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The Mitogen-Activated Protein Kinase Signal Transduction Pathways in *Alternaria* Species

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Mitogen-activated protein kinase (MAPK) cascades are conserved signaling modules in the eukaryotic cells. They are involved in many major cell processes in fungi such as stress responses, vegetative growth, pathogenicity, secondary metabolism and cell wall integrity. In this review, we summarized the advances of research on the MAPK signaling pathways in *Alternaria* species. As major phytopathogenic fungi, *Alternaria* species reduce crop production. In contrast to the five MAPK pathways known in yeast, only three MAPK pathways as *Fus3/Kss1*-type, *Hog1*-type, and *Slt2*-type have been characterized in *Alternaria*. The *Fus3/Kss1*-type MAPK pathway participates in regulation of vegetative growth, conidiation, production of some cell-wall-degrading enzymes and pathogenicity. The *Hog1*-type pathway is involved in osmotic and oxidative stress, fungicides susceptibility and pathogenicity. The *Slt2*-type MAP kinases play an important role on maintaining cell wall integrity, pathogenicity and conidiation. Although recent advances on the MAPK pathways in *Alternaria* spp. reveal many important features on the pathogenicity, there are many unsolved problems regarding to the unknown MAP kinase cascade components and network among other major signal transduction. Considering the economic loss induced by *Alternaria* spp., more researches on the MAPK pathways will need to control the *Alternaria* diseases.

Keywords **:** *Alternaria* species, mitogen-activated protein kinase (MAPK), pathogenicity, signal transduction

Fungi live in a continuous changing environment and receive a lot of external stimuli. In order to survive in this environment and colonize in their hosts, they have developed highly complicated survival strategies. By sensing stimuli-signals from outside and translating them into changes in gene expression, fungi thus respond to the environmental stimuli (Kiel et al., 2010). There are many signal transduction cascades in fungi, including mitogen-activated protein (MAP) kinase signaling cascades (MAPK pathways), G-protein regulated cyclic-AMP signaling pathways (PKA pathway), calcium-mediated signaling, Ras1 signaling and so on (Lengeler et al., 2000). These signal transduction pathways control a wide variety of processes in fungi, and furthermore, allow fungi cells to quickly adapt to a changing environment for the survival. Among them, the MAP kinase pathways are widely studied in different fungi and demonstrated their important roles on processes such as responding to biotic and abiotic stresses, mating and pathogenicity (Tables 1, 2 and 3). These diverse functions especially in fungal pathogenicity makes them potentially targets of future antifungal drugs, therefore receive more and more attentions (May et al., 2005).

The MAP kinases (EC 2.7.11.24) are a family of serine/threonine-specific protein kinases, which are evolutionarily conserved in eukaryotic organisms. These kinases are activated through the protein kinase cascades and respond to diverse external stimuli. For each cascade, there are at least three essential enzymes, a MAP kinase kinase kinase (MAPKKK, MKKK, MEKK or MAP3K), a MAP kinase kinase (MAPKK, MKK, MEK, or MAP2K) and a MAP kinase (MAPK) which are activated in series. By sequential phosphorylation, MAPKKKs receive environmental signals and deliver them to MAPKKs, then to MAPKs. Finally the phosphorylated MAPKs deliver these signals to downstream targets, including several kinases and transcription factors, which eventually regulate some genes gene expression responding to the external stimulus (Chen and Thorner, 2007).

Over the past decades extensive numbers of research have revealed remarkable functions of MAPK pathway in fungi, especially in *Saccharomyces cerevisiae* which have five MAPK pathways for pheromone response, filamentation/invasion, high osmolarity/growth, cell integrity, and spore cell assembly (Gustin et al., 1998). For phytopathogenic fungi, the most extensively studied organism is the

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rice blast fungus *Magnaporthe grisea*. Three MAPK signaling pathways named as *pmk1*, *mps1* and *osm1* play diverse roles in pathogenesis-related development (Dixon et al., 1999; Xu and Hamer, 1996; Xu et al., 1998). Other plant pathogens, e.g., *Colletotrichum lagenarium* (Kojima et al., 2002; Kojima et al., 2004; Takano et al., 2000), *Botrytis cinerea* (Liu et al., 2011; Rui and Hahn, 2007; Segmuller et al., 2006) and *Mycosphaerella graminicola* (Cousin et al., 2006; Mehrabi et al., 2006a; Moriwaki et al., 2006) have also been studied in depth on the functions of their MAPK signaling pathways.

Alternaria is a large genus of moulds that includes saprophytic, endophytic and pathogenic species. It contains about 50 species of which most are plant parasites. These plant pathogens can infect a large number of economically important plants, and significant reduce yields quantitatively and qualitatively (Verma and Saharan, 1994). *Alternaria* spp. cause at least 20% of agricultural spoilage and even cause many human health disorders (Sobiya et al., 2012). Major plant diseases caused by these fungi include early blight of potato by *A. alternata*, stem canker of tomato by *A. arborescens*, leaf lesions on Asian pear by *A. arbusti*, brassica dark leaf spot on most *Brassica* species by *A. brassicicola*, brown spot disease on tobacco by *A. longipes*, Alternaria blight of ginseng by *A. panax*, and early blight in potatoes and tomatoes by *A. solani*.

Previous studies on fungi reveal the important roles of MAPK signaling pathways in fungal development and virulence, but the processes regulated by same type of MAPK pathways in different fungi are as diverse as the fungi themselves (Tables 1, 2, 3). In recent years, a lot of work has been done to understand the roles of this conserved MAPK pathway cascades in *Alternaria* species. These extensive works have been resulted in isolation and functional characterization of many genes of the three MAPK pathways (*Fus3/Kss1*-type, *Hog1*-type, and *Slt2*-type) in *Alternaria* (Fig. 1), which includes three MAPK proteins of Amk1, AbSlt2 and AbHog1 in *A. brassicicola*

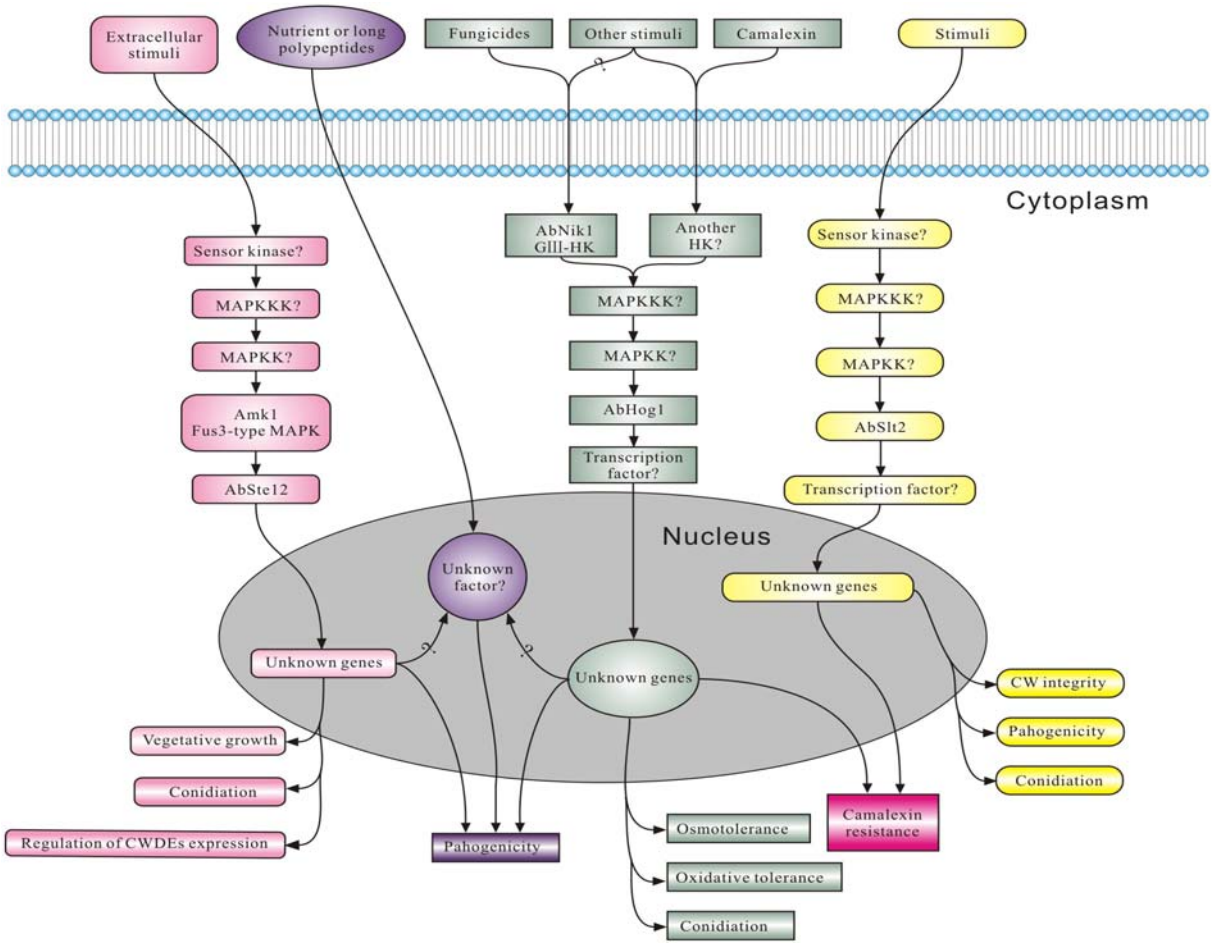

Fig. 1. The mitogen-activated protein kinase (MAPK) pathways in *A. brassicicola*. Only three MAPK pathways (Fus3/Kss1-type, Hog1-type, and Slt2-type) and the corresponding MAPK proteins such as Amk1, AbSlt2 and AbHog1 have been characterized in *A. brassicicola.* The upstream components of MAPKKK and MAPKK and downstream components except AbSte12 in the Fus3/Kss1-type pathway have not been characterized in Alternaria.

and *AaFus3*, AaSlt2 and AaHog1 in *A. alternata*. In Fus3/Kss1-type pathway, the extracellular stimuli pass the signals sequentially to senor kinases, MAPKKK, MAPKK, and MAPK Amk1. The Amk1 further transmits the signals to transcription factor AbSte12, and the AbSte12 turns on genes with the roles on vegetative growth, conidiation, and pathogenicity. In the Hog1-type pathway, AbNik1 detects extracellular fungicides and other stimuli and another histidine kinase may detect camalexin and external stimuli, and the signals are transmitted to AbHog1 through MAPKKK and MAPKK. The AbHog1 is transported to the nucleus and interacts with unknown transcription factors for tolerance induction of osmotic and oxidative stresses. The AbSlt2 protein transmits the signals transferred from the corresponding unkown MAPKK to nucleus by interacting with unknown transcription factors. The three MAPK signaling pathways work independently, or sometimes act coordinately to form signaling networks.

There is a limited resource delivering the recent advances of the research on MAPK signaling pathways in *Alternaria*

Table 1. *Fus3/Kss1* homologs and their functions

Fungal Species	Gene	Genebank accession no.	Functions	References
A. alternata	AaFus3	ACY73851.1	Vegetative growth, conidiation, pathogenicity, expression of CWDEs	(Lin and Chung, 2010)
A. brassicicola	Amk1	-	Vegetative growth, conidiation, pathogenicity, appresorium production, expression of CWDEs	(Cho et al. 2007)
Aspergillus nidulaus	MpkB	AAF12815	Post-karyogamy process, hyphal anastomosis, natural product biosynthesis	(Jun et al., 2011)
B. cinerea	Bmp1	AAG23132	Growth rate, pathogenicity, host penetration	(Zheng et al., 2000)
B. oryzae	Bmk1	BAD42855	Conidiation, pathogenicity	(Moriwaki et al., 2007)
Beauveria bassiana	Bbmpk1	AAQ01000	Appressorium formation, host penetration, adhesion to insect cuticles	(Zhang et al., 2010)
C. parasitica	Cpmk2	AAP86959	Conidiation, vegetative growth, virulence	(Choi et al., 2005)
C. purpurea	Cpmk1	CAC47939	Pathogenicity,	(Mey et al., 2002b)
C.heterostrophus	Chk1	AAF05913	Conidiation, appressorium formation, pathogenicity, regulation of cellulase-encoding genes, melanin biosynthesis	(Eliahu et al., 2007; Lev et al., 1999)
C.lagenarium	Cmk1	AAD50496	Appressorium formation, conidial germination, pathogenicity, melanin metabolism	(Takano et al., 2000)
Candida albicans	Cek1	P28869	Mating, filamentation, construction of the cell wall	(Csank et al., 1998; Eisman et al., 2006)
C. albicans	Cek2	AAG43110	Mating	(Chen et al., 2000)
F. oxysporum	Fmk1	AAG01162	Pathogenicity, invasive growth, root attachment, expression of CWDEs	(Di Pietro et al., 2001)
F. graminearum	Gpmk1	AAL73403	Pathogenicity, conidiation, expression of CWDEs	(Jenczmionka et al., 2003)
Gaeumannomyces graminis	Gmk1	AAG44657	Complement of *M. grisea Pmk1* mutants	(Dufresne and Osbourn, 2001)
M. grisea	Pmk1	AAC49521	Appressorium formation, plant infection, pathogenicity	(Xu and Hamer, 1996)
M.graminicola	MgFus3	AAX81518	Pycnidiation, pathogenicity, stomatal penetration	(Cousin et al., 2006)
N. crassa	Mak-2	AAK25816	Hyphal fusion, conidiation, mating and vegetative growth	(Li, 2005)
P. teres	Ptk1	AAK52840	Conidiation, appressorium formation, and pathogenicity	(Ruiz-Roldan et al., 2001)
Puccinia striiformis	PsMAPK1	HM535614	Regulation of plant penetration and infectious growth	(Guo et al., 2011)
S. cerevisiae	Fus3	NP_009537	Mating	(Gustin et al., 1998)
Sclerotinia sclerotiorum	Smk1	AAQ54908	Sclerotial development	(Chen et al., 2004)
Stagonospora nodorum	Mak2	AAX63387	Vegetative growth, virulence	(Solomon et al., 2005)
T. virens	TmkA	EHK23252	Biocontrol properties, conidiation, expression of CWDEs	(Mukherjee et al., 2003; Mendoza-Mendoza, 2003)
U. maydis	Kpp2	AAF15528	Mating, virulence	(Muller et al., 1999)
U. maydis	Kpp6	CAD43731	Host penetration, mating, virulence	(Brachmann et al., 2003)
Verticillium dahliae	Vmk1	AAW71477	Conidiation, microsclerotia formation, athogenicity	(Rauyaree et al., 2005)

species, although *Alternaria* is causing serious problems on crop production. Here, we summarize the recent achievements gained on the *Alternaria* MAPK signaling pathways, and discuss the significance for the regulation and function of these pathways by comparing the composing components with other phytopathogenic fungal species.

The *Fus3/Kss1* MAPK pathway

The *Fus3/Kss1* pathway is the most extensively studied MAPK cascades in fungal pathogens. A large number of *FUS3/KSS1* homologs have been isolated and characterized the functions (Table 1). They are involved in appressorium formation, pathogenicity, conidiation, virulence, vegetative growth, melanin metabolism and some other processes. *The Fus3/Kss1* pathways are somewhat functionally conserved but still have diverse roles in different fungi.

The functions of the published fungal *FUS3/KSS1* homologs are summarized in Table 1. As shown in *B. cinerea* (Zheng et al., 2000) and *Neurospora crassa* (Li, 2005), the *Fus3/Kss1* pathway has been shown to be required for vegetative growth in *Alternaria*. Disruption of *AaFus3*, a *S. cerevisiae Fus3* homologous gene in *A. alternata*, decreased the radial growth by 40 ± 8% compared to that of wild type on potato dextrose agar (PDA) media, and the growth rate was restored to the original wild type level by reintroducing the gene *AaFus3* into the mutants (Lin and Chung, 2010). A similar result was acquired in a study of *A. brassicicola* (Cho et al., 2007). In that study, deletion of *Amk1*, the *AaFus3* homologous gene, also significantly reduced the growth rate of *A. brassicicola* on different media. The regulation of vegetative growth by the *Fus3/Kss1* pathway is not universal in all plant pathogenic fungi. Several fungi including *M. grisea* and *Ustilago maydis(ubc3)* did not reduce the growth rate by the disruption of the pathway (Muller et al., 1999; Xu and Hamer, 1996).

The *Fus3/Kss1* pathway is also involved in conidiation of *Alternaria* spp.. In *A. alternata*, the *AaFUS3*-deficient mutants could not produce fully differentiated conidia, either in culture media or in plants. The mutants only produced highly melanized hyphae with distinct septae in chains on PDA media. The addition of cAMP, yeast extracts, or glucose could not induce conidiation at all (Lin and Chung, 2010). Similar conidiation defect was also reported in *Amk1*-deficient mutants of *A. brassicicola* (Cho et al., 2007). They could not produced fully developed conidia on many media, e.g., PDA, weak PDA (1/20 concentration), and glycerol yeast extract agar (Cho et al., 2007). There are two classifications of plant pathogenic fungi related to the role of the *Fus3/Kss1* pathway on conidiation. Mutants of *Fus3* with the defect in conidiation were found in many fungi such as *Cryphonectria parasitica* (Choi et al., 2005), *Fusarium graminearum* (Jenczmionka et al., 2003), *Cochliobolus heterostrophus* (Lev et al., 1999) and *Pyrenophora teres* (Ruiz-Roldan et al., 2001), whereas those *Fus3* mutants of *M. grisea* (Xu and Hamer, 1996), *B. cinerea* (Zheng et al., 2000), *Fusarium oxysporum* (Di Pietro et al., 2001) and *C. lagenarium* (Takano et al., 2000) could produce conidia normally. These results suggest that the *Fus3/Kss1* pathway plays distinct roles for conidiation among different fungal species.

The most important and conserved function of *Fus3/Kss1* pathway can be the regulation of pathogenicity of the fungi. Most of the reported mutants of *Fus3/Kss1* homologs showed reduced or even no pathogenicity on their hosts, but the mechanisms for the lowered pathogenicity varied among different fungi (Table 1). In *Alternaria* spp., disruption of *A. alternata AaFus3* or *A. brassicicola Amk1* (Fus3-type MAPK) also resulted in reduced pathogenicity. The *Amk1* mutants could not induce noticeable disease symptoms on *Brassica oleracea* even inoculations with 300 times more protoconidia than the wild type, though they were capable of infecting wounded leaves. Disruption of *Amk1* gene may cause the reduction even loss of the penetration ability. Further infection assay on the host leaf surface confirmed this hypothesis. Although the *Amk1* mutants could produce appresorium-like structure on the leaf surface, but this kind of appresorium could not penetrate leaf surface or cause tissue destruction as that of the wild type (Cho et al., 2007). The fault in appresorium production was also found in the similar mutants of *M. grisea* (Xu and Hamer, 1996), *C. lagenarium* (Takano et al., 2000), *C. heterostrophus* (Lev et al., 1999) and *P. teres* (Ruiz-Roldan et al., 2001), which indicated a conserved role of *Fus3/Kss1* pathway on appresorium production. Interestingly, the *Amk1* mutants partially restored their pathogenicity by addition of crushed host plant leaves or nutrients like tryptone, yeast extract and bovine serum albumin, which was never reported in other fungi. These results suggest that there is another nutrients-activated signaling pathway involving in pathogenesis. Similar to the *Amk1* mutants, the *AaFus3* mutants of *A. alternata* also behaved differently on intact leaves and wounded leaves havingonly pinpoint lesions on intact leaves but typical necrotic lesions on wounded leaves. The lesions on wounded leaves caused by the *AaFus3* mutants did not have significant difference in size with those caused by the wild-type strain (Lin and Chung, 2010). While in *P. teres* and *C. lagenarium*, *Fus3/Kss1*-type MAPK mutants are nonpathogenic both on intact and wounded hosts. The difference between *Alternaria* and other fungi indicated the diverse roles *Fus3/Kss1* homogies played on pathogenesis in different fungi.

In *Alternaria* the *Fus3/Kss1* pathway also played an important role in the production of some cell-wall-degrad-

ing enzymes (CWDE), which was only reported in *F. oxysporum* (Di Pietro et al., 2001), *C. heterostrophus* (Lev, 2003), *Trichoderma atroviride* (Olmedo-Monfil et al., 2002) and *Trichoderma virens* (Mendoza-Mendoza, 2003). Deletion the *AaFus3* gene resulted in higher activities of lipase alkaline phosphatase, and cutinase, which result suggest a negative regulation of these three genes by *AaFus3*. But for pectinase, xylanase, and cellulase activities, there were no noticeable differences between the wild type and *AaFus3* mutants (Lin and Chung, 2010). In *A. brassicicola*, *Amk1* also negatively regulated some CWDE such as chymotrypsin, glycosyl hydrolase, and N-acetyl glucosaminidase in the nutrient rich media (Cho et al., 2007). In the infection process, the genes of chymotrypsin, glycosyl hydrolase, and lipase showed a high expression in the wild type strain and in *Amk1* mutants on wounded plants, but not on the intact ones (Cho et al., 2007). These results coincided with the pathogenicity of *Amk1* mutants.

The High-Osmolarity Glycerol (*Hog1*) MAPK pathway

The *Hog1* pathway is intensively studied in the budding yeast *S. cerevisiae*. By inducing the production of glycerol, the *Hog1* pathway thus allows yeast to adapt to high osmolarity (Brewster et al., 1993). For long time the *Hog1* pathway was considered to be exclusively involved in the response to extracellular osmolarity. However, subsequent studies revealed that this signal route is responsible for diverse processes of *S. cerevisiae*, such as adaptation to heat stress (Winkler et al., 2002) and citric acid stress (Lawrence et al., 2004). This pathway is also responsive to arsenite (Sotelo and Rodriguez-Gabriel, 2006) and bacterial endotoxin (Marques et al., 2006). In filamentous fungi, the *Hog1*-type MAPKs function in adaptation to abiotic/fungicide stress, secondary metabolism, conidiation and pathogenicity (Table 2).

The homolog of *Hog1* (*AaHog1*) in *A. alternata* is also involved in cellular resistance to osmotic stresses as have been reported for many other fungi. *AaHog1* disruption mutants were highly sensitive to potassium chloride and sodium chloride, but not to glucose, sucrose, sorbitol or mannitol. *AaHog1* disruption mutants also showed sensitivity to oxidative stress caused by tert-butyl-hydroxyperoxide, H_2O_2, and menadione (Lin and Chung, 2010). While in *A. brassicicola*, the *Abhog1* mutants displayed sensitivity

Table 2. *Hog1* homologs and their functions

Fungal Species	Gene	Genebank accession no.	Functions	References
A.alternata	*AaHog1*	Q52PH6	Adaption to osmotic and oxidative stress, fungicides susceptibility, pathogenesis	(Lin and Chung, 2010)
A.brassicicola	*AbHog1*	AAX86000	Adaption to osmotic and oxidative stress, fungicides susceptibility, pathogenesis, resistance to camalexin and brassinin	(Joubert et al., 2011)
A. fumigatus	*sakA*	XP_752664	Conidial germination in response to nitrogen and carbon source starvation	(Xue et al., 2004)
A. fumigatus	*MpkC*	XP_753727	Carbon source utilization	(Reyes et al., 2006)
A. nidulans	*sakA*	-	stress signal transduction, sexual development and spore viability	(Kawasaki et al., 2002)
B. bassiana	*Bbhog1*	AAS77871	Abiotic stress resistance, pathogenicity, appressorium formation, regulation of hydrophobicity gene expression, adhesion to insect cuticles	(Zhang et al., 2009)
B. cinerea	*BcSak1*	CAJ85638	Conidiation, vegetative growth, sclerotial development, host penetration, oxidative stress, melanin biosynthesis	(Liu et al., 2011, Segmuller et al., 2006)
B. oryzae	*Srm1*	BAE48722	Response to hyperosmotic stress, oxidative and UV stress	(Moriwaki et al., 2006)
C. albicans	*Hog1*	XP_721016	Oxidative stress response, chlamydospore formation, morphogenesis, virulence, respiratory metabolism	(Alonso-Monge et al., 1999, 2003, 2009)
C. heterostrophus	*Hog1*	BAD99295	Melanin biosynthesis, resistance to hyperosmotic and oxidative stress, stress up-regulation of *G3PP1*gene, penetration	(Igbaria et al., 2008)
C. lagenarium	*Osc1*	BAD11137	Responses to hyperosmotic stress and sensitivity to fludioxonil	(Kojima et al., 2004)
C. parasitica	*Cpmk1*	AAO27796	Virulence, pigmentation, conidiation, laccase production and cryparin expression	(Park et al., 2004)
F. proliferatum	*Fphog1*	ABO46009	Different abiotic stresses response, fumonisin biosynthesis	(Ádám et al., 2008, Kohut et al., 2009)
M. grisea	*Osm1*	AAF09475	Osmotolerance, stress response	(Dixon et al., 1999)
M. graminicola	*Mghog1*	ABD92790	Pathogenicity, osmotolerance, fungicide resistance, yeast-like growth	(Mehrabi et al., 2006b)
S. cerevisiae	*Hog1*	NP_013214	Osmolyte synthesis	(Gustin et al., 1998)
Trichoderma harzianum	*ThHog1*	-	hyperosmotic stress response	(Delgado-Jarana et al., 2006)

to oxidant menadione, not to H_2O_2 (Joubert et al., 2011). In addition to osmotolerance and oxidative stress resistance, the *Hog1* homologs also exhibited adaption to other abiotic stresses such as heat and UV-irradiations (Ádám et al., 2008). Normally the *Hog1* pathway regulated accumulation of some carbohydrates in response to the hyperosmotic stresses, but the carbohydrates varies among different fungi. The accumulated carbohydrates includeglycerol in *S. cerevisiae* (Brewster et al., 1993), arabitol in *M. grisea* (Dixon et al., 1999), glycerol and arabitol in *Candida albicans* (Kayingo and Wong, 2005), erythritol and arabitol in *B. bassana* (Zhang et al., 2009). In *Alternaria* spp., there is no research on this topic and the accumulated carbohydratet is not clear.

The *Hog1* MAPK pathway also plays a pivotal role in fungicides susceptibility. *Hog1* mutants of fungi like *Fusarium proliferatum* (Ádám et al., 2008), *Aspergillus nidulans* (Kawasaki et al., 2002), *C. heterostrophus* (Igbaria et al., 2008) and *C. lagenarium* (Kojima et al., 2004) all showed tolerant to phenylpyrrole and dicarboximide fungicides. In *A. alternata*, the *AaHog1* mutants were also resistant to dicarboximide and phenylpyrrole fungicides, but the induced level of resistance was very small, implying another signaling pathway in response to these fungicides stress (Lin and Chung, 2010). Phenylpyrrole resistance was also found in the *Abhog1* mutants of *A. brassicicola*, and this resistance was much higher than that of *AaHog1* mutants (Joubert et al., 2011).

The Hog1 homologs play species-specific role in fungi pathogenesis. The Hog1-type mutants of *M. grisea* (Dixon et al., 1999), *C. lagenarium* (Kojima et al., 2004) and *B. oryzae* (Moriwaki et al., 2006) remained fully pathogenic on their own hosts. However, *MgHog1* disruption mutants of *M. graminicola* could not infect wheat leaves (Mehrabi et al., 2006b). In *C. parasitica*, knockout of the Hog1 homolog *cpmk1* induced some hypovirulence-associated symptoms including reduction in pigmentation, conidiation, laccase production, and virulence on chestnut tree (Park et al., 2004). The Hog1 signaling pathway is also involved in *Alternaria* pathogenesis. Inactivation of *AaHog1* resulted in reduced pathogenicity of *A. alternata* on the leaves of tangerine Minneola. Both point inoculation and spray inoculation with *AaHog1* conidial suspension produced no lesions on intact leaves, while characteristic necrotic symptom was observed on leaves inoculated with wild type strain. Loss or reduction of penetration capacity may not account for pathogenicity defect since the *AaHog1* mutants were also non-pathogenic on pre-wounded leaves. Inoculation with cell-free culture filtrates obtained from the *AaHog1* mutants or wild types showed similar results, which excluded the participation of the host-selective toxin in the reduced pathogenicity of *AaHog1* mutants. The detoxification of reactive oxygen species or resistance to phytoalexin may be (partially) responsible for the pathogenicity defect of the *AaHog1* mutants (Lin and Chung, 2010). The reduced pathogenicity was also found in *Abhog1* mutants of *A. brassicicola*. The *Abhog1* mutants were hypersensitive to camalexin and brassinin and had a low conidia germination rates. This defect may be partially responsible for the reduced pathogenicity (Joubert et al., 2011).

In *S. cerevisiae*, Sln1p, the histidine kinase (HK), works as an osmosensor and negatively regulates the HOG1 signaling pathway (Maeda et al., 1994). In filamentous fungi, the HK-mediated signal pathways were also connected with Hog1-type MAP kinase pathway, but the mode of regulation is varied in the different fungi. In *C. heterostrophus*, Dic-1, a Group III HK, positively regulates phosphorylation of the HOG1 MAPK (Yoshimi et al., 2005). While in *B. cinerea*, the histidine kinase Bos1 negatively regulated the HOG1-like MAP kinase Sak1 (Liu et al., 2008). As for *A. alternata*, both *AaHSK1* (a two-component histidine kinase) and *AaHog1* were involved in osmotic stress, but the *AaHSK1*-deletion mutants were hypersensitive to salt stress, but not to the high concentrations of sugar. In contrast, the *AaHog1* mutants were resistant to salt and sensitive to sugar. The *AaHSK1* mutants showed obvious resistance to oxidants and fungicides (dicarboximide and phenylpyrrole) while *AaHog1* mutants did not. These results suggested that the HK-mediated signal pathways work independently of the *Hog1* signaling pathway in regulating some processes in *A. alternata*. *AbNik1*, a two-component histidine kinase gene of *A. brassicicola*, upregulated the *Hog1* MAPK cascade which decreased fungicidal activity of ambruticin (Dongo et al., 2009). However, Joubert (2011) reported that camalexin-induced phosphorylation of AbHog1p was independent of the regulation of the *AbNik1*. These data suggested that the HK- and Hog1-mediated signaling pathway work differently in different fungi.

The Cell Wall Integrity MAPK pathway (Slt2 MAPK pathway)

The *Slt2* MAPK pathway in *S. cerevisiae* regulates cell wall integrity and is activated in response to external stimuli such as high temperature (Humberto et al., 1993), hypo-osmolarity (Davenport et al., 1995) and cell-wall-stress agents (Ketela et al., 1999). Unlike the extensively studied Fus3/Kss1 pathways, *Slt2* homologs are less well investigated in fungi. The *Slt2* homologys have been shown to beinvolved in regulating cell wall integrity, pathogenicity/virulence, conidiation, oxidative stress, melanin biosynthesis (Table 3).

As the primary function of *Slt2* pathway, its homologs in

Table 3. *Slt2* homologs and their functions

Fungal Species	Gene	Genebank accession no.	Functions	References
A. alternata	*AaSlt2*	ADC35363	Adaption to oxidative stress,accumulation of melanin and chitin, conidial formation, pathogenicity	(Yago et al., 2011)
A. brassicicola	*AbSlt2*	AAU11317	Adaption to oxidative stress, conidial germination, pathogenicity	(Joubert et al., 2011)
A. fumigatus	*MpkA*	XP_751459	Cell wall integrity, oxidative stress, pyomelanin formation, gliotoxin production and iron adaptation	(Valiante et al., 2008)
A. nidulans	*MpkA*	AAD24428	Conidial germination, polarized growth	(Bussink and Osmani, 1999)
B. cinerea	*Bmp3*	ABJ51957	Aprotrophic growth, conidiation, plant surface sensing, host colonization, melanin biosynthesis	(Rui and Hahn, 2007)
C. albicans	*Mkc1*	P43068	Cell wall construction, resistance to antifungals, virulence	(Diez-Orejas et al., 1997, Navarro-Garcia et al., 1995; 1998)
C. heterostrophus	*Mps1*	ABM54149	Melanin Biosynthesis, conidiation, virulence, female fertility	(Eliahu et al., 2007, Igbaria et al., 2008)
C. lagenarium	*Maf1*	AAL50116	Pathogenicity, appressorium formation, conidiation	(Kojima et al., 2002)
C. neoformans	*Mpk1*		Cell wall integrity, resistance to antifungals, calcineurin	(Kraus et al., 2003)
C. purpurea	*Cpmk2*	CAC87145	Pathogenicity, conidiation, cell wall structure, virulence	(Mey et al., 2002a)
F. graminearum	*Mgv1*	AAM13670	Vegetative growth, female fertility, plant infection, cell wall integrity	(Hou et al., 2002
M. grisea	*Mps1*	AAC63682	Pathogenicity, cell wall integrity, pathogen penetration, appressorium formation, conidiation, female fertility,	(Xu et al., 1998)
M. graminicola	*MgSlt2*	AAY98511	Pathogenicity, fungicide resisitance, pycnidia formation, virulence, melanisation	(Mehrabi et al., 2006a)
S. cerevisiae	*Slt2*	NP_011895	Cell wall remodeling	(Gustin et al., 1998)
T. virens	*TmkB*	ACD88751	Cell wall integrity, conidiation, vegetative growth, melanin biosynthesis	(Kumar et al., 2010)

many pathogenic fungi also had the role of maintaining cell wall integrity in *M. grisea* (Xu et al., 1998), *Claviceps purpurea* (Mey et al., 2002a), *Aspergillus fumigates* (Valiante et al., 2008), *A. nidulans* (Bussink and Osmani, 1999), *F. graminearum* (Hou et al., 2002) and *M. graminicola* (Mehrabi et al., 2006a). However, in *B. cinerea*, the Δbmp3 (*Slt2* homologs) mutant and the wild-type showed no difference of protoplast release when treated with β-glucanase, the chitin synthase inhibitor Nikkomycin Z, and the cell-wall formation inhibitor Calcofluor-White (Rui and Hahn, 2007). Similar results were also founded in *C. lagenarium* (Kojima et al., 2002). These results indicate that the *Slt2*-related pathway is not essential for maintaining cell wall integrity in these two fungi. In *A. alternata*, *AaSlt2* mutants were hypersensitive to cell wall-degrading enzymes and compounds. Further analysis showed that disruption of *AaSlt2* resulted in less accumulation of melanin and chitin (Yago et al., 2011). Melanin locates in the fungal cell wall (Jacobson, 2000) and affects cell wall thickness, therefore, it protects fungal pathogens against stresses such as oxidants, UV radiation (Jacobson, 2000, Romero-Martinez et al., 2000), and wall-degrading enzymes (Butler et al., 2001). Did the reduced amount of melanin and chitin cause the weak cell wall of *AaSlt2* mutant? This question needs further investigation. Deletion of an *SLT2*- homolog *AbSlt2* in *A. brassicicola* also increased sensitivity to oxidative stress, and the *AbSlt2* mutant showed hypersensitivity to while the *AaSlt2* mutants grew normally in potato dextrose agar (PDA) containing 30 mM H_2O_2 (Joubert et al., 2011; Yago et al., 2011).

As have been shown in *C. lagenarium* (Kojima et al., 2002), *C. purpurea* (Mey et al., 2002a), *M. grisea* (Xu et al., 1998) and *B. cinerea* (Rui and Hahn, 2007), the *Slt2* pathway was also involved in conidiation in *Alternaria*. In *A. alternata*, deletion of *AaSlt2* signiticantly reduced conidial formation, but did not affect the conidial germination. Further light microscopy examination showed that the conidia of the *AaSlt2* mutants displayed an aberrant shape as less melanized, few distinct vertical and transverse septa (Yago et al., 2011). The *AbSlt2* disruption mutant of *A. brassicicola* also produced deformed conidia, but unlike *AaSlt2* mutants, they had normal conidiation rates and delayed germination rate (Joubert et al., 2011). Conidia of similar mutants in *M. grisea* had normal morphology and germination rates (Xu et al., 1998). These findings suggest that the *Slt2* pathway takes different effects on conidiation in different fungi, although thet *Slt2* pathway in several fungi is involved directly in the conidiation.

Like the *Fus3/Kss1* pathway, the *Slt2* pathway is also involved in pathogenicity of *Alternaria*, which has been shown in other phytopathogenic fungi (Table 3). In *A. alternata*, spraying inoculation with conidial suspension of the wild type or the *A. alternata Slt2* mutant induced different symptoms on tangerine Minneola leaves. The lesions

induced by the *Slt2* mutant were smaller and progressed more slowly than that induced by the wild type strain, which may be a comprehensive result of reduced production of ACT (*Alternaria citri* tangerine pathotype) -toxin, growth retardation and deformation of hyphal extension (Yago et al., 2011). Disruption of *AbSlt2* gene also reduced the virulence of *A. brassicicola*. However, there were two different results in two independent reports as for the cause of the attenuated pathogenicity of *AbSlt2* mutants. Intact and pre-wounded leaves of *B. oleracea* were inoculated with spore suspension of wild type strain and *AbSlt2* mutants. On intact leaves, these two reports showed similar result as large typical necrosis on leaves with the wild type and small lesions on leaves with the *AbSlt2* mutants. However, on pre-wounded leaves, Joubert (2011) reported that the *AbSlt2* mutant still showed reduced pathogenicity compared with the wild type strain, similar results with that on intact leaves. While in the Scott's report, the *AbSlt2* mutant recovered their pathogenicity on wounded leaves (Scott, 2008). There was no difference in the size of the lesions caused by the *AbSlt2* mutants and the wild type strain. These results on intact leaves suggested that the reduced penetration capacity is partially responsible for the pathogenicity defect in the *AbSlt2* mutants. Microscopic observations showed that conidia of the *AbSlt2* mutant only produced excessive elongation hyphae on onion epidermis and cabbage leaf surfaces and rarely formed appressoria (Joubert et al., 2011). Even the formed appressoria seemed to not be fully developed or immature (Scott, 2008). But why the pathogenicity of the *AbSlt2* mutants on pre-wounded leaves behaved so differently is still questionable, which question needs to be further investigated.

An important function for *AbSlt2* can be the protection of *A. brassicicola* cells from cell wall stress caused by indolic phytoalexins. As mentioned earlier, the *Abhog1*-mediated signaling pathway was also involved in phytoalexins stress (Joubert et al., 2011). This feature may be a cause of the reduced virulence in *AbSlt2* and *Abhog1* mutants; however, the mechanisms of the regulations by these genes are still not investigated.

Concluding remarks

There have been many researches to understand the roles of genes on the MAPK signaling pathway of *Alternaria* spp., especially for *A. alternata* and *A. brassicicola*. The MAPK genes, *Amk1/AbSlt2/AbHog1* in *A. brassicicola* and *AaFus3/AaSlt2/AaHog1* in *A. alternata*, have been characterized and the functions have been also analyzed. Like their homologs in other plant pathogenic fungi, these MAPK pathways have some conserved roles, such as the *Fus3/Kss1*-type pathway on regulation of vegetative growth, conidiation and pathogenicity, the *Hog1*-type pathway on adaptation to osmotic stress and fungicides susceptibility, and the *Slt2*-type pathway on maintenance of cell wall integrity. These MAPK pathways in *Alternaria* , however, also have unique functions shown in the adaptation to brassicaceous indolic phytoalexins by the *Slt2*-type pathway.

Although some achievements have already been obtained, the research on *Alternaria* MAPK signaling networks is still at the beginning compared to the wealthy data of other fungi such as *S. cerevisiae* and *M. grisea*. No MAPKK and MAPKKK gene in *Alternaria* species has been reported, therefore, the underlying mechanism for the regulatory functions is still unknown. In the future, more studies will be required to identify the unknown MAPK cascade components, upstream activators and downstream target transcription factors, and also elucidate the regulatory networks with other signaling pathways.

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