

MaFKS, a β -1,3-glucan synthase, is involved in cell wall integrity, hyperosmotic pressure tolerance and conidiation in *Metarhizium acridum*

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Abstract β -Glucan is an essential cell wall structural component in most fungi and its helical structure is important for maintenance of cell wall elasticity in fungi. The gene encoding β -1,3-glucan synthase in the entomopathogenic fungus *Metarhizium acridum* (*MaFKS*; HQ441252) was cloned. The function of *MaFKS* was analyzed by RNA interference (RNAi). FKS-RNAi transformants were more sensitive to agents that disturb the cell wall or cell membrane and to hyperosmotic stress than the wild type. In comparison with the wild type, aerial hyphae and conidial yield were obviously reduced in FKS-RNAi transformants on potato dextrose agar plates with Congo red, calcofluor white, sodium dodecyl sulfate, KCl, sorbitol or mannitol. The β -1,3-glucan content significantly decreased in FKS-RNAi transformants, indicating that *MaFKS* affects the synthesis of β -1,3-glucan in the fungal cell wall and confirming its role in the maintenance of cell wall integrity, hyperosmotic pressure tolerance and conidiation.

Keywords *MaFKS* · β -1,3-glucan synthase · Cell wall · Entomopathogenic fungus

Introduction

Metarhizium, one of the most important genera of entomopathogenic fungi, can kill insects directly by penetration of the cuticle and has emerged as an excellent model organism for exploring many questions at the molecular and biochemical levels (Clarkson and Charnley 1996; Gao et al. 2011). In Australia, Africa and China, *M. acridum* has been exploited in commercial biological insecticides for grasshoppers and locusts (Langewald and Kooyman 2007; Milner et al. 2002; Peng et al. 2008). As with other entomopathogenic fungi, *Metarhizium* has been considered as a promising alternative or supplement to chemical pesticides because of its environmental safety, high insect specificity and low likelihood of the development of insect resistance (Charnley and Collins 2007). However, some disadvantages have retarded widespread application, including short-term storage, sensitivity to environmental conditions and the resources required for mass production of conidia (Daoust and Roberts 1983; Ekesi et al. 2003). Therefore, an understanding of the mechanism of resistance to adversity and conidia production in *M. acridum* is necessary for commercial development and improvement of the biocontrol fungus.

The fungal cell wall, which comprises a polysaccharide–protein complex, can protect the cell from harsh environments and plays a prominent role in resisting host response (Latgé 2007). β -1,3-Glucan and chitin are the major polysaccharides in the basic framework of the fungal cell wall (Lesage and Bussey 2006). Thus, β -1,3-glucan synthesis is crucial for cell wall formation and fungal development (Bowman and Free 2006). β -1,3-Glucan chains are synthesized by a plasma membrane-localized enzyme, β -1,3-glucan synthase (GS; EC 2.4.1.34), which can use UDP-glucose as a substrate and transfer glucose to linear

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β -1,3-glucan chains (Douglas et al. 1994). The GS complex can be divided into two parts: a catalytic subunit (*FKS*) and a regulatory subunit (*RHO1*). *FKS* encodes a large protein, which is greater than 200 kDa and has 10–16 potential transmembrane helices (Beauvais et al. 2001). In yeast, there are usually multiple *FKS* genes, which are essential for normal spore wall formation and proper assembly (Ishihara et al. 2007; Martín et al. 2000). In filamentous fungi, such as *Aspergillus nidulans*, *Cordyceps militaris* and *A. fumigatus*, only one *FKS* homolog gene has been identified (Kelly et al. 1996; Beauvais et al. 2001; Ujita et al. 2006). A colony of *A. fumigatus* on potato dextrose agar (PDA) containing 1 μ g/ml caspofungin showed no filamentous growth and the mycelium was deformed around the edges (Kahn et al. 2006). In *Fusarium solani*, depleted expression of *FsFKS1* reduced spore viability and caused lysis of spores and hyphae (Ha et al. 2006). The heterokaryon Δ *FKS1::hygB* of *Coccidioides posadasii*, which has a null *FKS1* allele, has an abnormal appearance, with swelling of hyphal elements (Kellner et al. 2005). Nevertheless, the characteristics of FKS in entomopathogenic fungi have not been investigated and its exact function is still far from clear.

In the present study, we cloned a single gene from *M. acridum* with similarity to other filamentous ascomycete *FKS* genes. To investigate its function, the expression level of *MaFKS* was down-regulated by RNA interference (RNAi). Phenotypic examinations of FKS-RNAi transformants demonstrated that *MaFKS* is involved in cell wall integrity, hyperosmotic pressure tolerance and conidiation in *M. acridum*.

Materials and methods

Strain, media and growth conditions

Metarhizium acridum strain CQMa102 was as previously described (Peng et al. 2009). For the stress tolerance test, 2 μ l of conidial suspension (5×10^5 conidia/ml) was inoculated onto PDA plates containing KCl (1 M), sorbitol (1 M) or mannitol (1 M) and incubated for 9 days or onto PDA plates containing sodium dodecyl sulfate (SDS; 0.01% w/v), calcofluor white (CFW; 50 μ g/ml) or Congo red (CR; 500 μ g/ml) and incubated at 28°C for 4 days. *Agrobacterium tumefaciens* AGL1 was used for fungal transformation. *Escherichia coli* XL-blue was used for routine bacterial transformations and maintenance of plasmids.

Gene cloning and phylogenetic analysis

From the whole genomic sequence of *M. acridum* (Gao et al. 2011), we found a gene with high similarity to the

glucan synthase gene of *C. militaris* (BAE94194.1). The full cDNA sequence was acquired using RACE PCR. First-strand cDNA was synthesized with reverse transcriptase and the oligo dT-3sites adaptor primer (Takara, China). The 3'-end fragment of the *MaFKS* cDNA sequence was amplified using a 3'-Full RACE kit (Takara, China) according to the manufacturer's instructions with the three sites adaptor primer and FKS-3S (5'-GCT TGC TGT ATG GGT CCC GTA TT-3'). 5'-RACE PCR was performed according to the manufacturer's instructions (5'-Full RACE Core Set, Takara, China). The primers were RT-Prime (5'-AAG CAG TGG TAT CAA CGC AGA GT-3' and FKS-5A (5'-CGA CCG TAG TAG TCC ATT TCA GC-3'). The full-length *MaFKS* cDNA sequence (5,820 bp) was amplified with the gene-specific forward primers MaFKS-F2 (5'-TTT GGG CAA CAA TGT CGG-3') and MaFKS-R2 (5'-GTC AGT TCA CCT AGG ATG TC-3'). Each PCR product was subcloned into the pMD19-T vector and transformed into *E. coli* XL-blue for determination by GenScript (Nanjing, China). MEGA v4.1 (<http://www.megasoftware.net>) was used to construct phylogenetic dendrograms. Analysis of the transmembrane domains was performed using DAS (<http://www.expasy.ch>).

Construction of an RNAi vector

To study the function of *MaFKS*, an RNAi vector was constructed. Plasmid pPK₂-pB was generated by inserting a bar (herbicide resistance gene) cassette into *EcoRI* and *HindIII* sites of the binary vector pPK₂ (McCluskey 2003) in which an *hph* (hygromycin B resistance gene) cassette was deleted. A partial sequence of *MaFKS* (4,660–4,924 bp) was amplified using FKSF (5'-CGG AAT TCC ACC AAT TCG CAT GGA AC-3') and FKSR primers (5'-GCG ATA TCC GAG GTA CGG TCT CAA GGT C-3') from the cDNA of *MaFKS*. The resultant PCR product was digested with *EcoRI/EcoRV* and inserted into the dual promoter vector pDPB. Then the *MaFKS* cassette with dual promoters was excised from pDPB by *HindIII* and *XbaI* digestion and inserted into pPK₂-pB to form pPK₂-pB-*MaFKS*-RNAi.

Transformation of *M. acridum* and transformant screening

Agrobacterium-mediated transformation was carried out as described by dos Reis et al. (2004). To confirm the expression level of *MaFKS*, wild-type (WT) cells and FKS-RNAi transformants were grown in 1/4 SDAY (1% dextrose, 0.25% peptone, 0.5% yeast extract and 2% agar) for 3 days and the mycelia were collected and washed with sterile water. Then the mycelium (2 g fresh wt.) was incubated in 20 ml of PDA liquid medium containing

500 µg/ml CR at 28°C for 6 h. Total RNA was isolated using an SV Total RNA Isolation System (Promega, USA). Synthesis of cDNA and real-time RT-PCR were performed using the method described by Peng et al. (2009). Primers FKSRF (5'-TGG TGA AGC ATC GGC AAA AC-3') and FKSRR (5'-GTA CGG TCT CAA GGT CCA G-3') were used to detect *MaFKS* expression levels. *Gpdh* encoding glyceraldehyde-3-phosphate dehydrogenase was selected as the reference gene and primers 5'-AGA TGG AGG AGT TGG TGT TG-3' and 5'-GAC TGC CCG CAT TGA GAA G-3' were used.

Microscopy

Aliquots of 100 µl of conidial suspension (2×10^7 conidia/ml) for various strains were inoculated into 20 ml of 1/4 SDAY liquid medium and 1/4 SDAY containing 500 µg/ml CR, 50 µg/ml CFW or 0.01% SDS and incubated at 28°C on a rotary shaker (100 rpm) for 48 h to observe hyphal bodies with a microscope. Mycelium of WT cells and FKS-RNAi transformants was harvested from 1/4 SDAY liquid medium and stained with aniline blue for detection of β -1,3-glucans as previously described (Nicholas et al. 1994).

Measurement of β -1,3-glucan

The β -1,3-glucan content of the hyphal wall was measured using the silkworm larvae plasma (SLP) reagent set (Wako Pure Chemical Industries Ltd, Osaka), as previously described (Tsuchiya et al. 1996). The hyphal wall was isolated from hyphae cultured in liquid 1/4 SDAY medium for 60 h, as described by Dallies et al. (1998). The β -1,3-glucan content was normalized to the fresh weight of mycelium.

Conidial yield of transformants

Conidial yield was determined using the method described by Fang et al. (2004). Conidia were spread onto PDA and incubated on PDA plates containing CR (500 µg/ml), CFW (50 µg/ml), SDS (0.01%), KCl (1 M), sorbitol (1 M) or mannitol (1 M) for 12 days.

Statistical analysis

All experiments were repeated at least three times, and data for RNAi efficiency, conidial production and β -1,3-glucan content were analyzed by one-way analysis of variance (ANOVA). The Tukey's post hoc test was used to compare the means. SAS 2004 for Windows was used for all statistical analyses.

Results

Cloning *MaFKS* from *M. acridum*

Using information on the whole *M. acridum* genome, we cloned one homolog to the *FKS1* gene of *Saccharomyces cerevisiae* and other fungi, named *MaFKS* (HQ441252). Alignment with the cDNA sequence demonstrated that *MaFKS* contains a 5,820 bp open reading frame (ORF) and two introns located at the N terminus (177–238 bp) and the C terminus (5,539–5,600 bp). The complete ORF of *MaFKS* encodes a predicted protein of 1938 amino acids (aa) with a molecular mass of 221.7 kDa and a *pI* of 7.94. The predicted *MaFKS* protein has the highest degree of similarity to the putative Fksp of *C. militaris* (88% identity, BAE94194.1). In comparison with the putative FKS protein from *A. fumigatus*, the putative β -1,3-glucan synthase domain (aa 852–1,683) contains the putative UDP-glucose binding consensus sequence K/RXTG at the C terminus (*MaFKS*, RITG: aa 1573–1576) (Inoue et al. 1996; Beauvais et al. 2001). Analysis of the transmembrane domains showed that the deduced *MaFKS* protein has 16 transmembrane helices like FKS from *A. nidulans* (Kelly et al. 1996) (Fig. 1a). We also found two domains (domains 1 and 2) in *M. acridum* FKS that are homologous (44 and 29% identity, respectively) to cellulose synthase of *Aerobacter xylinum* (SP19449) as described by Kelly et al. (1996) (Fig. 1b). A phylogenetic tree was constructed using the neighbor-joining method and shows the relationship of FKS-like proteins from *S. cerevisiae* and other fungi. The putative *MaFKS* protein is most closely related to the putative FKS protein from two other entomopathogenic fungi, *Beauveria bassiana* and *C. militaris* (Fig. 1c).

RNAi effectively down-regulates *MaFKS* expression

To study the function of *MaFKS*, an RNAi strategy was applied. Phosphinothricin-resistant transformants of *M. acridum* were generated by transformation with the vector pPK₂-pB-*MaFKS*-RNAi (Fig. 2a). Transcripts of *MaFKS* were detected by real-time RT-PCR. In comparison with the WT, *MaFKS* transcription was inhibited in RNAi transformants, with down-regulation ranging from 40 to 64.2% (RNAi-73, -55, -80, -71 and -69) ($F_{5,17} = 21.353$; $P = 0.0001$) (Fig. 2b).

MaFKS is involved in cell wall integrity

To determine the cell wall integrity of FKS-RNAi transformants, the sensitivity of two transformants (RNAi-69 and RNAi-71) and the WT to agents that disturb the cell wall or cell membrane was investigated. A 2 µl aliquot of conidial suspension, containing approximately 10,000

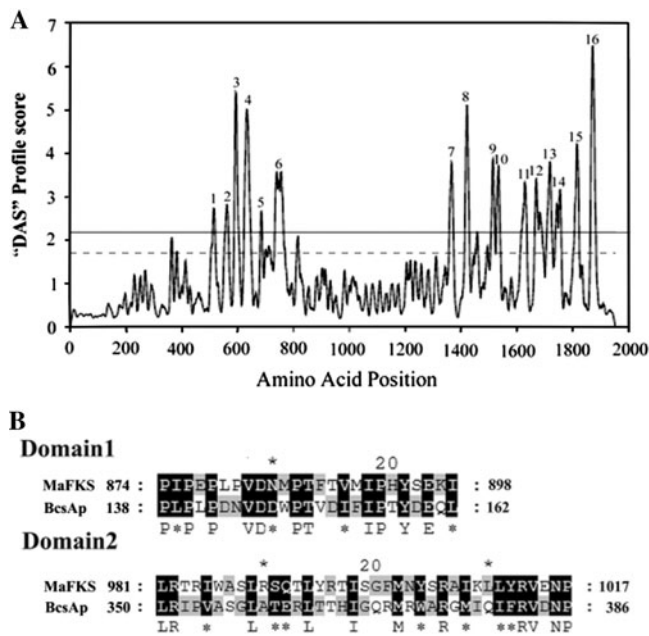


Fig. 1 Analysis of putative FKS from *M. acridum*. **a** Plot of *M. acridum* FKS transmembrane regions. The solid line indicates a strict cutoff and the dashed line a loose cutoff. The numbers above each peak correspond to the predicted transmembrane domains. **b** Domains 1 and 2 are conserved regions between putative MaFKS and BcsAp (cellulose synthase of *A. xylinum*). Asterisks denote similar amino acids. **c** Neighbor-joining tree inferred from FKS protein sequence alignments. Numbers above and below the nodes

represent the results of bootstrap analyses (100 replicates) using the neighbor-joining method. The tree shows that the *M. acridum* cloned gene is closely related to homologous sequences obtained from entomopathogenic fungi. Fungal species: Af, *A. fumigatus*; An, *A. nidulans*; Ba, *B. bassiana*; Cm, *C. militaris*; Cn, *C. neoformans*; Cp, *C. posadasii*; Ed, *Exophiala dermatitidis*; Ma, *M. acridum*; Nc, *N. crassa*; Pb, *Paracoccidioides brasiliensis*; Pm, *Penicillium marneffeii*; Sc, *S. cerevisiae*; Sp, *S. pombe*

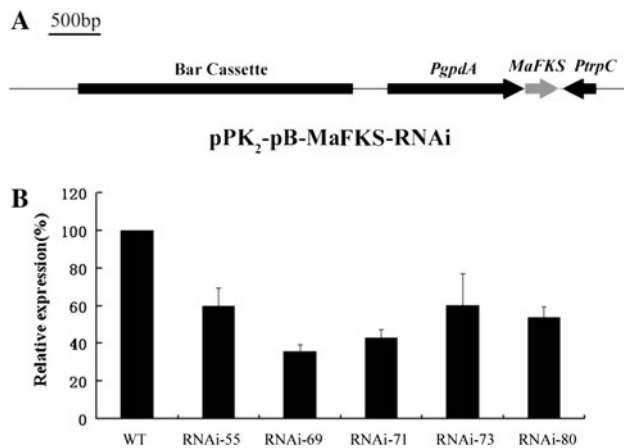


Fig. 2 Effect of RNAi on *MaFKS* expression in *M. acridum*. **a** Maps of pPK₂-pB-MaFKS-RNAi, the silencing vector for *MaFKS*. *PgpdA* is the promoter of *gpd* from *A. nidulans*, *bar* is the herbicide resistance gene, *PtprC* is the promoter of *trpC* from *A. nidulans*, and *MaFKS* (4,660–4,924 bp) is the partial sequence of the β -1,3-glucan synthase gene in *M. acridum*. **b** Quantitative RT-PCR. Analysis of *MaFKS* expression on PDA containing CR for transformants RNAi-55, -69, -71, -73 and -80 compared to the WT ($F_{5,17} = 21.353$; $P = 0.0001$). Results were first standardized against actin, with WT expression arbitrarily set to 100

conidia, was spotted with a micropipette onto PDA plates containing CR, CFW or SDS. In comparison with WT, FKS-RNAi transformants had much less aerial mycelium on PDA plates with or without CR (500 μ g/ml), CFW (50 μ g/ml) or SDS (0.01%) after 4 days of incubation (Fig. 3a). A viability test for conidia demonstrated that there was no significant difference in germination between the conidia on PDA with and without CR, CFW or SDS (data not shown). The two transformants showed a similar phenotype, and thus RNAi-69 was further examined.

To further clarify the effect of agents that disturb the cell wall or cell membrane, we observed mycelial growth in 1/4 SDAY liquid medium supplemented with CR, CFW or SDS. Most of the mycelium elements in the transformants were indistinguishable from WT hyphae in liquid medium. However, closer inspection revealed some regions of the mycelium with frequent aberrant conformations, such as blowing out of cell wall elements at hyphal tips and internal compartments in the presence of CR (Fig. 3b). However, this phenotype was not observed in 1/4 SDAY liquid medium and 1/4 SDAY supplemented with CFW or SDS (data not shown). All the results demonstrate that depleted *MaFKS* expression affected mycelium growth and

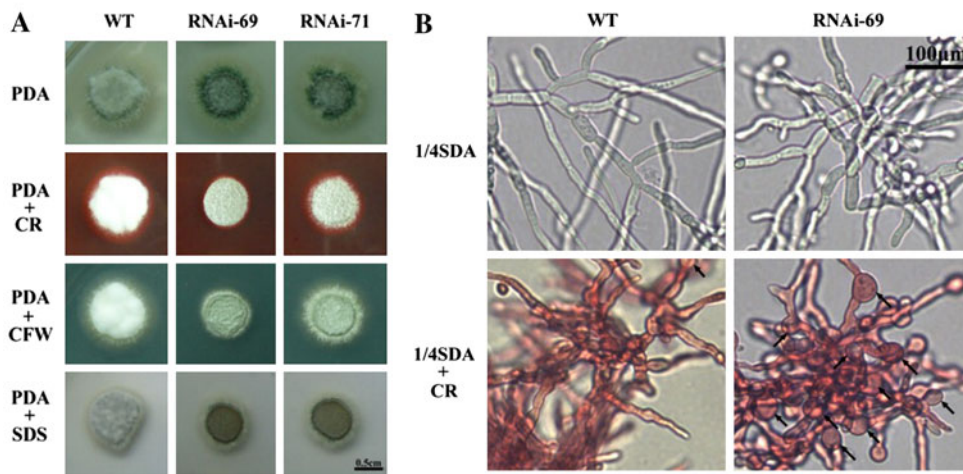


Fig. 3 Growth characteristics of FKS-RNAi transformants. **a** Fungal growth on solid media. Colonies of WT and FKS-RNAi transformants were cultured for 4 days on PDA or PDA plates containing 500 µg/ml CR, 50 µg/ml CFW or 0.01% SDS. **b** Mycelium development in

liquid media. The WT and FKS-RNAi transformants were inoculated into 1/4 SDAY liquid medium and 1/4 SDAY supplemented with 500 µg/ml CR and incubated at 28°C on a rotary shaker (100 rpm) for 48 h. *Arrows* indicate abnormally shaped hyphal compartments

increased sensitivity to the agents used, which indicates that *MaFKS* plays an important role in cell wall integrity in *M. acridum*.

Depleted *MaFKS* expression increases the sensitivity to hyperosmotic pressure in *M. acridum*

Under hyperosmotic pressure, growth of both WT and FKS-RNAi transformants was partially inhibited, but *MaFKS*-RNAi made *M. acridum* more sensitive to hyperosmotic pressure (Fig. 4). Substrate hyphae of the transformants on PDA plates containing 1 M KCl were much fewer than for the WT. Moreover, the colony surface for transformants was smooth and tawny, while that for the WT was typically wrinkled and green. On PDA plates containing 1 M sorbitol or mannitol, both transformants and WT were wrinkled and green, but the transformants had sparse aerial hyphae compared with WT (Fig. 4). These results reveal that *MaFKS* influences the hyperosmotic pressure tolerance of *M. acridum*.

MaFKS is involved in conidiation in *M. acridum*

Conidial yield was measured on PDA and PDA plates containing CR, CFW, SDS, KCl, sorbitol or mannitol after 12 days of incubation. There was no significant difference in conidial yield between WT and transformants on PDA plates ($F_{1,5} = 0.883$; $P = 0.401$), but conidial yield/mm² was significantly higher for WT than for FKS-RNAi transformants on PDA plates containing CR ($F_{1,5} = 24.874$; $P = 0.0076$), CFW ($F_{1,5} = 113.032$; $P = 0.0004$), SDS ($F_{1,5} = 248.914$; $P = 0.0001$), KCl ($F_{1,5} = 122.129$; $P = 0.0004$), sorbitol ($F_{1,5} = 197.936$; $P = 0.0001$) or

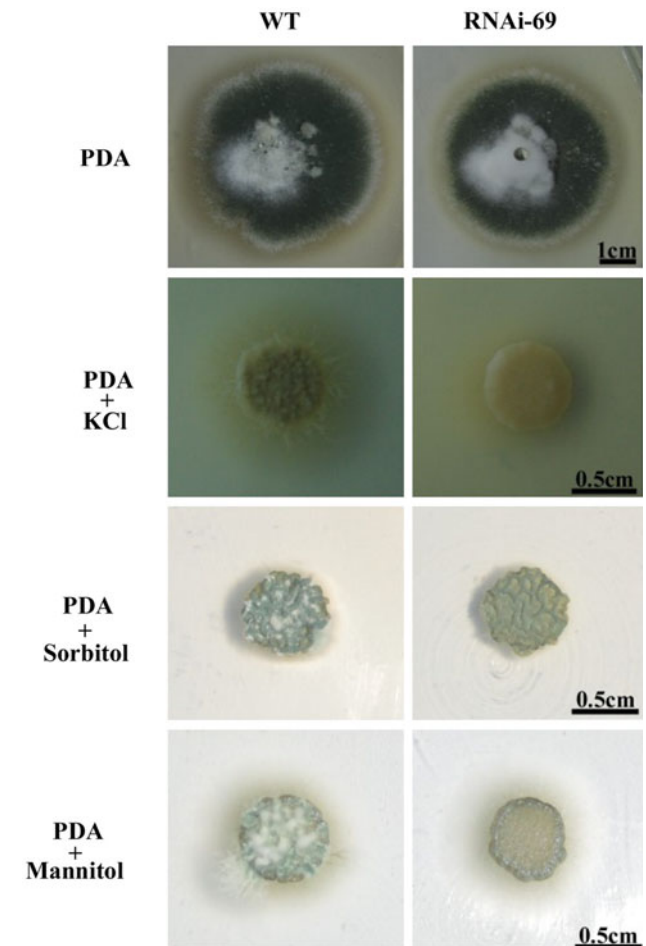


Fig. 4 Sensitivity of FKS-RNAi transformants to hyperosmotic pressure. Colonies of WT and FKS-RNAi transformants were cultured for 9 days on PDA plates with 1 M KCl, 1 M sorbitol or 1 M mannitol

mannitol ($F_{1,5} = 33.314$; $P = 0.0045$) (Fig. 5). The results show that the expression *MaFKS* level influences conidial yield in *M. acridum* under certain adverse conditions. In addition, we observed much fewer aerial hyphae for interference transformants than for WT (data not shown); thus, the reduced conidial yield may be caused by a direct effect on conidial yield and/or an indirect one resulting merely from reduced mycelia growth.

FKS-RNAi transformant are defective in β -1,3-glucan synthesis

The β -1,3-glucan content of hyphal wall in mycelium was quantified using the SLP reagent set and the results demonstrate that the β -1,3-glucan content in FKS-RNAi transformants was only 51.32% of the WT content ($F_{1,5} = 34.105$; $P = 0.0043$) (Fig. 6a). To further investigate changes in β -1,3-glucan distribution, mycelia were stained with the β -1,3-glucan-specific fluorochrome aniline blue. Fluorescence microscopy revealed that WT and

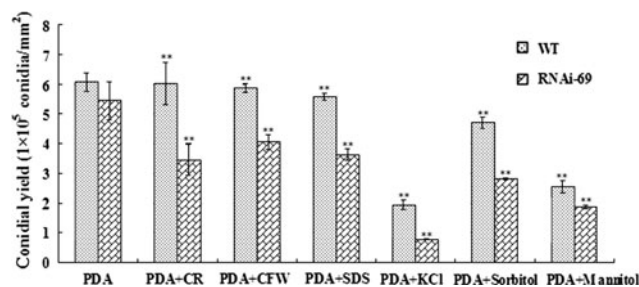
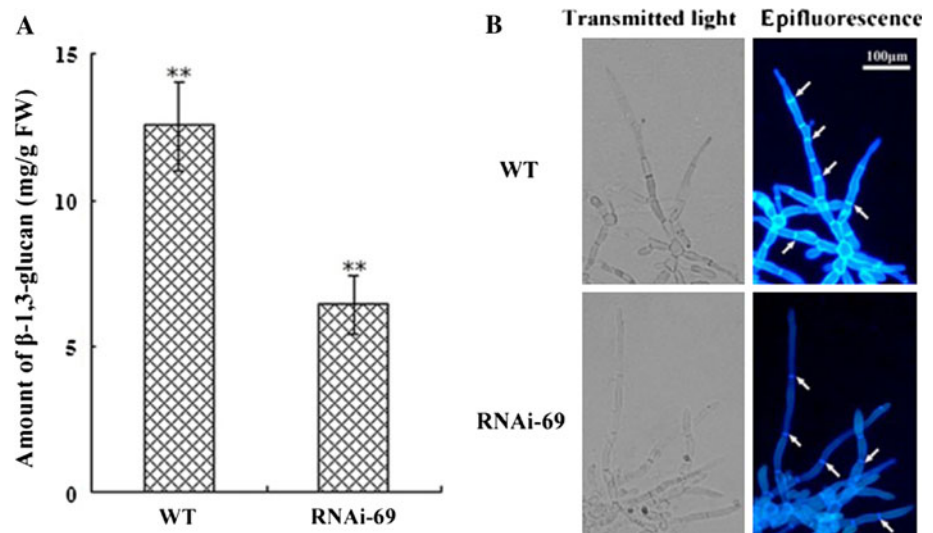


Fig. 5 Conidial yield of WT and FKS-RNAi transformants. The conidia were spread onto PDA and PDA plates containing 500 μ g/ml CR, 50 μ g/ml CFW, 0.01% SDS, 1 M KCl, 1 M sorbitol or 1 M mannitol and incubated for 12 days. Error bars show the standard deviation for three trials (** $P < 0.01$)

Fig. 6 β -1,3-Glucan content and staining of mycelium of WT and transformants. **a** β -1,3-Glucan content in hyphae of WT and RNAi-69 transformants was measured using the SLP reagent set (** $P < 0.01$). **b** WT and FKS-RNAi transformants were inoculated into 1/4 SDAY liquid medium, incubated at 28°C on a rotary shaker (100 rpm) for 48 h, and then stained with the β -1,3-glucan-specific fluorochrome aniline blue



transformant hyphae fluoresced over the majority of the hyphal surfaces, but staining in septal regions was greatly decreased in transformant compared to WT hyphae (Fig. 6b). All these results indicate that depleted *MaFKS* transcription decreases the β -1,3-glucan content in hyphal walls in *M. acridum*, especially in septal regions.

Discussion

β -1,3-Glucan synthesis is essential for life in fungi examined to date (Latgé 2007; Kellner et al. 2005). The β -1,3-glucan synthase catalytic subunit plays an important role in cell wall assembly and growth in fungi (Ishihara et al. 2007; Bernard and Latgé 2001). In this study, we identified a single gene, *MaFKS*, in *M. acridum* with significant similarity to known fungal *FKS* genes. As with other Fksp proteins, the predicted MaFKS protein has 16 transmembrane domains, the putative UDP-glucose binding consensus sequence R/KXTG at the C terminus (RITG) (Beauvais et al. 2001; Kelly et al. 1996; Thompson et al. 1999; Mazur et al. 1995; Mio et al. 1997) and two conserved domains (Kelly et al. 1996; Pereira et al. 2000). All the data presented support the hypothesis that the cloned *MaFKS* gene encodes β -1,3-glucan synthase.

Genes encoding the β -1,3-glucan synthase catalytic subunit have been well studied in *S. cerevisiae* (Douglas et al. 1994) and several other fungi (Thompson et al. 1999; Beauvais et al. 2001; Kellner et al. 2005; Ujita et al. 2006). In this study, we constructed RNAi transformants of *MaFKS*. Microscopic examination of mycelia from transformants grown in the presence of CR revealed some hyphal swelling and blowing out of cell walls. This morphology is similar to that related to the effects of inhibitors of β -1,3-glucan synthase, which cause hyphal swelling in

A. fumigatus and *C. posadasii* germings (Kurtz et al. 1994; Kellner et al. 2005). In addition, previous research has demonstrated that mutations in genes involved in cell wall biosynthesis, such as *Rho1*, *chsV* and *ChsVb* in *Fusarium oxysporum*, leads to increased sensitivity to cell wall-disturbing agents (Madrid et al. 2003; Martínez-Rocha et al. 2008; Martín-Urdíroz et al. 2008). The phenotype is consistent with our transformants on solid media containing CR or CFW. This indicates that MaFKS plays an important role in cell wall organization and maintains cell wall integrity, and suggests that the cloned *MaFKS* gene encodes β -1,3-glucan synthase. In comparison with WT, β -1,3-glucan content in FKS-RNAi transformants was significantly decreased, particularly in septal regions, which confirms that *MaFKS* encodes β -1,3-glucan synthase.

Interestingly, unlike disruption of other genes related to cell wall biosynthesis, including *rho1* (Martínez-Rocha et al. 2008) and *gas1* (Caracuel et al. 2005) null mutants of *F. oxysporum*, the growth of FKS-RNAi *M. acridum* transformants could not be relieved under hyperosmotic pressure, which suggests that MaFKS plays a role not only in maintaining cell wall integrity, but also in hyperosmotic pressure tolerance. A possible explanation is that β -1,3-glucan is a helical structure that can assume various states by extension (Rees et al. 1982). In *S. cerevisiae*, the mechanical strength of the cell wall is mainly due to β -1,3-glucan (Klis et al. 2002). A decrease in *MaFKS* transcript levels can affect the amount of β -1,3-glucan in the hyphal wall. This makes the cell wall fragile, which influences the sensitivity to hyperosmotic pressure in *M. acridum*.

Increased cell wall synthesis is also a hallmark of conidiation (Peng et al. 2009). For instance, *Ecm33* and *GEL1* in *A. fumigatus* and *csmB* in *A. nidulans* are involved in fungal cell wall biosynthesis, but also play a role in filamentous fungal conidiation (Mouyna et al. 2005; Romano et al. 2006; Takeshita et al. 2006). Conidial yield analysis demonstrated that conidial production by *MaFKS*-RNAi transformants was reduced on PDA plates containing disturbing agents or KCl, sorbitol or mannitol. This suggests that *MaFKS* is important during conidiation under certain conditions. As with *csmB* mutants of *A. nidulans* (Takeshita et al. 2006), conidiation of these transformants might be repressed in response to certain signals derived from cell wall defects.

As an alternative to chemical pesticides, *M. acridum* has been intensively investigated as a commercial biopesticide for grasshoppers and locusts (Langewald and Kooyman 2007; Milner et al. 2002; Peng et al. 2008). Stress tolerance and conidiation are two important factors that could determine the success of entomopathogenic fungi for insect pest management. The dynamic cell wall of fungi is an intermediary between the fungus and the environment and can change, depending on the life cycle stage and

environmental conditions (Latgé 2007). This study demonstrated that FKS is involved in cell wall integrity, hyperosmotic pressure tolerance and conidiation in *M. acridum*. Thus, our data should help in elucidating the mechanism of resistance to adversity and the conidiation process in entomopathogenic fungi.

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References

- Beauvais A, Bruneau JM, Mol PC, Buitrago MJ, Legrand R, Latgé JP (2001) Glucan synthase complex of *Aspergillus fumigatus*. J Bacteriol 183:2273–2279
- Bernard M, Latgé JP (2001) *Aspergillus fumigatus* cell wall: composition and biosynthesis. Med Mycol 39:9–17
- Bowman SM, Free SJ (2006) The structure and synthesis of the fungal cell wall. Bioessays 28:799–808
- Caracuel Z, Martínez-Rocha AL, Pietro AD, Madrid MP, Roncero MIG (2005) *Fusarium oxysporum* *gas1* encodes a putative beta-1, 3-glucanoyltransferase required for virulence on tomato plants. Mol Plant Microbe Interact 18:1140–1147
- Charnley A, Collins SA (2007) Entomopathogenic fungi and their role in pest control. In: Kubicek CP, Druzhinina IS (eds) Environmental and microbial relationships, 2nd edn. Springer, Berlin, pp 159–187
- Clarkson JM, Charnley AK (1996) New insights into the mechanisms of fungal pathogenesis in insects. Trends Microbiol 4:197–203
- Dallies N, Francois J, Paquet V (1998) A new method for quantitative determination of polysaccharides in the yeast cell wall. Application to the cell wall defective mutants of *Saccharomyces cerevisiae*. Yeast 14:1297–1306
- Daoust RA, Roberts DW (1983) Studies on the prolonged storage of *Metarhizium anisopliae* conidia: effect of temperature and relative humidity on conidial viability and virulence against mosquitoes. J Invertebr Pathol 41:143–150
- dos Reis MC, Pelegrinelli Fungaro MH, Delgado Duarte RT, Furlaneto L, Furlaneto MC (2004) *Agrobacterium tumefaciens*-mediated genetic transformation of the entomopathogenic fungus *Beauveria bassiana*. J Microbiol Methods 58:197–202
- Douglas CM, Foor F, Marrinan JA et al (1994) The *Saccharomyces cerevisiae* FKS1 (ETG1) gene encodes an integral membrane protein which is a subunit of 1, 3-beta-D-glucan synthase. Proc Natl Acad Sci USA 91:12907–12911
- Ekeshi S, Maniania NK, Lux SA (2003) Effect of soil temperature and moisture on survival and infectivity of *Metarhizium anisopliae* to four tephritid fruit fly puparia. J Invertebr Pathol 83:157–167
- Fang WG, Zhang YJ, Yang XY, Zheng XL, Duan H, Li Y, Pei Y (2004) *Agrobacterium tumefaciens*-mediated transformation of *Beauveria bassiana* using an herbicide resistance gene as a selection marker. J Invertebr Pathol 85:18–24
- Gao Q, Jin K, Ying SH et al (2011) Genome sequencing and comparative transcriptomics of the model entomopathogenic fungi *Metarhizium anisopliae* and *M. acridum*. PLoS Genet 7:e1001264
- Ha Y, Covert SF, Momany M (2006) FFKS1, the 1, 3- β -glucan synthase from the caspofungin-resistant fungus *Fusarium solani*. Eukaryot Cell 5:1036–1042

- Inoue SB, Qadota H, Arisawa M, Anraku Y, Watanabe T, Ohya Y (1996) Signaling toward Yeast 1, 3- β -glucan synthase. *Cell Struct Funct* 21:395–402
- Ishihara S, Hirata A, Nogami S, Beauvais A, Latge JP, Ohya Y (2007) Homologous subunits of 1,3-beta-glucan synthase are important for spore wall assembly in *Saccharomyces cerevisiae*. *Eukaryot Cell* 6:143–156
- Kahn JN, Hsu MJ, Racine F, Giacobbe R, Motyl M (2006) Caspofungin susceptibility in *Aspergillus* and non-*Aspergillus* molds: inhibition of glucan synthase and reduction of beta-D-1, 3 glucan levels in culture. *Antimicrob Agents Ch* 50:2214–2216
- Kellner EM, Orsborn KI, Siegel EM, Mandel MA, Orbach MJ, Galgiani JN (2005) *Coccidioides posadasii* contains a single 1,3-beta-glucan synthase gene that appears to be essential for growth. *Eukaryot Cell* 4:111–120
- Kelly R, Register E, Ming-Jo HSU, Kurtz M, Nielsen J (1996) Isolation of a gene involved in 1,3-b-glucan synthesis in *Aspergillus nidulans* and purification of the corresponding protein. *J Bacteriol* 178:4381–4391
- Klis FM, Mol P, Hellingwerf K, Brul S (2002) Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* 26:239–256
- Kurtz MB, Heath IB, Marrinan J, Dreikorn S, Onishi J, Douglas C (1994) Morphological effects of lipopeptides against *Aspergillus fumigatus* correlate with activities against (1,3)-beta-D-glucan synthase. *Antimicrob Agents Ch* 38:1480–1489
- Langewald J, Kooyman C (2007) Green muscle, a fungal biopesticide for control of grasshoppers and locusts in Africa. In: Vincent C, Goettel MS, Lazarovitis G (eds) *Biological control, a global perspective*. CABI, Oxfordshire, pp 311–327
- Latgé JP (2007) The cell wall: a carbohydrate armour for the fungal cell. *Mol Microbiol* 66:279–290
- Lesage G, Bussey H (2006) Cell wall assembly in *Saccharomyces cerevisiae*. *Microbiol Mol Biol Rev* 70:317–343
- Madrid MP, Di Pietro A, Roncero MI (2003) Class V chitin synthase determines pathogenesis in the vascular wilt fungus *Fusarium oxysporum* and mediates resistance to plant defence compounds. *Mol Microbiol* 47:257–266
- Martín V, Ribas JC, Carnero E, Durán A, Sánchez Y (2000) *bgs2*, a sporulation-specific glucan synthase homologue is required for proper ascospore wall maturation in fission yeast. *Mol Microbiol* 38:308–321
- Martínez-Rocha AL, Roncero MI, López-Ramírez A, Mariné M, Guarro J, Martínez-Cadena G, Di Pietro A (2008) *Rho1* has distinct functions in morphogenesis, cell wall biosynthesis and virulence of *Fusarium oxysporum*. *Cell Microbiol* 10:1339–1951
- Martín-Urdíroz M, Roncero MI, González-Reyes JA, Ruiz-Roldán C (2008) ChsVb, a class VII chitin synthase involved in septation, is critical for pathogenicity in *Fusarium oxysporum*. *Eukaryot Cell* 7:112–121
- Mazur P, Morin N, Baginsky W, EL-Shebeini M, Clemas JA, Nielsen JB, Foor F (1995) Differential expression and function of two homologous subunits of yeast 1,3-b-D-glucan synthase. *Mol Cell Biol* 15:5671–5681
- McCluskey K (2003) The Fungal Genetics Stock Center: from molds to molecules. *Adv Appl Microbiol* 52:245–262
- Milner RJ, Lim RP, Hunter DM (2002) Risks to the aquatic ecosystem from the application of *Metarhizium anisopliae* for locust control in Australia. *Pest Manag Sci* 58:718–723
- Mio T, Adachi-shimizu M, Tachibana Y, Tabuchi H, Inoue SB, Yabe T, Yamada-Okabe T, Arisawa M, Watanabe T, Yamada-Okabe H (1997) Cloning of the *Candida albicans* homolog of *Saccharomyces cerevisiae* GSC1/FKS1 and its involvement in b-1,3-glucan synthesis. *J Bacteriol* 179:4096–4105
- Mouyna I, Morelle W, Vai M et al (2005) Deletion of *GEL2* encoding for a $\beta(1-3)$ glucanoyltransferase affects morphogenesis and virulence in *Aspergillus fumigatus*. *Mol Microbiol* 56:1675–1688
- Nicholas R, Williams D, Hunter P (1994) Investigation of the value of β -glucan-specific fluorochromes for predicting the β -glucan content of cell walls of zoopathogenic fungi. *Mycol Res* 98:694–698
- Peng GX, Wang ZK, Yin YP, Zeng DY, Xia YX (2008) Field trials of *Metarhizium anisopliae* var. *acridum* (Ascomycota: Hypocreales) against oriental migratory locusts, *Locusta migratoria manilensis* (Meyen) in Northern China. *Crop Prot* 27:1244–1250
- Peng GX, Xie L, Hu J, Xia YX (2009) Identification of genes that are preferentially expressed in conidiogenous cell development of *Metarhizium anisopliae* by suppression subtractive hybridization. *Curr Genet* 55:263–271
- Pereira M, Felipe MS, Brígido MM, Soares CMA, Azevedo MO (2000) Molecular cloning and characterization of a glucan synthase gene from the human pathogenic fungus *Paracoccidioides brasiliensis*. *Yeast* 16:451–462
- Rees DA, Morris ER, Thom D, Madden JK (1982) Shapes and interactions of carbohydrate chains. In: Aspinall GO (ed) *The polysaccharides*, vol I. Academic Press, New York, pp 196–290
- Romano J, Nimrod G, Ben-Tal N, Shadkchan Y, Baruch K, Sharon H, Oshero N (2006) Disruption of the *Aspergillus fumigatus* *ECM33* homologue results in rapid conidial germination, antifungal resistance and hypervirulence. *Microbiology* 152:1919–1928
- Takeshita N, Yamashita S, Ohta A, Horiuchi H (2006) *Aspergillus nidulans* class V and VI chitin synthases CsmA and CsmB, each with a myosin motor-like domain, perform compensatory functions that are essential for hyphal tip growth. *Mol Microbiol* 59:1380–1394
- Thompson JR, Douglas CM, Li WL, Jue CK, Pramanik B, Yuan X, Rude TH, Toffaletti DL, Perfect JR, Kurtz M (1999) A glucan synthase *FKS1* homolog in *Cryptococcus neoformans* is single copy and encodes an essential function. *J Bacteriol* 181:444–453
- Tsuchiya M, Asahi N, Suzuoki F, Ashida M, Matsuura S (1996) Detection of peptidoglycan and beta-glucan with silkworm larvae plasma test. *FEMS Immunol Med Microbiol* 15:129–134
- Ujita M, Katsuno Y, Suzuki K, Sugiyama K, Takeda E, Yokoyama E, Hara A (2006) Molecular cloning and sequence analysis of the β -1,3-glucan synthase catalytic subunit gene from a medicinal fungus. *Cordyceps militaris*. *Mycoscience* 47:98–105