Research in Microbiology 166 (2015) 132–142

www.elsevier.com/locate/resmic

Original article

α1-Tubulin FaTuA1 plays crucial roles in vegetative growth and conidiation in *Fusarium asiaticum*

Weiqun Hu, Xiaoping Zhang, Xiang Chen, Jingwu Zheng, Yanni Yin*, Zhonghua Ma

Institute of Biotechnology, Zhejiang University, 866 Yuhangtang Road, Hangzhou 310058, China

Received 11 October 2014; accepted 8 January 2015
Available online 7 February 2015

Abstract

The filamentous ascomycete *Fusarium asiaticum* contains two homologous genes *FaTUA1* and *FaTUA2* encoding α-tubulins. In this study, we found that *FaTUA2* was dispensable for vegetative growth and sporulation in *F. asiaticum*. The deletion of *FaTUA1* however led to dramatically reduced mycelial growth, twisted hyphae and abnormal nuclei in apical cells of hyphae. The *FaTUA1* deletion mutant (ΔFaTuA1-5) also showed a significant decrease in conidiation, and produced abnormal conidia. Pathogenicity assays showed that ΔFaTuA1-5 exhibited decreased virulence on wheat head. Unexpectedly, the deletion of *FaTUA1* led to resistance to high temperatures. In addition, ΔFaTuA2 showed increased sensitivity to carbendazim. Furthermore, increased *FaTUA2* expression in ΔFaTuA1-5 partially restored the defects of the mutant in mycelial growth, conidial production and virulence, *vice versa*, increased *FaTUA1* expression in the *FaTUA2* deletion mutant also partially relieved the defect of the mutant in the delay of conidial germination. Taken together, these results indicate that FaTuA1 plays crucial roles in vegetative growth and development, and the functions of FaTuA1 and FaTuA2 are partially interchangeable in *F. asiaticum*.

Keywords: Alpha-tubulin; Conidiation; *Fusarium asiaticum*; Fusarium head blight; Vegetative growth

1. Introduction

Fusarium head blight (FHB) is a devastating disease of wheat and other cereal crops, which can cause huge economical losses worldwide in epidemic years [1,2]. In addition to the yield losses, mycotoxins produced by the causal agents in infected wheat pose a serious threat to food safety. FHB is caused by several *Fusarium* spp., amongst *Fusarium graminearum* species complex (*Fg* complex) are the most important etiological agents [3,4]. Owing to different cropping systems and climatic conditions, the distributions of *Fusarium* spp. associated with FHB vary significantly in different regions of the world. In China, phylogenetic species *Fusarium asiaticum* and *F. graminearum* (sensu stricto) are the major causal agents of FHB, although other *Fusarium* spp. (such as *Fusarium culmorum*, *Fusarium avenaceum* and *Fusarium tricinctum*) were also isolated occasionally from infected wheat heads [5].

Because most wheat cultivars planted throughout the world are susceptible to *Fusarium* spp. [6], application of synthetic fungicides remains one of the major tools for the control of FHB [7]. Currently, effective fungicides against FHB are limited [7]. Moreover, *Fusarium* spp. have developed resistance to several fungicides, including methyl benzimidazole carbamate (MBC) and sterol demethylation inhibitors [8–12]. Therefore, the exploration of new compounds is desperately in need for the management of FHB effectively.

The α, β-tubulin heterodimer polymerizes into microtubules which play a central role in many cellular processes, including cell divisions, intracellular transports, and the establishment of cell polarity. Most ascomycetes such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Aspergillus nidulans* contain two homologous α-tubulin genes [13]. The α1-tubulin gene is essential and required during

* Corresponding author.
E-mail address: ynyin@zju.edu.cn (Y. Yin).

http://dx.doi.org/10.1016/j.resmic.2015.01.001

vegetative growth for mitosis and nuclear migration, whereas α2-tubulin gene is dispensable for vegetative growth and asexual sporulation [14–16]. Disruption of α2-tubulin gene led to hypersensitivity to the antimicrotubule drugs in *S. cerevisiae* and *S. pombe* [14,16], reduced viability in *S. cerevisiae* [16], and delay of conidial germination and defects in the production of ascospores in *A. nidulans* [17,18].

Re-sequencing of *F. asiaticum* genome showed that this fungus contains two putative α- and two β-tubulin genes. Several studies have been conducted to explore the functions of β-tubulins in this pathogen. The resistance to MBC fungicides is caused by point mutations in the β2-tubulin [19,20]. Deletion of the β2-tubulin resulted in reduced conidiation, vegetative growth and pathogenicity, but increased sensitivity to carbendazim [19,21]. In addition, the β1-tubulin deletion mutants displayed also reduced vegetative growth and pathogenicity, produced shorten spores, and were resistant to carbendazim [22], indicating both β1-and β2-tubulins play important roles in vegetative growth, conidiation, and responses to fungicides in *F. asiaticum*. However, to date, the functions of α-tubulins have not been documented in any phytopathogenic fungi, including *Fusarium* spp. Thus, this study was initiated to investigate the roles of two α-tubulin genes in *F. asiaticum* by using target gene deletion strategy.

2. Materials and methods

2.1. Strains and culture conditions

F. asiaticum strain GJ33 collected from wheat field in Jiangsu province, China was used as a wild-type strain. The wild type and the resulting transformants were grown on potato dextrose agar (PDA; 200 g potato, 20 g dextrose, 20 g agar and 1.l water), complete medium (CM; 1% glucose, 0.2% peptone, 0.1% yeast extract, 0.1% casamino acids, nitrate salts, trace elements, 0.01% vitamins and 1.l water, pH 6.5), minimal medium (MM; 10 mM K_2HPO_4, 10 mM KH_2PO_4, 4 mM $(NH4)_2SO_4$, 2.5 mM NaCl, 2 mM $MgSO_4$, 0.45 mM $CaCl_2$, 9 mM $FeSO_4$, 10 mM glucose and 1.l water, pH 6.9), or yeast extract peptone dextrose broth (YEPD; 10 g peptone, 3 g yeast extract, 20 g glucose and 1 l water, pH 6.7) for mycelial growth tests, and in mung bean liquid medium (MBL; 40 g mung beans boiled in 1.l water for 20 min, and then filtered through cheesecloth), or carboxymethyl cellulose liquid medium (CMC; 15 g carboxylmethyl cellulose, 1 g yeast extract, 0.5 g $MgSO_4$, 1 g NH_4NO_3, 1 g KH_2PO_4 and 1.l water) for sporulation tests. Each experiment was repeated three times.

2.2. Sequence analyses of FaTUA1 and FaTUA2 genes from F. asiaticum

Based on the sequences of *TUA1* (FGSG_00639.3) and *TUA2* (FGSG_00397.3) genes in *F. graminearum*, the pairs of primers A–F + A-R and B–F + B–R (Table S1) were designated to amplify *FaTUA1* and *FaTUA2*, respectively. PCR amplifications were purified, cloned, and sequenced. The sequences of *FaTUA1* and *FaTUA2* were deposited in GenBank under accession numbers KM078599 and KM078600. The phylogenic tree was constructed by Mega 4.1 (http://www.megasoftware.net/method) using the neighbor-joining method [23].

Total RNA was extracted from hyphae and conidia of the wild-type GJ33 using a TaKaRa RNAiso Reagent (TaKaRa Biotech. Co., Dalian, China), and used for reverse transcription with the primer oligo$(dT)_{18}$ using a RevertAid H Minus First Strand cDNA Synthesis kit (Fermentas Life Sciences, Burlington, Canada). Transcripts of two *FaTUA* in *F. asiaticum* were detected by the reverse transcription PCR with primer pairs A1 + A2 and B1 + B2 (Table S1), respectively. Each primer pair spans an intron in *FaTUA1* or *FaTUA2*.

2.3. Construction of FaTUA1 and FaTUA2 deletion mutants

FaTUA1 deletion vector pBS-*FaTUA1*-Del was constructed by inserting two flanking sequences of *FaTUA1* into left and right sides of *HPH* (hygromycin resistance gene) in the pBS-HPH1 vector [24]. Briefly, by using primer pair A3 + A4 (Table S1), a 478 bp upstream flanking sequence of *FaTUA1* was amplified from GJ33 genomic DNA, and was inserted into *Xho*I-*Sal*I sites of the pBS-HPH1 vector to generate a plasmid pBS-*FaTUA1*-Up. Subsequently, a 600 bp downstream flanking sequence of *FaTUA1* amplified from GJ33 genomic DNA using the primers A5 + A6 (Table S1) was inserted into *Pst*I-*Bam*HI sites of the pBS-*FaTUA1*-Up vector to generate a plasmid pBS-*FaTUA1*-Del. Finally, the 2581 bp fragment containing *FaTUA1*-upstream-HPH-*FaTUA1*-downstream cassette was obtained by PCR amplification with primer pair A3 + A6 from the pBS-*FaTUA1*-Del. The resultant PCR product was purified and used for protoplast transformation. Using the similar strategy, the *FaTUA2*-upstream-HPH-*FaTUA2*-downstream cassette was also constructed. The PEG-mediated protoplast fungal transformation was performed as described previously [25]. For selective growth of transformants, PDA medium supplemented with hygromycin (100 mg/l) were used. After single spore isolation, transformants were kept at 4 °C for further following experiments.

2.4. Genetic complementation and increased expression of FaTUA

To confirm that the phenotype of *FaTUA1* deletion mutant is due to disruption of the gene, genetic complementation was performed. The *FaTUA1* complement plasmid pCA-*FaTUA1*-Com was constructed using the backbone of pCAMBIA1300. First, a *Xho*I-*Kpn*I *neo* cassette containing a *trpC* promoter was amplified from plasmid pBS-RP-Red-A8-NEO [26] with primers Neo-F + Neo-R (Table S1), and cloned into the *Xho*I-*Kpn*I site of pCAMBIA1300 to create plasmid pCA-neo. Then, a 3250 bp of full length *FaTUA1* gene including 1532 bp promoter region was amplified using primer pair A9 + A10 (Table S1) from genomic DNA of the wild-type GJ33, and subsequently cloned into the *Kpn*I-*Xba*I sites of pCAMBIA1300 to generate the complement plasmid pCA-*FaTUA1*-Com. Transformation of ΔFaTuA1-5 with the full-