acid manipulation. Restriction enzyme digestion, cloning, and Southern hybridization analysis were performed according to the description of standard protocols (Sambrook and Russell, 2001). Purification of plasmid DNA was prepared using a plasmid extraction kit (Exprep™ Plasmid SV; GeneAll Biotechnology, Seoul, Korea). The extraction of genomic DNA was done as previously described (Kim et al., 2005). The total RNA was extracted using an RNA extraction kit (easy-spin™ Total RNA Extraction Kit; iNtRON Biotechnology, Sungnam, Korea). PCR primers were obtained from the Bioneer oligonucleotide synthesis facility (Bioneer Corporation, Daejeon, Korea), and used as previously described (Kim et al., 2008). The quantitative real-time PCR (qRT-PCR) analysis was performed with subtle modifications of Kim et al. (2005). SYBR®GREEN PCR Master Mix (Applied Biosystems, Warrington, UK), and 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) were used for precise quantification of the transcript level. The PCR reactions were repeated at least twice independently with three replicates and a representative set of data is presented. Cyclophilin (*CYP1*, MGG_10447.5) was used as endogenous control gene for normalization in the qRT-PCR analysis and the sequences of the primer pairs (CYPqF/CYPqR, MHF16qF/MHF16qR, and MHF21qF/MHF21qR) were listed in Table 1. Semi-quantitative RT-PCR was performed using cDNA prepared according to the method mentioned above after heat shock treatment at 42°C for 45 min on the wild type and the *Δmhf16* mutant. The PCR reaction was performed with GeneAmp™ PCR system 2700 (Applied Biosystems, Foster City, CA, USA) and PCR conditions were set at 94°C for 3 min, and cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min for 22 or 27 cycles with final extension step at 72°C for 10 min. All the primers used for semi-quantitative RT-PCR (DK1Fa/DK1R for *MHS1*, DK2F/DK2R for *MHS2*, Xh_DK9F/DK9R for *MHS3*, DK3F/DK3R for *MHS4*, DK4F/DK4R for *MHS5*, DK5F/DK5R for *MHS6*, DK6F/DK6R for *MHS7*, and MTF/MTR for β-tubulin-encoding gene as endogenous control) were listed in Table 1.

Disruption of *MHF16* and *MHF21* and complementation. Based on the flanking sequences of MGG_06766.5 (*MHF16*) and MGG_08180.5 (*MHF21*) on the *M. oryzae* genome data (http://www.broad.mit.edu/annotation/fungi/magnaporthe), PCR primers were designed for construction of knockout vectors. The 1 kb fragments of the 5' and 3' flanking sequences of *MHF16* were amplified with *Kpn*I_DJ16_5F/*Bgl*II_DJ16_5R and *Sac*I_DJ16_3F/*Xba*I_DJ16_3R from the genomic DNA of the wild type strain KJ201, and cloned into pGEM-T-easy vector (Shanghai Promega, Shanghai, China). Each of the fragments was prepared with restriction enzyme digestion with combinations for ligation. Flanking fragments amplified (1 kb each) and the *hph* cassette from pSK597 (1.4 kb) were ligated into pSK1440 to generate pDJ005. The plasmids pSK597 and pSK1440 were gift from Dr. Seogchan Kang (The Pennsylvania State

Table 1. Primers used in this study

Gene name (Locus)	Primer name	Sequences
MHF16 (MGG_06766.5)	*Kpn*I_DJ16_5F	5'-gggtaccCAAGCGACTGGGATTTAT-3'
	*Bgl*II_DJ16_5R	5'-ggaagatctCACAGTGGCGTTTTGAG-3'
	*Sac*I_DJ16_3F	5'-cgagctcGCTTATGCGGTATTATTTTC-3'
	*Xba*I_DJ16_3R	5'-gctctgaaATTTATTGCGGAGAGTCG-3'
	MHF16qF	5'-ATCTGCCACTGAGAAGGTCT-3'
	MHF16qR	5'-TTTCCGGAATGTATGATGAA-3'
	Nc_DJ16F	5'-ccatggCCAACATGTTAGAACCGTTACC-3'
	DJ16R	5'-TCAATAGTCGTAAGACGCCG-3'
MHF21 (MGG_08180.5)	DJ21_5F	5'-AAGCTTGTTCGTCTTTCCTT-3'
	DJ21_5R	5'-TTCAGCTTGAGGGATGTCTA-3'
	DJ21_3F	5'-AGTTCCCCACCTACTTGACA-3'
	*Xba*I_DJ21_3R	5'-ctctagaAAATCAAGGACTGGCAGAAG-3'
	MHF21qF	5'-TGTTTGACGACTCAGGAAAG-3'
	MHF21qR	5'-GCGAGTGTAGAGTGCATGTT-3'
	Ps_DJ21F	5'-ctgcagGCGCGAATATCTAATACACCT-3'
	DJ21R	5'-CGCCTCTAAGCCAAATTACA-3'
CYP1 (MGG_10447.5)	CYPqF	5'-GCCTAACGTTTTCTTCGACATTTC-3'
	CYPqR	5'-GTTCTCGTCGGCAAACTTCTC-3'
MHS1 (MGG_02503.5)	Nc2_DK1Fa	5'-ccatggCGATGAAGTCAGGCTCAAG-3'
	DK1R	5'-TCATAGCTCATCGTGGGAAC-3'
	DK1Fa	5'-ACGATGAAGTCAGGCTCAAG-3'
MHS2 (MGG_02842.5)	2_DK2F	5'-gaGCTTTCCAAATGAGCGAAAA-3'
	DK2R	5'-TCACAATGTACCCCTGACACC-3'
	DK2F	5'-GCTTTCCAAATGAGCGAAAA-3'
MHS3 (MGG_03039.5)	DK9F	5'-CTCTCAACCAAGGTCAGAATC-3'
	Xh_DK9R	5'-ctcgagTCCTTCCCTTGTCTATCGGA-3'
MHS4 (MGG_04191.5)	Bg2_DK3F	5'-cagatctgATGCTCGCGTCAAGACTTTC-3'
	DK3R	5'-TTAAGGCTTGGTCTCGTCTTTC-3'
	DK3F	5'-ATGCTCGCGTCAAGACTTTC-3'
MHS5 (MGG_06065.5)	Bg2_DK4F	5'-aagatctgGCCACCATGTCTGTAGTTGG-3'
	DK4R	5'-TTATGCGCCATTAGCTTTAGC-3'
	DK4F	5'-GCCACCATGTCTGTAGTTGG-3'
MHS6 (MGG_06648.5)	Sm_DK5F	5'-cccgggAACACAGACGAGCATAGCAA-3'
	Sm_DK5R	5'-cccgggAAGTCTCGCATGTGAATCGT-3'
	DK5F	5'-GCCATGTCGCCTCTTATAAA-3'
	DK5R	5'-CTCAGCGATACTCTTCTTGATG-3'
MHS7 (MGG_06958.5)	DK6F	5'-AACATATTCAAGATGGCGCC-3'
	DK6R	5'-CCAGCACCGTAGAATTTCAT-3'
B-tubulin (MGG_00604.5)	MTF	5'-CTCCAGGGTTTCCAGATCAC-3'
	MTR	5'-CCTCACCAGTGTACCAATGC-3'

Lower-case letters indicate extended sequences used for cloning.

University, PA, USA). The plasmid was transformed to *Agrobacterium tumefaciens* strain AGL-1 and used for fungal transformation by *A. tumefaciens*-mediated transformation (ATMT). Similar processes were conducted for *MHF21*. The 5' flanking (1.7 kb) and 3' flanking (1.3 kb) fragments were amplified with primer combinations of DJ21_5F/DJ21_5R and DJ21_3F/*Xba*I_DJ21_3R, respectively, and cloned into pGEM-T-easy vector. Flanking fragments were prepared by restriction enzyme digestion with *Kpn*I and *Hind*III (for 1.3 kb 5' flanking fragment), *Bgl*II and *Xba*I (for 1.3 kb 5' flanking fragment) from the clones, and *hph* cassette (1.4 kb) was taken from pEMP1-3 (Ahn et al., 2004) by digestion with *Hind*III and *Bam*HI. The fragments were ligated to the pSK1440, which resulted in the KO vector of pSis203. The fungal transformation was performed by ATMT again with pSis203. The fungal transformants were selected in the medium including 200 ppm hygromycin and then dual selection using both of the media containing 5 μM 5-fuoro-2'-deoxyuridine (F2dU) as a final concentration, and the medium with hygromycin was

applied for the second selection (Khang et al., 2005). To construct complementation vectors, PCR was performed using the *Kpn*I_DJ16_5F and *Xba*I_DJ16_3R pair (for *MHF16*) and DJ21_5F and *Xba*I_DJ21_3R pair (for *MHF21*) with the genomic DNA of the wild type strain KJ201. Amplicons of 3.9 kb and 4.1 kb covering the coding regions with flanking regions of *MHF16* and *MHF21* were cloned to pCR®2.1-TOPO® vector (Invitrogen, Carlsbad, CA, USA). Each fragment was ligated to the multi-cloning site of pSK615 including the geneticin marker gene after restriction enzyme digestion with *Kpn*I, and *Kpn*I and *Xba*I combinations, respectively. The resulting clones (pDJ008 and pSIS205) were transformed to AGL-1 strain of *A. tumefaciens*. Complementation of the Δ*mhf16* mutant and the Δ*mhf21* mutant by wild type alleles were achieved by ATMT separately, and the transformants were selected on the selection medium containing 800 ppm geneticin. The candidate transformants were screened by PCR and further confirmed by Southern hybridization analysis.

Fungal development assays. For conidiation assay, mycelial discs of individual strains were grown on OMA at 25°C with continuous fluorescent light for 12 days with 3-4 replicates. Conidia were collected in 5ml of sterile distilled water (S.D.W.) and counted the number of conidia under hemacytometer. Three droplets of conidial suspension of 2×10^4 conidia ml^{-1} were placed on plastic cover slips (Marienfeld, Lauda-Königshofen, Germany) for conidial germination and appressorium formation analyses. The abilities of conidial germination and appressorium formation were observed under a light microscope after 12 h incubation in a moistened box at room temperature.

Pathogenicity assay. Conidia collected from 2-week-old OMA culture were resuspended in S.D.W. and the concentration was adjusted to 1×10^5 conidia ml^{-1} with Tween20 (250 ppm, final concentration). Five mililiter of spore suspensions from each strain were inoculated on 3 to 4-leaf-stage rice plants (susceptible rice cultivar Nagdongbyeo) by spraying. The inoculated plants were maintained in a dew chamber overnight at dark and moved back to the growth chamber. Disease severity was assessed 7 days after inoculation. For invasive growth assay, droplets of conidial suspension (2×10^4 conidia ml^{-1} in S.D.W.) were placed on onion epidermal cells floated on S.D.W. and the infection hyphae were observed 48 h after inoculation under a light microscope. The samples were stained with either Rose Bengal (0.5%) or Aniline Blue (0.5%).

Yeast two-hybrid assay. The cDNAs of putative ORFs encoding heat shock protein 70s (*MHS*s, *M. oryzae* heat shock protein seventy) were individually cloned into either pGEM-T-easy vector (*MHS1*, *3*, *4*, *5* and *MHF21* amplified with Nc2_DK1Fa/DK1R, Xh_DK9F/DK9R, Bg2_DK3F/DK3R, Bg2_DK4F/DK4R, and Ps_DJ22F/DJ22R in order) or pCR®2.1-TOPO® vector (Invitrogen) (*MHS2*, *6*, *7*, and *MHF16* amplified with DK2F/DK2R, Sm_DK5F/Sm_DK5R, DK6F/DK6R, and Nc_DJ17F/DJ17R, respectively) by PCR amplification using the primers listed in Table 1. Each fragment was subject to restriction enzyme digestion (*Nco*I and *Eco*RI for *MHS1* and *MHF16*, *Bam*HI and *Xho*I for *MHS2* and *MHS7*, *Eco*RI and *Xho*I for *MHS3*, *Bgl*II and *Eco*RI for *MHS4* and *MHS5* (introduced to *BamH*I and *EcoR*I sites in pACT2), *Sma*I for *MHS6*, *Pst*I for *MHF21*) and it was cloned to the yeast two-hybrid plasmids (pAS2-1 or pACT2; Clontech Laboratories, Palo Alto, CA, USA). The MHF16 and MHF21 were fused to GAL4-BD (pAS2-1), and the 7 MHSs were fused to GAL4-AD (pACT2). The clones in combinations were introduced into the *S. cerevisiae* strain AH109 (*MATa*, *trp1-901*, *leu2-3*, *ura3-52*, *his3-200*, *gal4D*, *gal80D*) cells by co-transformation. Subsequently, the transformants were selected on synthetic dropout (SD) agar medium lacking leucine and tryptophan (SD-LT). The selected colonies were screened on the SD agar medium lacking leucine, tryptophan, and histidine (SD-LTH) and the SD agar medium lacking leucine and tryptophan, histidine and adenine (SD-LTHA). All media and buffers for the yeast two-hybrid assay were prepared by the methods from the Matchmaker System 2 (Clontech Laboratories, Palo Alto, CA, USA).

Bioinformatic analysis. All bioinformatic analysis was performed at the Comparative Fungal Genomics Platform (CFGP; http://cfgp.snu.ac.kr/), a web-based bioinformatic database, which contains annotated genome sequence information of various organisms and bioinformatic analysis tools used in this study such as BLAST, ClustalW, InterProScan, and BLASTMatrix (Park et al., 2008). BLASTMatrix tool implemented in CFGP was used to find homologue proteins of Hsp40 family from 5 fungi with InterProScan (Mulder et al., 2005) results stored in CFGP. Amino acid sequences of Hsp40 J-domains from MHF16 and MHF21 homologues were aligned by ClustalW algorithm (Thompson et al., 1994) with default options and phylogenetic trees were constructed with 1,000 bootstrap repeats.

Results

Identification of genes encoding putative Hsp40 family proteins in *M. oryzae*. We searched among the annotated genes within the *M. oryzae* genome using the InterProScan program from the Comparative Fungal Genomics Platform database (Park et al., 2008). Six of the InterProScan terms

contained the description of DnaJ: Heat shock protein DnaJ, N-terminal (IPR001623); DnaJ central region (IPR-001305); Chaperone DnaJ, C-terminal (IPR002939); Heat shock protein DnaJ (IPR003095); Hsp40/DnaJ peptide binding (IPR008971); and Chaperone DnaJ (IPR012724). Twenty-eight genes were identified that contained one or more of the above terms, but none of the predicted proteins contained the Chaperone DnaJ (IPR012724) domain. All but MGG_11144.5 possessed the IPR001623, also called J-domain. Instead, the MGG_11144.5 contained only Chaperone DnaJ, C-terminal (IPR002939) in its predicted coding sequence based on the genomic information for *M. oryzae* strain 70-15. However, we detected an N-terminal domain (IPR001623) containing the conserved tripeptide HPD and Heat shock protein DnaJ (IPR003095) domain in a translation of the 5' flanking region by comparing it to the best-matched homologue from *Gibberella zeae* FGSG_09768.3. In the case of MGG_04462.5, we failed to detect a HPD motif in the J-domain, despite a high level of conservation in the nascent portion. We also detected the presence of an intron at the expected tripeptide site, and we attempted to adjust the intron by comparing it to matching sequences in *M. oryzae* strain KJ201 (unpublished data). A tripeptide motif was found, suggesting a strong possibility of mis-prediction for the intron in the *M. oryzae* strain 70-15 gene annotation. Neither MGG_07377.5, MGG_03709.5 nor MGG_00406.5 had the HPD tripeptide in their J-domains. The matched yeast homologue of MGG_00406.5 is PAM16, which was designated J-like protein 3 (Jlp3) in *S. cerevisiae* (Walsh et al., 2004); thus, we designated the above 3 proteins as J-like, and not Hsp40, proteins. The remaining 25 genes, which fulfilled the Hsp40 criteria,

Table 2. Identification of *MHF*s in the genome of *M. oryzae*

M. oryzae		*G. zeae*		*N. crassa*		*A. nidulans*		*S. cerevisiae*		
Gene name	Locus name	Locus name	e-value	Locus name	e-value	Locus name	e-value	Locus name	e-value	Gene name
–	MGG_00406.5	FGSG_08333.3	2.00E-41	NCU05515.2	2.00E-44	AN5693.3	5.00E-41	–	–	–
MHF1	MGG_00967.5	FGSG_09932.3	9.00E-56	NCU04305.2	2.00E-43	AN4403.3	3.00E-34	YFL016C	1.00E-12	MDJ1
MHF2	MGG_01587.5	–	–	–	–	AN10224.3	2.00E-36	–	–	–
MHF3	MGG_01834.5	FGSG_05098.3	1.00E-131	NCU05199.2	1.00E-111	AN0892.3	1.00E-41	YNL227C	4.00E-11	JJJ1
MHF4	MGG_03389.5	FGSG_06080.3	3.00E-44	NCU06150.2	1.00E-46	–	–	YPR061C	8.00E-09	JID1
MHF5	MGG_03565.5	FGSG_09848.3	1.00E-163	NCU02424.2	1.00E-180	AN3463.3	1.00E-122	YMR214W	6.00E-14	SCJ1
MHF6	MGG_03581.5	FGSG_09857.3	1.00E-160	NCU02432.2	1.00E-172	AN9060.3	1.00E-103	YNL227C	7.00E-63	JJJ1
–	MGG_03709.5	FGSG_00939.3	2.00E-94	–	–	AN8179.3	8.00E-23	–	–	–
MHF7	MGG_03907.5	FGSG_01620.3	5.00E-96	NCU03335.2	9.00E-97	AN4441.3	5.00E-87	YMR161W	1.00E-22	HLJ1
MHF8	MGG_04080.5	FGSG_05978.3	0	NCU03622.2	0	AN8798.3	0	YDR320C	9.00E-47	SWA2
MHF9	MGG_04180.5	FGSG_09767.3	3.00E-90	NCU07713.2	8.00E-80	AN0590.3	4.00E-75	YMR161W	1.00E-06	HLJ1
MHF10	MGG_04462.5	FGSG_06825.3	1.00E-170	NCU07414.2	1.00E-166	AN2731.3	1.00E-144	YNL064C	2.00E-98	YDJ1
MHF11	MGG_05249.5	FGSG_04121.3	1.00E-154	NCU00465.2	1.00E-159	AN7360.3	1.00E-117	YNL064C	7.00E-66	YDJ1
MHF12	MGG_05319.5	FGSG_06678.3	0	NCU00170.2	0	AN4192.3	1.00E-157	YMR214W	4.00E-22	SCJ1
MHF13	MGG_05320.5	FGSG_06644.3	0	NCU00169.2	0	AN0834.3	0	YOR254C	2.00E-52	SEC63
MHF14	MGG_05820.5	FGSG_06080.3	8.00E-23	NCU06150.2	6.00E-26	–	–	–	–	–
MHF15	MGG_06486.5	FGSG_07301.3	1.00E-32	NCU06415.2	4.00E-32	AN0059.3	3.00E-23	YNL064C	9.00E-10	YDJ1
MHF16	**MGG_06766.5**	FGSG_02004.3	1.00E-31	NCU04145.2	2.00E-30	–	–	–	–	–
MHF17	MGG_07295.5	FGSG_10185.3	1.00E-113	NCU01284.2	1.00E-115	AN9101.3	2.00E-62	YMR214W	6.00E-09	SCJ1
–	MGG_07377.5	–	–	–	–	–	–	–	–	–
MHF18	MGG_07502.5	FGSG_10390.3	1.00E-170	NCU11102.2	1.00E-173	AN6170.3	1.00E-146	YMR214W	5.00E-76	SCJ1
MHF19	MGG_07712.5	FGSG_01083.3	5.00E-96	NCU02491.2	2.00E-66	AN10706.3	3.00E-60	YER048C	2.00E-10	CAJ1
MHF20	MGG_07716.5	FGSG_07359.3	1.00E-133	NCU02490.2	1.00E-143	AN5770.3	1.00E-103	YFR041C	3.00E-09	ERJ5
MHF21	**MGG_08180.5**	FGSG_05133.3	1.00E-137	NCU03732.2	1.00E-149	AN2238.3	1.00E-118	YNL007C	1.00E-75	SIS1
MHF22	MGG_09235.5	FGSG_09380.3	0	NCU06052.2	0	AN3375.3	1.00E-150	YIR004W	5.00E-45	DJP1
MHF23	MGG_10159.5	FGSG_06723.3	8.00E-45	NCU00075.2	8.00E-40	AN4559.3	9.00E-39	YLR008C	2.00E-22	PAM18
MHF24	MGG_10565.5	FGSG_02785.3	0	NCU03009.2	0	AN7143.3	1.00E-176	YGR285C	5.00E-98	ZUO1
MHF25	MGG_11144.5	FGSG_09768.3	5.00E-60	NCU05196.2	4.00E-57	AN10778.3	8.00E-51	YFL016C	1.00E-13	MDJ1

Genes encoding putative Hsp40s in *M. oryzae* (*MHF*s) were identified using the InterProScan program and a sequence-based strategy; they are listed with other fungal homologues matched by the BLASTMatrix in CFGP (http:// www.cfgp.snu.ac.kr/). *G. zeae*, *Gibberella zeae*; *N. crassa*, *Neurospora crassa*; *A. nidulans*, *Aspergillus nidulans*; *S. cerevisiae*, *Saccharomyces cerevisiae*.

were designated *MHF* (for *M. oryzae* heat shock protein forty) genes and were numbered according to the locus name (Table 2).

We next classified the putative MHF proteins based on their predicted domain composition and peptide sequences, similar to the sequence-based classification system used in a previous study of yeast Hsp40s (Cheetham and Caplan, 1998). MHF10, MHF11, and MHF18 were categorized as type I proteins, meaning that they contained all of the conserved domains, including a J-domain in the N-terminal region, a zinc finger (-like) domain, and a G/F-rich domain. MHF21 and MHF7 were categorized as type II proteins because they possessed all of the above domains except the zinc finger (-like) domain. MHF5, MHF8, MHF12, and

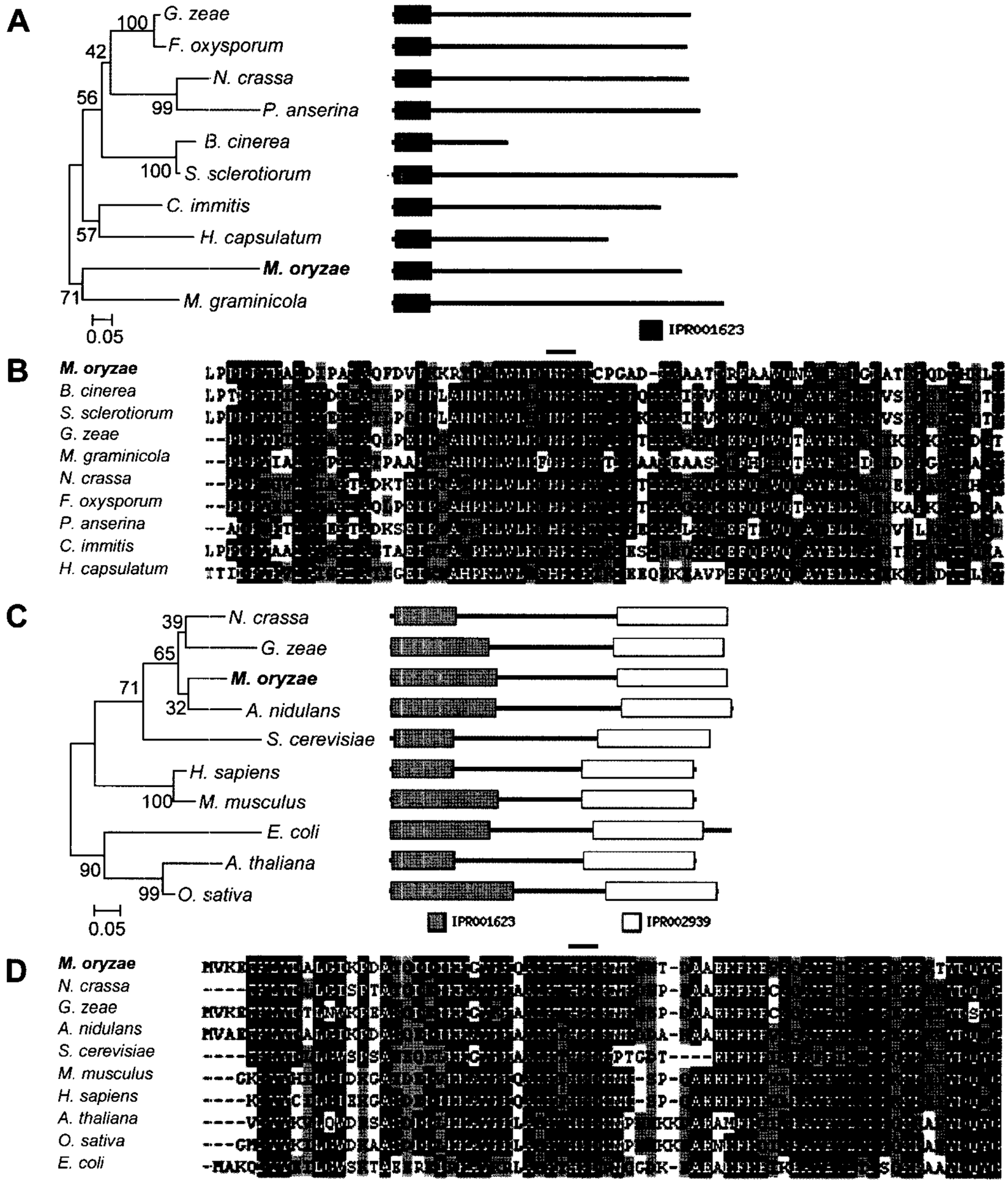

Fig. 1. Sequence alignment and phylogenetic analysis of the J-domains in MHF16 and MHF21 and their putative homologues. (A) and (C) A phylogenetic tree was generated from an alignment of the J-domains predicted using InterProScan program with the best-matched homologues of MHF16 and MHF21, respectively, which were retrieved by BLASTMatrix from the CFGP (http://www.cfgp.snu.ac.kr/). IPR001623, Heat shock protein DnaJ, N-terminal; IPR002939, Chaperone DnaJ, C-terminal. (B) and (D) Sequence alignment of the J-domains from homologues of MHF16 and MHF 21, respectively, by ClustalW. The essential tripeptide, HPD, is indicated by a black bar. *G. zeae*, *Gibberella zeae*; *F. oxysporum*, *Fusarium oxysporum*; *N. crassa*, *Neurospora crassa*; *P. anserina*, *Podospora anserina*; *B. cinerea*, *Botrytis cinerea*; *S. Sclerotiorum*, *Sclerotinia sclerotiorum*; *C. immitis*, *Coccidioides immitis*; *H. capsulatum*, *Histoplasma capsulatum*; *M. graminicola*, *Mycosphaerella graminicola*. *A. nidulans*, *Aspergillus nidulans*; *S. cerevisiae*, *Saccharomyces cerevisiae*; *H. sapiens*, *Homo sapiens*; *M. musculus*, *Mus musculus*; *A. thaliana*, *Arabidopsis thaliana*; *O. sativa*, *Oryzae sativa*.

MHF23 were grouped together as type III proteins due to the location of the J-domain in the C-terminal region, instead of at the N-terminus. The others were classified as type III, meaning that they possessed a single J-domain with weak conservation in the other domains.

Potential homologues of the MHFs in other fungi were identified using the BLASTMatrix tool in the CFGP database. The criterion for the e-value was set at <1e-5, and those matched proteins without Hsp40-/DnaJ-related domains were excluded from the list (Table 2). A homologue of MHF2 was identified only in *Aspergillus nidulans*, whereas no homologues of MHF14 or MHF16 were found in *A. nidulans* or *S. cerevisiae*. Both MHF4 and MHF14 were matched to the same proteins in *G. zeae* and *Neurospora crass* (FGSC_06080.3 and NCU06150.2), respectively. In addition, MHF18, MHF12, MHF5, and MHF17 were matched to SCJ1; MHF10, MHF11, and MHF15 were matched to YDJ1; MHF7 and MHF9 were matched to HLJ1; MHF25 and MHF1 were matched to MDJ1; and MHF6 and MHF3 were matched to JJJ1 in *S. cerevisiae* (Table 2).

We next decided to functionally characterize two of the Hsp40-encoding genes, *MHF16* and *MHF21*. MHF16 (locus MGG_06766.5), which was defined as a type III protein was weakly conserved in other species, whereas MHF21 (locus MGG_08180.5), which was classified as a type II protein, was well conserved in all of the organisms considered (Fig. 1 and Table 2). The predicted aa sequences of the J-domains in MHF16 and MHF21, which were determined using the InterProScan program, were extracted and trimmed. The domain sequences were then aligned and phylogenetically analyzed with their respective potential homologues using the ClustalW algorithm (Fig. 1).

Disruption of two Hsp40-encoding genes in *M. oryzae*. The *MHF16* and *MHF21* genes were deleted by targeted gene replacement using *Agrobacterium tumefaciens*-mediated transformation (ATMT) with dual selection. *MHF16* consists of a 1,692 bp ORF with no introns and encodes a protein with 563 aa, while *MHF21* is composed of a 1,287 bp ORF with two introns (102 and 69 bp) and encodes a protein of 371 aa according to the genomic information for *M. oryzae* strain 70-15 (http://www.broad.mit.edu/annotation/fungi/magnaporthe/). The cDNA sequence of *MHF21* from *M. oryzae* strain KJ201 coincided with that from strain 70-15; however, a single nucleotide difference (A to C at nucleotide 999, producing an E to D substitution) was found between the coding sequences of *MHF16* and strain 70-15 (data not shown). Fungal transformation was performed using *M. oryzae* strain KJ201 with knockout vectors containing both a hygromycin-resistance gene and a dual selection marker (*HSVtk*). Out of the more than 1,000 transformants obtained for each gene replacement, 14 Δ*mhf16* and 7 Δ*mhf21* candidates were screened by PCR and Southern hybridization analysis (Fig. 2). Each of the confirmed deletion mutants (Δ*mhf16*-13 and Δ*mhf21*-2) was used in our subsequent experiments as a representative mutant strain since no difference was observed among the confirmed mutants.

For complementation of the mutants, vectors were constructed by PCR that contained fragments of the ORFs and flanking regions. Fungal transformation was performed by ATMT, and those transformants with a single-copy insertion were identified by PCR and Southern hybridization analysis (Fig. 2). Recovery of the transcription of *MHF16* and *MHF21* was verified by Northern hybridization analysis (data not shown).

Expression profiles of *MHF16* and *MHF21* under heat and cold shock conditions. The transcriptional regulation of *MHF16* and *MHF21* under cold shock and various heat shock conditions was explored by quantitative real-time PCR (qRT-PCR) analysis. Under cold shock conditions (4°C for 40 or 80 min), a reduction occurred in the expression of *MHF16* and *MHF21* compared to that under the control condition (25°C; Fig. 3A). In contrast, the expression of *MHF16* under various heat shock conditions (42°C for 5, 10, 20, 40, or 80 min) was down-regulated, while the expression of *MHF21* was not significantly altered (Fig. 3A). The mRNA expression of *MHF16* and *MHF21* was also analyzed during various developmental stages, including conidial germination, appressorium formation,

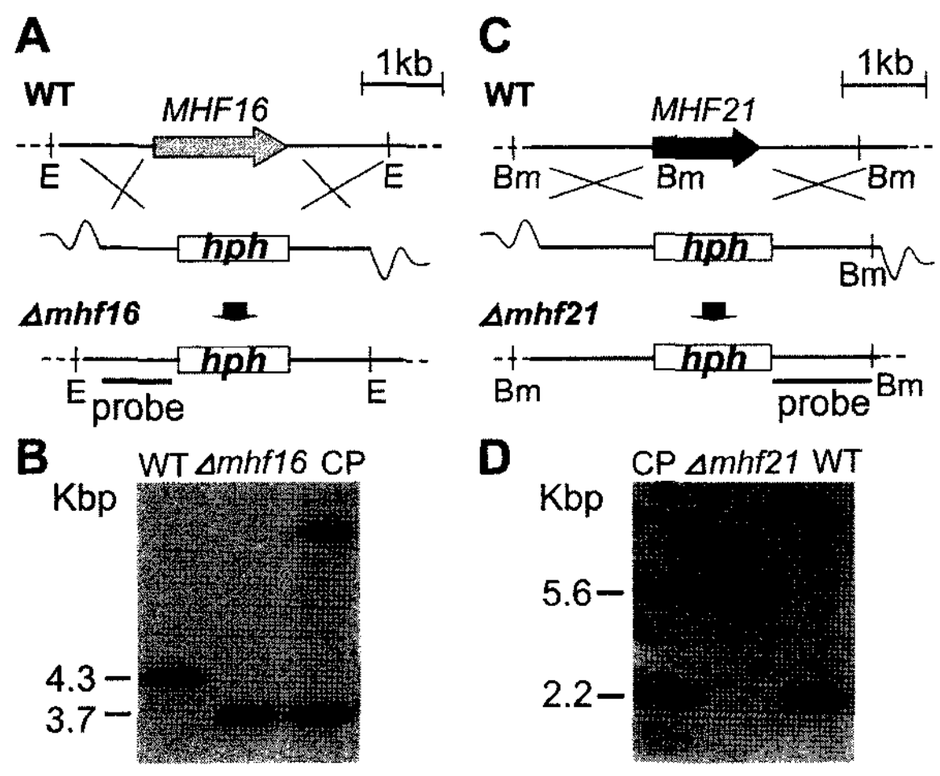

Fig. 2. Generation of the deletion mutants and confirmation by Southern hybridization analysis. (A) and (C) Generation of the deletion mutants by homologous recombination; the location of the probes for Southern hybridization analysis is marked with a bar. E, *Eco*RI; Bm, *Bam*HI. (B) and (D) Genomic DNA from each strain was digested with *Eco*RI and *Bam*HI, respectively, and probed with the fragment marked in (A) and (C). WT, wild type; CP, complementation transformant.

conidiation, and infection *in planta* by qRT-PCR. Significant up-regulation of *MHF16* during conidiation (2.7-fold) was evident, while down-regulation of *MHF21* during appressorium formation (0.1-fold) was detected (Fig. 3B).

Developmental defects in the Δ*mhf16* and Δ*mhf21* mutants. While the wild type strain grew evenly with dense aerial mycelia, the colonies of the Δ*mhf16* mutant exhibited random changes in aerial mycelia density and pigmentation, although their morphology was similar to that of the wild type in some cases (Fig. 4A and 4B). This phenomenon was also observed within a single plate, and the altered morphology of the colonies was randomly repeated upon transfer to fresh plates (Fig. 4B). We referred to this type of altering colony morphology as "sectoring." Each of the altered sectors grew well on hygromycin, and the mutated allele with hygromycin cassette was found to be preserved by Southern hybridization analysis (data not shown). Sectoring was observed during at least 3 successive transfers to fresh media. In contrast, the morphology of the Δ*mhf21* mutant was the same as that of the wild type (i.e., without sectoring; data not shown).

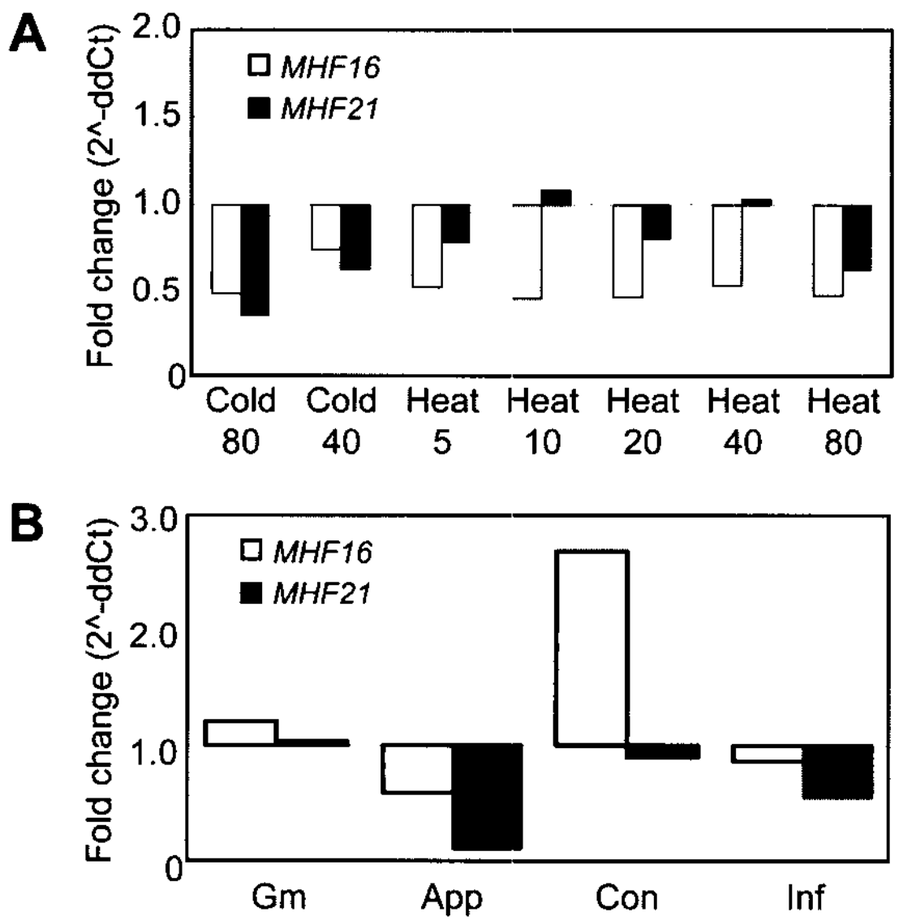

Fig. 3. Expression profiles of *MHF16* and *MHF21*. The transcriptional expression of *MHF16* and *MHF21* was analyzed by quantitative real-time PCR. The values were calculated against those for an untreated sample grown in liquid complete medium after normalization with *CYP1* (endogenous control gene). (A) Mycelia grown in liquid complete medium for 3 days were cold shocked at 4°C for 40 or 80 min or heat shocked at 42°C for 5, 10, 20, 40, and 80 min. (B) Expression analysis during conidial germination, appressorium formation, conidiation, and infection *in planta*. Gm, conidial germination (2 h); App, appressorium formation (4 h on a hydrophobic surface); Con, conidiation (15 days on oatmeal agar medium); Inf, infection *in planta* (150 h after inoculation on the susceptible rice cultivar Nagdongbyeo).

Conidiation in the Δ*mhf16* and Δ*mhf21* mutants decreased significantly on oatmeal agar medium (OMA) in terms of the number of conidia generated. The conidiation rates in the mutants were reduced to 7% and 15%, respectively, of the level in the wild type strain (Table 3). Reduced and slow conidiophore development was also observed in the mutants compared to the wild type. The number of sympodially generated conidia on the conidiophore in the mutants (1-3 after 24 h) was less than that in the wild type (3-5 or more after 24 h). However, no change occurred in the shape of the conidia, and all of the conidia were viable in both mutants. The defects in conidiation in the Δ*mhf16* and Δ*mhf21* mutants and the sectoring observed in Δ*mhf16* were successfully complemented when the wild type alleles were reintroduced (Table 3 and Fig. 4A).

Pathogenicity of the Δ*mhf16* and Δ*mhf21* mutants. Conidial germination and appressorium formation were

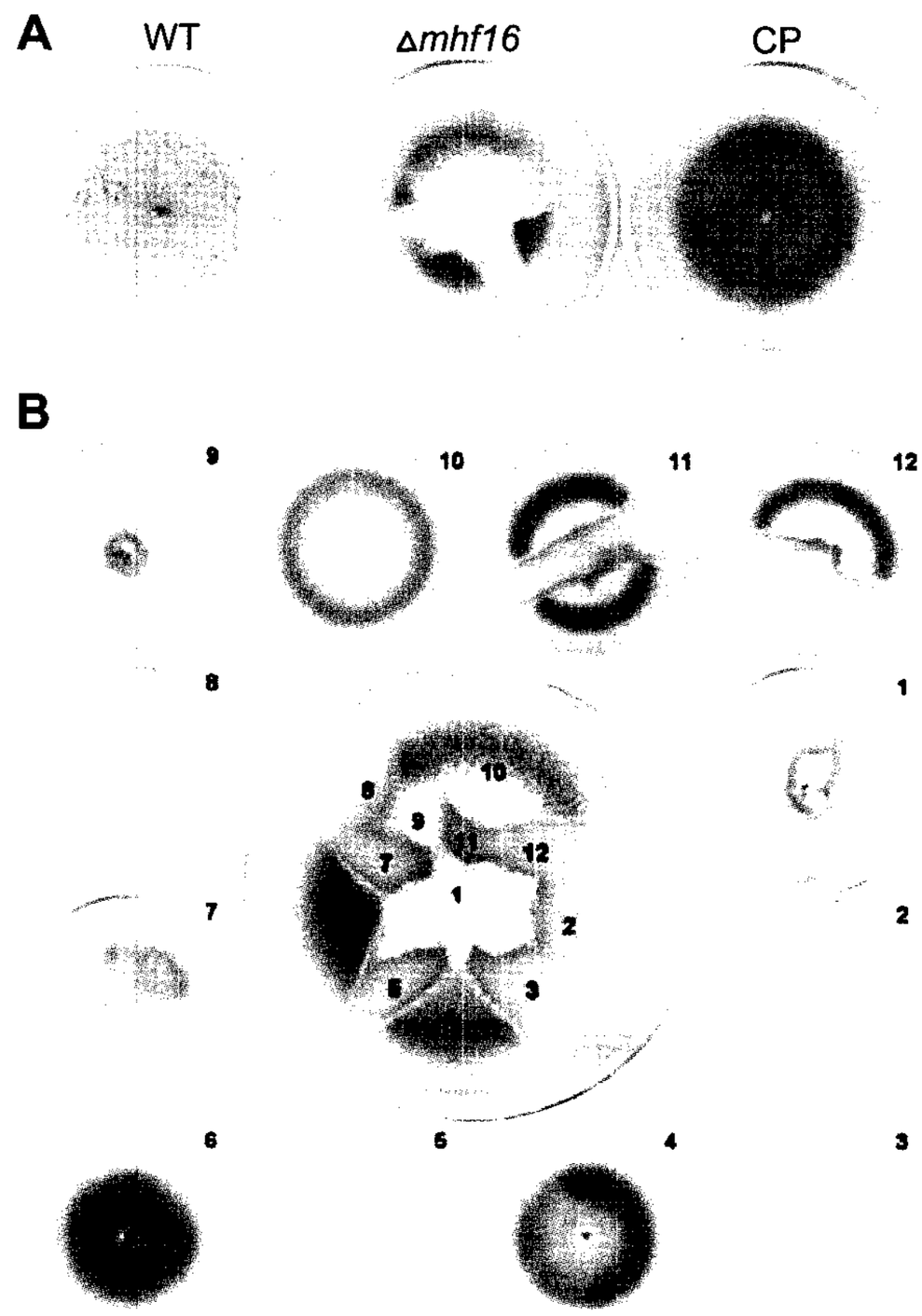

Fig. 4. Sectoring morphology of Δ*mhf16*. (A) Each strain was grown on oatmeal agar medium (OMA) for 2 weeks. WT, wild type; CP, complementation transformant. (B) Using one of the plates showing severe sectoring (in the middle), each sector was transferred to fresh OMA and photos were taken 2 weeks later. The corresponding number on the original plate is shown for each sector.

Table 3. Defects in conidiation development in the Δ*mhf16* and the Δ*mhf21* mutants

	Conidiation rate ($\times 10^4$ conidia ml^{-1})[a]	Conidial germination rate (%)[b]	Appressorium formation rate (%)[b]
Wild type	455.0 ± 94.0	99.1 ± 0.8	98.8 ± 0.6
Δ*mhf16*	33.0 ± 17.0	99.1 ± 1.0	95.5 ± 3.7
Δ*mhf16/MHF16*	225.0 ± 101.0	96.0 ± 0.7	91.6 ± 0.6
Wild type	182.0 ± 49.0	99.7 ± 0.5	98.8 ± 0.5
Δ*mhf21*	59.0 ± 5.0	99.4 ± 0.5	99.1 ± 0
Δ*mhf21/MHF21*	173.0 ± 2.0	97.6 ± 0.5	98.8 ± 0.6

[a]The conidiation rate of each strain on oatmeal agar medium after 12 days of growth was compared.
[b]Droplets of spore suspensions from each strain were placed onto the hydrophobic plastic coverslips in a humidity box at 25°C and conidial germination and the appressorium formation rate were observed under a light microscope 12 h later.

unaffected by the deletion of *MHF16* or *MHF21* (Table 3). The conidia of the Δ*mhf16* and Δ*mhf21* mutants germinated, formed appressoria, and successfully penetrated onion epidermal cells. The invasive growth of the mutants was similar to that of the wild type when observed 72 h after inoculation (Fig. 5A and 5B). They also successfully infected the susceptible rice cultivar Nagdongbyeo when inoculated by spraying. Typical diamond-shaped lesions surrounded by a yellow hollow were observed on the leaves sprayed with the wild type and mutants (Fig. 5C and 5D).

Interaction of MHF16 and MHF21 with MHSs. To find the physically interacting partners of MHF16 and MHF21, we performed a yeast two-hybrid analysis using the putative homologues of Hsp70 in *M. oryzae*. We searched the genomic sequence of *M. oryzae* using InterProScan from the CFGP, and found 9 proteins with Hsp70-related domains (IPR001023, Heat shock protein Hsp70; IPR013126, Heat shock protein 70; and IPR012725, Chaperone DNAK). Seven putative *Hsp70* transcripts were confirmed by RT-PCR, which were subsequently designated as *MHS*s (*M. oryzae* heat shock protein seventy) according to the locus number (*MHS1-7*). The cDNAs of the 7 *MHS*s were fused to the activation domain (AD) in pACT2, while *MHF16* and *MHF21* were fused to the binding domain (BD) in pAS2-1. No positive interaction was observed, regardless of the combination, on SD-LTH or SD-LTHA media (Fig. 6). Moreover, none of the *MHS*s was induced by heat shock when examined by semi-quantitative RT-PCR. Most of the *MHS*s (*MHS3-7*) were down-regulated in response to heat shock conditions, and this pattern of gene expression was unaltered even in the Δ*mhf16* mutant (Fig. 7).

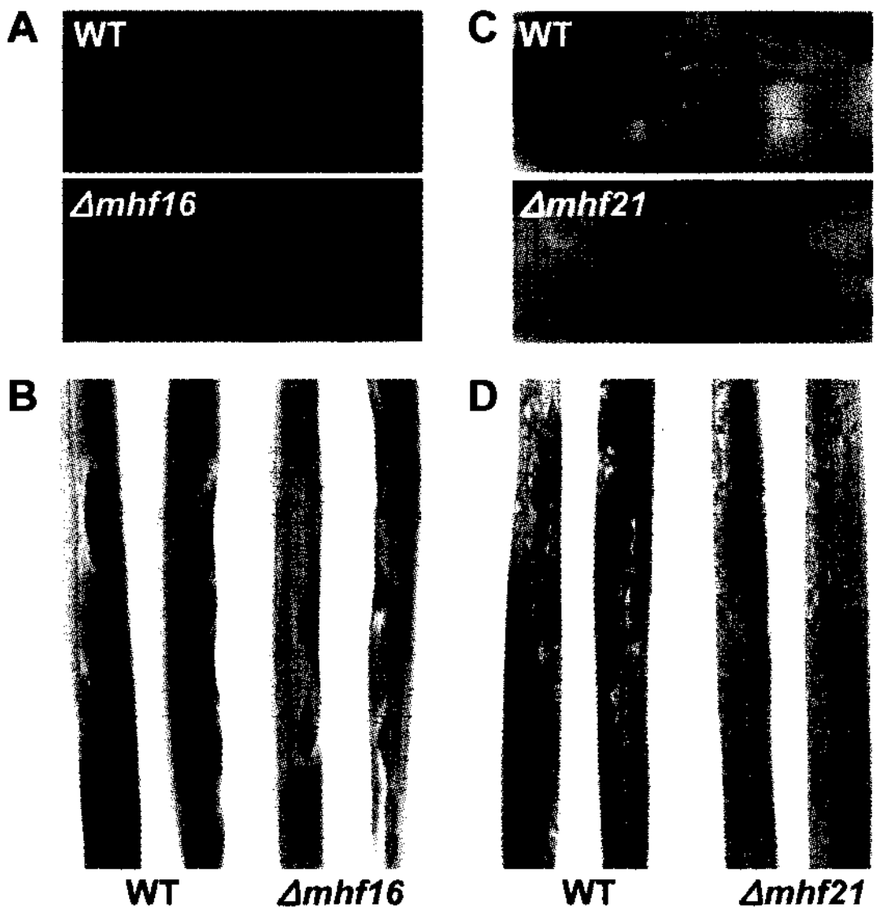

Fig. 5. Pathogenicity of Δ*mhf16* and Δ*mhf21*. (A) and (C) A spore suspension from each strain was placed on onion epidermal strips; infectious growth was observed 72 h after inoculation under a light microscope. The samples were stained with 0.5% Rose Bengal (A) and 0.5% Aniline Blue (C). (B) and (D) Each strain was spray-inoculated onto the susceptible rice cultivar Nagdongbyeo, and photos were taken 7 days after inoculation.

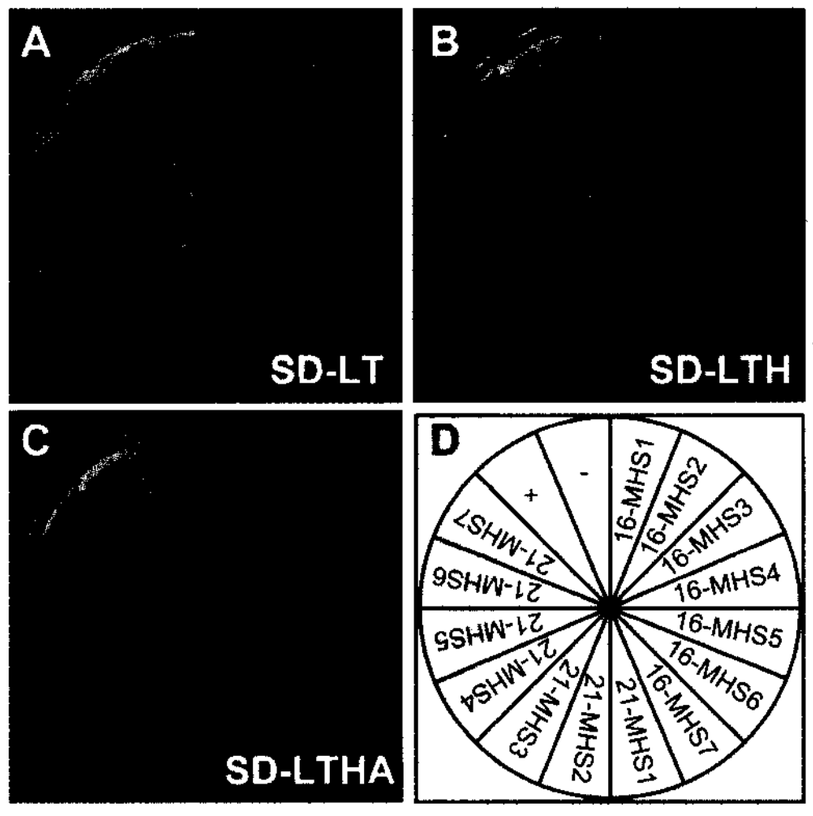

Fig. 6. Physical interactions among MHF16, MHF21, and the MHSs. MHF16 and MHF21 were fused to the binding domain in pAS2-1, then tested against MHSs fused to the activation domain in pACT2 by co-transformation into yeast cells. SV40 large T antigen (pTD1-1) with murine p53 (pVA3-1) and human lamin C (pLAM5'-1) were used as positive (+) and negative (–) controls, respectively.

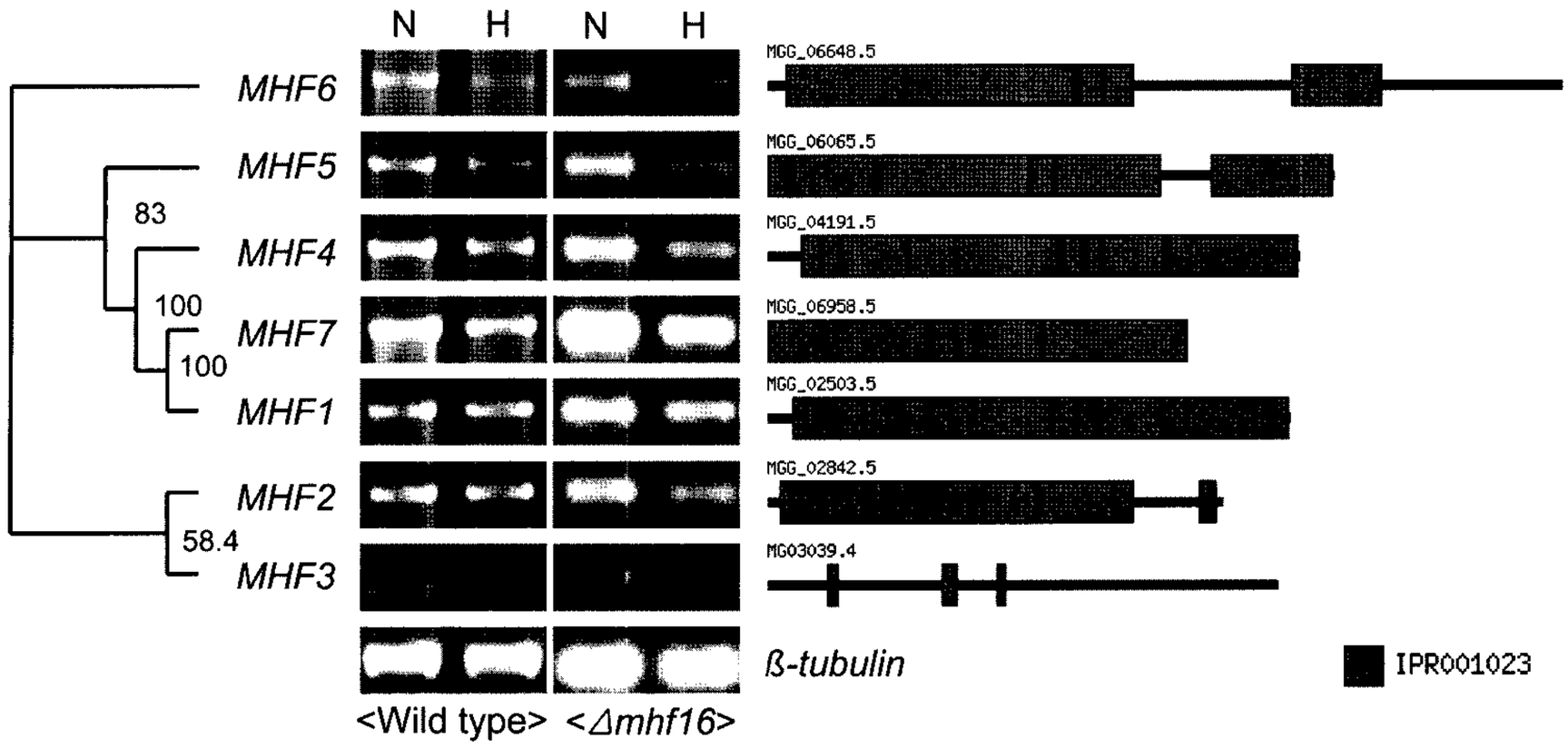

Fig. 7. Expression profiles of the *MHS*s under conditions of heat shock and after the deletion of *MHF16*. *MHF* expression in response to heat shock was analyzed by semi-quantitative RT-PCR in the wild type and the Δ*mhf16* mutant. Heat shock treatment at 42°C for 45 min (H) was applied to both the wild type and the Δ*mhf16* mutant and compared to the no treatment (control) condition (N). The domain architecture of each *MHS* is depicted with IRP001023 (Heat shock protein Hsp70); the phylogenetic tree was constructed after alignment with ClustalW.

Discussion

We identified 25 *Hsp40* genes in the genome of *M. oryzae* based on an InterProScan analysis (Mulder et al., 2005) and the definition of a J-domain (Walsh et al., 2004). All of the putative Hsp40s (i.e., MHFs) had a J-domain, which includes the conserved tripeptide HPD and several additional conserved residues (Fig. 1). The 25 MHFs from *M. oryzae* were grouped into 3 subtypes (Cheetham and Caplan, 1998) as follows: 3 type I (MHF10, MFH11, and MHF18), 2 type II (MHF7 and MHF21), and 20 type III (the others) Hsp40s. The type I Hsp40s were the most conserved, whereas the type III Hsp40s were less conserved among the various organisms considered (Table 2). Fewer type I and type II proteins were predicted for *M. oryzae* compared to *S. cerevisiae* (5 and 4, respectively) among the 22 Hsp40s. The type III Hsp40s included several proteins of unknown function in yeast; thus they can be described as a distinct group in terms of their cellular functions, whereas the type I and type II Hsp40s included classical chaperones known to function in protein folding by presenting substrates to their partner Hsp70s (Kelly, 1998; Walsh et al., 2004). The larger number of *Hsp40*s and the presence of a greater number of type III proteins may be due to variation in the genes encoding Hsp40 in *M. oryzae* with specialized functions as a plant pathogen.

We performed a series of biological assays to elucidate the functions of two of the *Hsp40* genes, *MHF16* and *MHF21*, in the rice blast fungus. First, deletion mutants of the *MHF*s were successfully generated by targeted fungal transformation using ATMT (Rho et al., 2001). The application of a dual selection marker system with a negative selection marker for the exclusion of ectopic transformants (Khang et al., 2005) was very helpful in terms of screening the transformants. This strategy will accelerate the functional characterization of the other *Hsp40*s in *M. oryzae*. In the case of *S. cerevisiae*, the deletion of *SIS1*, a homologue of *MHF21*, resulted in a lethal phenotype due to defects in translation initiation (Luke et al., 1991; Zhong and Arndt, 1993). The Δ*mhf21* mutant in *M. oryzae*, however, was viable and grew well vegetatively. Another Hsp40 or a different pathway, may exist that can mediate the functions of *MHF21* in *M. oryzae*, unlike in *S. cerevisiae*, because the total number of *Hsp40*s in *M. oryzae* is greater than that in yeast and the identification of multiple *MHF* matches to yeast *Hsp40*. No matching *Hsp40* homologue of *MHF16* was found in *S. cerevisiae*, and the Δ*mhf16* mutant showed a unique growth pattern on solid agar medium, which we called sectoring. The sectoring differed from a suppressor mutation because the degree of sectoring and the patterns were random. It was also different from the morphology of the *buf* mutant (Chumley and Valent, 1990) because the *buf*-like sectors in the Δ*mhf16* mutant were able to produce a wild type morphology in some cases by re-sectoring, whereas the *buf* mutation is stable and cannot produce a wild type revertant. No loss of the *MHF16* deletion alleles occurred in the sectors, as determined by Southern hybridization analysis (data not shown). Normal colonies with circular margins, an evenly pigmented mycelial mat, and dense conidiation were observed in the complementation transformants of the Δ*mhf16* mutant. These results imply that sectoring was not

caused by genetic alteration in other alleles. The unstable morphology of the colonies may be due to improper folding or the modification of a broad range of proteins. Additional experiments at the proteome level may explain the mechanism of sectoring due to the loss of *MHF16*.

The expression of *MHF16* and *MHF21* was not induced by heat shock conditions; instead the expression of *MHF16* was down-regulated by about 50%, while that of *MHF21* was not greatly altered. In addition, the mRNA expression of the *MHS*s was not appreciably up-regulated by heat shock conditions, and this pattern of expression was unaltered in the Δ*mhf16* mutant as shown by semi-quantitative RT-PCR (Fig. 7). Not all heat shock proteins are heat-inducible, although the first Hsps to be identified exhibited extreme inducibility under heat shock conditions (Craig, 1985; Werner-Washburne et al., 1989). In terms of development, the expression of *MHF16* was induced considerably during conidiation, and this may explain the defective phenotype of Δ*mhf16* during conidiation. The expression of *MHF21*, however, was not greatly altered during conidiation and was down-regulated during the appressorium formation stage, although the Δ*mhf21* mutant showed a significant reduction in conidiation. MHF21 expression may be regulated translationally or post-translationally rather than during transcription.

No physical interaction was detected between MHF16 or MHF21 and the 7 MHSs based on the yeast two-hybrid assay. MHF16 or MHF21 may be functionally independent of any interaction with Hsp70s. However, the interaction may have been very weak under the conditions used in this study, or some other component may be required as a mediator.

The virulence of the Δ*mhf16* and Δ*mhf21* mutants was equal to that of the wild type strain. Conidiation was severely affected in these mutants, but the conidia were functional and pathogenic on rice leaves. Still, considering the polycyclic nature of the fungus in the field, defects in conidiation may be critical for the rice blast epidemics. Since conidia are important secondary inoculums as well as primary inoculums in many plant pathogenic fungi, knowing whether other Hsps are involved in conidiation would be extremely useful.

In this study, we identified 25 MHF-encoding genes in the genome of *M. oryzae*, and revealed the functions of *MHF16* and *MHF21* during conidiation by functional analysis. Our results imply that Hsp40s appear to play important roles in the development of the rice blast fungus despite their redundancy. This is the first report on Hsp40s by a genome-wide approach and their biological functions in plant pathogenic fungi. Assessing the biological functions of the other 23 Hsp40s is warranted to decipher the function of the Hsp40 family in *M. oryzae*.

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References

Ahn, N., Kim, S., Choi, W., Im, K. H. and Lee, Y. H. 2004. Extracellular matrix protein gene, *EMP1*, is required for appressorium formation and pathogenicity of the rice blast fungus, *Magnaporthe grisea*. *Mol. Cells* 17:166-173.

Bonato, M. C., Silva, A. M., Gomes, S. L., Maia, J. C. and Juliani, M. H. 1987. Differential expression of heat-shock proteins and spontaneous synthesis of HSP70 during the life cycle of *Blastocladiella emersonii*. *Eur. J. Biochem.* 163:211-220.

Burnie, J. P., Carter, T. L., Hodgetts, S. J. and Matthews, R. C. 2006. Fungal heat-shock proteins in human disease. *FEMS Microbiol. Rev.* 30:53-88.

Caplan, A. J. and Douglas, M. G. 1991. Characterization of YDJ1: a yeast homologue of the bacterial dnaJ protein. *J. Cell Biol.* 114:609-621.

Cheetham, M. E. and Caplan, A. J. 1998. Structure, function and evolution of DnaJ: conservation and adaptation of chaperone function. *Cell Stress Chaperones* 3:28-36.

Chumley, F.G. and Valent, B. 1990. Genetic analysis of melanin-deficient, nonpathogenic mutants of *Magnaporthe grisea*. *Mol. Plant-Microbe. Interact.* 3:135-143.

Craig, E. A. 1985. The heat shock response. *CRC Crit. Rev. Biochem.* 18:239-280.

Dean, R. A., Talbot, N. J., Ebbole, D. J., Farman, M. L., Mitchell, T. K., Orbach, M. J., Thon, M., Kulkarni, R., Xu, J. R., Pan, H., Read, N. D., Lee, Y. H., Carbone, I., Brown, D., Oh, Y. Y., Donofrio, N., Jeong, J. S., Soanes, D. M., Djonovic, S., Kolomiets, E., Rehmeyer, C., Li, W., Harding, M., Kim, S., Lebrun, M. H., Bohnert, H., Coughlan, S., Butler, J., Calvo, S., Ma, L. J., Nicol, R., Purcell, S., Nusbaum, C., Galagan, J. E. and Birren, B. W. 2005. The genome sequence of the rice blast fungus *Magnaporthe grisea*. *Nature* 434:980-986.

Ebbole, D. J. 2007. *Magnaporthe* as a model for understanding host-pathogen interactions. *Annu. Rev. Phytopathol.* 45:437-456.

Fan, C. Y., Lee, S., Ren, H. Y. and Cyr, D. M. 2004. Exchangeable chaperone modules contribute to specification of type I and type II Hsp40 cellular function. *Mol. Biol. Cell.* 15:761-773.

Gething, M. J. 1997. Protein folding. The difference with prokaryotes. *Nature* 388:329-331.

Gething, M. J. and Sambrook, J. 1992. Protein folding in the cell. *Nature* 355:33-45.

Greene, M. K., Maskos, K. and Landry, S. J. 1998. Role of the J-domain in the cooperation of Hsp40 with Hsp70. *Proc. Natl.*

Acad. Sci. USA 95:6108-6113.
Heikkila, J. J. 1993. Heat shock gene expression and development. I. An overview of fungal, plant, and poikilothermic animal developmental systems. *Dev. Genet.* 14:1-5.
Jeon, J., Park, S. Y., Chi, M. H., Choi, J., Park, J., Rho, H. S., Kim, S., Goh, J., Yoo, S., Park, J. Y., Yi, M., Yang, S., Kwon, M. J., Han, S. S., Kim, B. R., Khang, C. H., Park, B., Lim, S. E., Jung, K., Kong, S., Karunakaran, M., Oh, H. S., Kim, H., Kang, S., Choi, W. B. and Lee, Y. H. 2007. Genome-wide functional analysis of pathogenicity genes in the rice blast fungus. *Nat. Genet.* 39:561-565.
Kaufmann, S. H. 1990. Heat shock proteins and the immune response. *Immunol. Today* 11:129-136.
Kelly, William L. 1998. The J-domain family and the recruitment of chaperone power. *Trends Biochem. Sci.* 23:222-227.
Khang, C. H., Park, S. Y., Lee, Y. H. and Kang, S. 2005. A dual selection based, targeted gene replacement tool for *Magnaporthe grisea* and *Fusarium oxysporum*. *Fungal Genet. Biol.* 42:483-492.
Kim, J. E., Kim, J. C., Jin, J., Yun, S. H. and Lee, Y. W. 2008. Functional characterization of genes located at the aurofusarin biosynthesis gene cluster in *Gibberella zeae*. *Plant Pathol. J.* 24:8-16.
Kim, S., Ahn, I. P., Rho, H. S. and Lee, Y. H. 2005. *MHP1*, a *Magnaporthe grisea* hydrophobin gene, is required for fungal development and plant colonization. *Mol. Microbiol.* 57:1224-1237.
Luke, M. M., Sutton, A. and Arndt, K. T. 1991. Characterization of SIS1, a *Saccharomyces cerevisiae* homologue of bacterial dnaJ proteins. *J. Cell Biol.* 114:623-638.
Martineau, C. N., Beckerich, J. M. and Kabani, M. 2007. Flo11p-independent control of "mat" formation by hsp70 molecular chaperones and nucleotide exchange factors in yeast. *Genetics* 177:1679-1689.
Meacham, G. C., Browne, B. L., Zhang, W., Kellermayer, R., Bedwell, D. M. and Cyr, D. M. 1999. Mutations in the yeast Hsp40 chaperone protein Ydj1 cause defects in Axl1 biogenesis and pro-a-factor processing. *J. Biol. Chem.* 274:34396-34402.
Mulder, N. J., Apweiler, R., Attwood, T. K., Bairoch, A., Bateman, A., Binns, D., Bradley, P., Bork, P., Bucher, P., Cerutti, L., Copley, R., Courcelle, E., Das, U., Durbin, R., Fleischmann, W., Gough, J., Haft, D., Harte, N., Hulo, N., Kahn, D., Kanapin, A., Krestyaninova, M., Lonsdale, D., Lopez, R., Letunic, I., Madera, M., Maslen, J., McDowall, J., Mitchell, A., Nikolskaya, A. N., Orchard, S., Pagni, M., Ponting, C. P., Quevillon, E., Selengut, J., Sigrist, C. J., Silventoinen, V., Studholme, D. J., Vaughan, R. and Wu, C. H. 2005. InterPro, progress and status in 2005. *Nucleic Acids Res.* 33:D201-205.
Nishikawa, S. and Endo, T. 1997. The yeast JEM1p is a DnaJ-like protein of the endoplasmic reticulum membrane required for nuclear fusion. *J. Biol. Chem.* 272:12889-12892.
Ou, S. H. 1985. Rice Diseases. Kew, England: Commonwealth Mycological Institute.
Park, J., Park, B., Jung, K., Jang, S., Yu, K., Choi, J., Kong, S., Kim, S., Kim, H., Kim, J. F., Blair, J. E., Lee, K., Kang, S. and Lee, Y. H. 2008. CFGP: a web-based, comparative fungal genomics platform. *Nucleic Acids Res.* 36:D562-571.
Rensing, L., Monnerjahn, C. and Meyer, U. 1998. Differential stress gene expression during the development of *Neurospora crassa* and other fungi. *FEMS Microbiol. Lett.* 168:159-166.
Rho, H. S., Kang, S. and Lee, Y. H. 2001. *Agrobacterium tumefaciens*-mediated transformation of the plant pathogenic fungus, *Magnaporthe grisea*. *Mol. Cells* 12:407-411.
Rowley, N., Prip-Buus, C., Westermann, B., Brown, C., Schwarz, E., Barrell, B. and Neupert, W. 1994. Mdj1p, a novel chaperone of the DnaJ family, is involved in mitochondrial biogenesis and protein folding. *Cell* 77:249-259.
Sambrook, J. and Russell, D. W. 2001. Molecular Cloning: a laboratory manual. 3rd ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
Talbot, N. J., Ebbole, D. J. and Hamer, J. E. 1993. Identification and characterization of *MPG1*, a gene involved in pathogenicity from the rice blast fungus *Magnaporthe grisea*. *Plant Cell* 5:1575-1590.
Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22:4673-4680.
Valent, B. 1990. Rice blast as a model system for plant pathology. *Phytopathology* 80:33-36.
Walsh, P., Bursac, D., Law, Y. C., Cyr, D. and Lithgow, T. 2004. The J-protein family: modulating protein assembly, disassembly and translocation. *EMBO Rep.* 5:567-571.
Werner-Washburne, M., Becker, J., Kosic-Smithers, J. and Craig, E. A. 1989. Yeast Hsp70 RNA levels vary in response to the physiological status of the cell. *J. Bacteriol.* 171:2680-2688.
Westermann, B. and Neupert, W. 1997. Mdj2p, a novel DnaJ homolog in the mitochondrial inner membrane of the yeast. *J. Mol. Biol.* 272:477-483.
Wright, C. M., Fewell, S. W., Sullivan, M. L., Pipas, J. M., Watkins, S. C. and Brodsky, J. L. 2007. The Hsp40 molecular chaperone Ydj1p, along with the protein kinase C pathway, affects cell-wall integrity in the yeast *Saccharomyces cerevisiae*. *Genetics* 175:1649-1664.
Yu, J., Hu, S., Wang, J., Wong, G. K., Li, S., Liu, B., Deng, Y., Dai, L., Zhou, Y., Zhang, X., Cao, M., Liu, J., Sun, J., Tang, J., Chen, Y., Huang, X., Lin, W., Ye, C., Tong, W., Cong, L., Geng, J., Han, Y., Li, L., Li, W., Hu, G., Li, J., Liu, Z., Qi, Q., Li, T., Wang, X., Lu, H., Wu, T., Zhu, M., Ni, P., Han, H., Dong, W., Ren, X., Feng, X., Cui, P., Li, X., Wang, H., Xu, X., Zhai, W., Xu, Z., Zhang, J., He, S., Xu, J., Zhang, K., Zheng, X., Dong, J., Zeng, W., Tao, L., Ye, J., Tan, J., Chen, X., He, J., Liu, D., Tian, W., Tian, C., Xia, H., Bao, Q., Li, G., Gao, H., Cao, T., Zhao, W., Li, P., Chen, W., Zhang, Y., Hu, J., Liu, S., Yang, J., Zhang, G., Xiong, Y., Li, Z., Mao, L., Zhou, C., Zhu, Z., Chen, R., Hao, B., Zheng, W., Chen, S., Guo, W., Tao, M., Zhu, L., Yuan, L. and Yang, H. 2002. A draft sequence of the rice genome (Oryza sativa L. ssp. indica). *Science* 296:79-92.
Zhong, T. and Arndt, K. T. 1993. The yeast SIS1 protein, a DnaJ homolog, is required for the initiation of translation. *Cell* 73:1175-1186.