重庆市基础研究与前沿探索项目：转录因子 MaSom1 调控绿僵菌微循环产孢的分子机制（cstc2018jcyjAX0554）

研究成果清单：

1、发表研究论文 8 篇：

(1) Zhao Tingting, Wen Zhiqiong, Xia Yuxian, Jin Kai (2020) The transmembrane protein MaSho1 negatively regulates conidial yield by shifting the conidiation pattern in *Metarhizium acridum*. Applied Microbiology and Biotechnology, 104 (9): 4005－4015.（中科院分区 2 区；JCR 分区 Q2）

(2) Wen Zhiqiong, Tian Huiting, Xia Yuxian, Jin Kai (2020) MaPmt1, a protein *O*-mannosyltransferase, contributes to virulence through governing the appressorium turgor pressure in *Metarhizium acridum*. Fungal Genetics and Biology, 145: 103480.（中科院分区 3 区；JCR 分区 Q2）

(3) Zhang Maoge, Wei Qinglv, Xia Yuxian, Jin Kai (2020) MaPacC, a pH-responsive transcription factor, negatively regulates thermotolerance and contributes to conidiation and virulence in *Metarhizium acridum*. Current Genetics, 66(2): 397－408.（中科院分区 3 区）

(4) Gao Pingping, Jin Kai, Xia Yuxian (2020) The phosphatase gene *MaCdc14* negatively regulates UV-B tolerance by mediating the transcription of melanin synthesis-related genes and contributes to conidiation in *Metarhizium acridum*. Current Genetics, 66(1):141－153.（中科院分区 3 区）

(5) Zhao Tingting, Tian Huiting, Xia Yuxian, Jin Kai (2019) MaPmt4, a protein *O*-mannosyltransferase, contributes to cell wall integrity, stress tolerance and virulence in *Metarhizium acridum*. Current Genetics, 65(4): 1025－1040.（中科院分区 3 区）

(6) Zhang Junjie, Jiang Hui, Du Yanru, Keyhani NO, Xia Yuxian, Jin Kai (2019) Members of chitin synthase family in *Metarhizium acridum* differentially affect fungal growth, stress tolerances, cell wall integrity and virulence. PLoS Pathogens. 15(8): e1007964. （中科院分区 2 区；JCR 分区 Q1）

(7) Gao Pingping, Li Muchun, Jin Kai, Xia Yuxian (2019) The homeobox gene *MaHl* governs microcycle conidiation for increased conidial yield by mediating transcription of conidiation pattern shift-related genes in *Metarhizium acridum*. Applied Microbiology and Biotechnology, 103(5): 2251－2262（中科院分区 2 区；JCR 分区 Q2）

(8) Zhang Jie, Wang Zhenglong, Nemat O. Keyhani, Peng Guoxiong, Jin Kai, Xia Yuxian (2019) The protein phosphatase gene *MaPpt1* acts as a programmer of microcycle conidiation and a negative regulator of UV-B tolerance in *Metarhizium acridum*. Applied Microbiology and Biotechnology, 103: 1351－1362.（中科院分区 2 区；JCR 分区 Q2）

2、获权发明专利 1 项：

金凯、夏玉先、杜彦茹. 一种真菌表达载体及其构建方法，专利号：ZL2017 1 0725033.6

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APPLIED GENETICS AND MOLECULAR BIOTECHNOLOGY

The transmembrane protein MaSho1 negatively regulates conidial yield by shifting the conidiation pattern in *Metarhizium acridum*

Tingting Zhao [1,2,3] · Zhiqiong Wen [1,2,3] · Yuxian Xia [1,2,3] · Kai Jin [1,2,3]

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Abstract

Sho1 is an important membrane sensor upstream of the HOG-MAPK signaling pathway, which plays critical roles in osmotic pressure response, growth, and virulence in fungi. Here, a Sho1 homolog (MaSho1), containing four transmembrane domains and one Src homology (SH3) domain, was characterized in *Metarhizium acridum*, a fungal pathogen of locusts. Targeted gene disruption of *MaSho1* impaired cell wall integrity, virulence, and tolerances to UV-B and oxidative stresses, while none of them was affected when the SH3 domain was deleted. Intriguingly, disruption of *MaSho1* significantly increased conidial yield, which was not affected in the SH3 domain mutant. Furthermore, it was found that deletion of *MaSho1* led to microcycle conidiation of *M. acridum* on the normal conidiation medium. Deletion of *MaSho1* significantly shortened the hyphal cells but had no effect on conidial germination. Digital gene expression profiling during conidiation indicated that differential expression of genes was associated with mycelial development, cell division, and differentiation between the wild type and the *MaSho1* mutant. These data suggested that disruption of *MaSho1* shifted the conidiation pattern by altering the transcription of genes to inhibit mycelial growth, thereby promoting the conidiation of *M. acridum*.

Keywords *Metarhizium acridum* · HOG-MAPK pathway · Sho1 · Conidiation pattern · Microcycle conidiation

Introduction

In most filamentous fungi, two kinds of asexual conidiation patterns, normal conidiation and microcycle conidiation, are found (Hanlin 1994; Jung et al. 2014). Normal conidiation is a basic part in the life cycle of the fungus, which sporulates after proper mycelial growth (Anderson and Smith 1971). During microcycle conidiation, however, conidia can bypass or shorten mycelial development and directly generate conidia (Bosch and Yantorno 1999; Lapaire and Dunkle 2003; Ahearm et al. 2007; Zhang et al. 2010; Pintye et al. 2011). Many environmental stresses can shift the conidiation pattern (Anderson and Smith 1971; Hanlin 1994; Jung et al. 2014; Wang et al. 2016). *Metarhizium acridum*, a locust-specific fungal pathogen, also has two conidiation patterns, and microcycle conidiation has some advantages in biocontrol potential compared with normal conidiation (Zhang et al. 2010). Therefore, exploring the underlying molecular causing conidiation pattern shift in *M. acridum* is helpful to improve the conidiation capacity and insecticidal efficiency.

The HOG-MAPK (high osmolarity glycerol mitogen-activated protein kinase) signaling pathway plays important roles in fungal adaptation to the changes of external environments (temperature, pH, and hyperosmotic stress, etc.) and participates in governing the cell growth, cell wall integrity, and invasive growth (Hohmann 2009; Chen and Thorner 2007; Saito and Posas 2012). In *Saccharomyces cerevisiae*, the HOG pathway comprises the two upstream branches of

Tingting Zhao and Zhiqiong Wen contributed equally to this work.

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✉ Yuxian Xia
yuxianxia@cqu.edu.cn

✉ Kai Jin
jinkai@cqu.edu.cn

[1] Genetic Engineering Research Center, School of Life Sciences, Chongqing University, Chongqing 401331, People's Republic of China

[2] Chongqing Engineering Research Center for Fungal Insecticide, Chongqing 401331, People's Republic of China

[3] Key Laboratory of Gene Function and Regulation Technologies under Chongqing Municipal Education Commission, Chongqing 401331, People's Republic of China

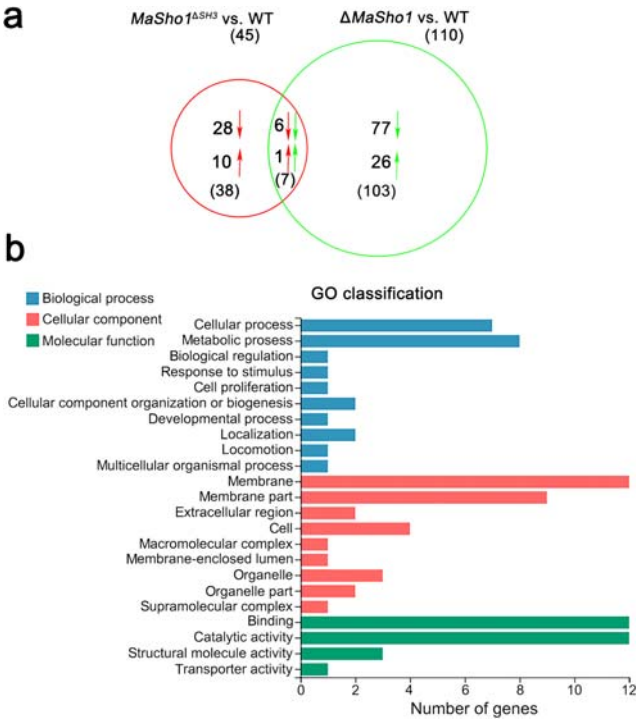

Fig. 6 Identification and GO annotation of DEGs. **a** The number of shared DEGs between Δ*MaSho1* vs. WT and *MaSho1*$^{\Delta SH3}$ vs. WT. *Up arrows* upregulated genes; *down arrows* downregulated genes. **b** Functional enrichment analysis by GO annotation for the 103 DEGs from Δ*MaSho1* vs. WT.

pattern, suggesting that the functions of MaSho1 might be independent of MaHog1 during the conidiation pattern shift. These mechanisms need to be clarified in future investigations.

Two conidiation patterns, normal conidiation and microcycle conidiation, are found in most filamentous fungi (Hanlin 1994). The two patterns are interconvertible under some specific conditions (Anderson and Smith 1971; Bosch and Yantorno 1999; Wang et al. 2016). Compared with normal conidiation, microcycle conidiation typically bypassed or simplified the hyphal growth (Jung et al. 2014). Thus, genes involved in cell polarity and hyphal growth are crucial for conidiation pattern shift. In *A. fumigatus*, deletion of *Sho1* led to decreased Spitzenkörper body size and affected fungal hyphal morphogenesis (Yang et al. 2011), suggesting the hyphal growth reduction in Δ*MaSho1* may result from a cell polarity defect. Consistently, our DGE data showed that a putative UDP-glucose 4-epimerase gene (MAC_08917), which is required for hyphal growth in *A. nidulans* (El-Ganiny et al. 2010), was remarkably downregulated in Δ*MaSho1*. A gene for cation-transporting ATPase 4 (MAC_09130), which plays an important role in cell polarity and division (Façanha et al. 2002), was also significantly downregulated when *MaSho1* was deleted. In addition, a downregulated DEG in Δ*MaSho1*, for a stomatin-like protein (MAC_03596), was crucial for hyphal polarized growth and morphology in *A. nidulans* (Takeshita et al. 2012). A glucose-methanol-choline oxidoreductase gene (MAC_02934), which was involved in hyphal development of *A. nidulans* and associated with FluG sporulation signaling pathways (Etxebeste et al. 2012), was significantly upregulated in Δ*MaSho1*. In yeast, cell membrane proteins and cell wall proteins are critical during cell division (Tiwari et al. 2016; Sethi et al. 2016). In our DGE data, genes encoding these proteins were downregulated in Δ*MaSho1*, including genes for a putative cell surface protein (MAC_08122), exo-1,3-β-D-glucanase (MAC_08796), and integral membrane protein (MAC_06062). Moreover, a gene for PRO1A C6 Zinc-finger protein (MAC_00376) playing important roles in conidial morphology and conidial yield (Masloff et al. 2002) was upregulated in Δ*MaSho1*.

In summary, *MaSho1* makes contributions to stress tolerances and virulence of *M. acridum*. Interestingly, the *MaSho1* deletion increases the conidial yield due to shifting the conidiation pattern of *M. acridum*, which is valuable for industrial production of mycopesticides. *MaSho1* and the DEGs in Δ*MaSho1* will be potentially useful to enhance the conidiation capacity of *M. acridum*.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

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MaPmt1, a protein *O*-mannosyltransferase, contributes to virulence through governing the appressorium turgor pressure in *Metarhizium acridum*

Zhiqiong Wen, Huiting Tian, Yuxian Xia *, Kai Jin *

Genetic Engineering Research Center, School of Life Sciences, Chongqing University, Chongqing 401331, PR China
Chongqing Engineering Research Center for Fungal Insecticide, Chongqing 401331, PR China
Key Laboratory of Gene Function and Regulation Technologies Under Chongqing Municipal Education Commission, Chongqing 401331, PR China

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ABSTRACT

O-glycosylation is a very important post-translational modification of protein and involved in many cell processes in fungi. There exist three protein *O*-manosyltransferanse genes (*MaPmt1*, *MaPmt2*, *MaPmt4*) in *Metarhizium acridum* based on sequence homology. Here, *MaPmt1*, a gene for Pmt1 *O*-manosyltransferanse in *M. acridum*, was characterized and functionally analyzed through targeted gene disruption and complementation methods. Deletion of *MaPmt1* had no effect on conidial germination, but slightly increased the conidial yield and significantly impaired fungal tolerances to UV-B radiation and wet-heat. Deletion of *MaPmt1* made the fungus become more sensitive to cell wall disturbing agents and exhibit a thinner cell wall with changed components. Insect bioassays showed that disruption of *MaPmt1* attenuated the fungal virulence significantly by topical inoculation but not by injection, indicating that *MaPmt1* is required for penetration during the infection of *M. acridum*. Interestingly, deletion of *MaPmt1* did not affect appressorium formation but significantly decreased appressorium turgor pressure. Moreover, the decreased virulence of *MaPmt1* disruptant is mainly due to the reduced appressorium turgor pressure, which may be resulted from the declined glycerol concentration, combined with the weakened cell wall that could not hold the normal appressorium turgor pressure to penetrate the host cuticle.

1. Introduction

Entomopathogenic fungi are environmentally friendly and have attracted much attention as an alternative to chemical insecticides (Glare et al., 2012) and can infect their insect hosts directly through the exoskeleton or cuticle (Wang and Wang, 2017). The infection process of fungi mainly involves the following steps: first, the fungi attach to host insect cuticle, followed by germination of conidia and germ tube formation, then developed into appressoria, known as the penetration structures (Holder and Keyhani, 2005). The process of fungi to penetrate the insect cuticle or skeleton mainly related to some cuticle degrading enzymes and turgor pressure (Freimoser et al., 2005; St Leger et al., 1992). To date, however, the molecular mechanism of infection of entomopathogenic fungi have not been fully elucidated. Thus, further understanding the pathogenesis of insect pathogenic fungi is helpful to tap their biological control potential.

Protein *O*-mannosylation, a widespread post-translational modification, is evolutionarily conserved from bacteria to fungi, as well as in human (Lommel and Strahl, 2009) and initiated by a family of protein *O*-mannosyltransferases (PMTs) (Abu-Qarn et al., 2008; Strahl-Bolsinger et al., 1993), and plays important roles in regulating function and secretion of proteins (Bourdineaud et al., 1998). Over the past decades, many functions of fungal *O*-mannosylation have been elucidated, i.e. cell wall integrity and stability, cell morphology, as well as protein sorting and localization (Hirayama et al., 2008; Lommel and Strahl, 2009). In *Saccharomyces cerevisiae*, seven members (ScPmt1-7) are included in PMT family which is divided into three subfamilies according to their homology: the Pmt1 subfamily including Pmt1, Pmt 5 and Pmt7, the Pmt2 subfamily including Pmt2, Pmt3 and Pmt6 and the Pmt4 subfamily containing a member of Pmt4 (Gentzsch and Tanner, 1997; Willer et al., 2002). In the human fungal pathogen *Candida albicans*, the PMT family includes five isoforms (Prill et al., 2005). But in

Abbreviations: CR, Congo red; CFW, calcofluor white; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ORF, open reading frame; TEM, transmission electron microscope.

* Corresponding authors at: Genetic Engineering Research Center, School of Life Sciences, Chongqing University, Chongqing 401331, PR China.
E-mail addresses: yuxianxia@cqu.edu.cn (Y. Xia), jinkai@cqu.edu.cn (K. Jin).

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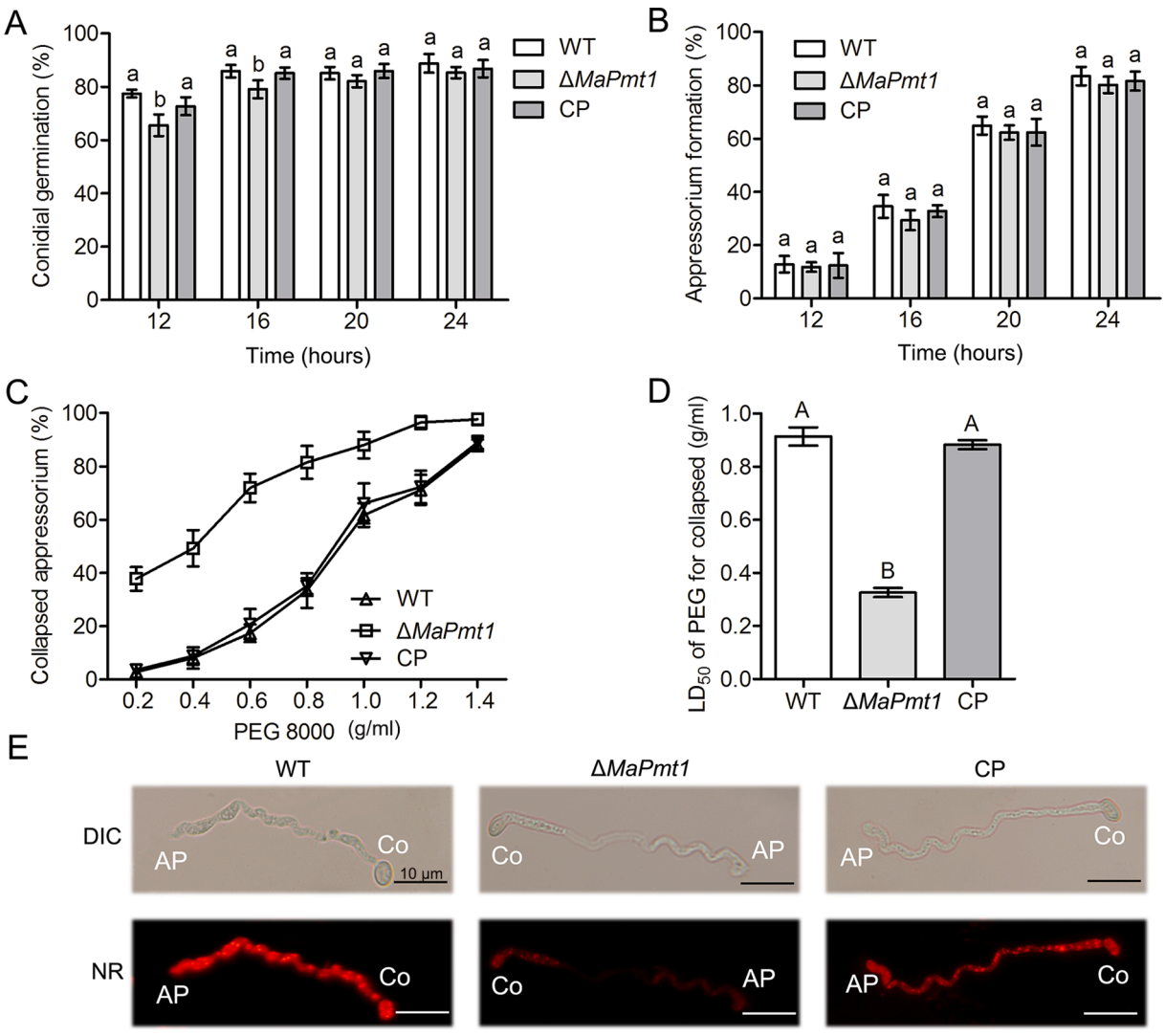

Fig. 5. Appressorium formation of fungal strains on locust wings. (A) Germination rates of conidia cultivated on locust wings for 12 h, 16 h, 20 h and 24 h. (B) Appressorium formation rates of fungal strains at 12 h, 16 h, 20 h and 24 h. (C) Collapsed appressoria in PEG-8000 with different concentrations of fungal strains. (D) The LD_{50}s of PEG-8000 to make 50% of appressoria collapsed. (E) Lipid droplets stained with Nile red in appressorium and mycelia. Error bars indicated standard deviations of biological triplicates. Bar = 10 μm. A, B: significant differences at $P < 0.01$. a, b: significant differences at $P < 0.05$. The same letters indicated no statistical significance ($P > 0.05$).

cell wall that can not bear a huge pressure in *M. acridum*.

5. Conclusions

As discussed above, *MaPmt1* was not only involved in stress tolerances and cell wall integrity, but also contributed to virulence in *M. acridum*. Notably, *MaPmt1* disruption decreased the appressorium turgor pressure by impairing the glycerol synthesis and/or changing the cell wall structure and compotents in *M. acridum*.

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Compliance with ethical standards

Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

CRediT authorship contribution statement

Zhiqiong Wen: Data curation, Formal analysis, Investigation, Software, Writing - original draft. **Huiting Tian:** Data curation, Formal analysis, Investigation, Software. **Yuxian Xia:** Supervision, Funding acquisition, Project administration. **Kai Jin:** Methodology, Resources, Supervision, Conceptualization, Funding acquisition, Project administration, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fgb.2020.103480.

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ORIGINAL ARTICLE

MaPacC, a pH-responsive transcription factor, negatively regulates thermotolerance and contributes to conidiation and virulence in *Metarhizium acridum*

Maoge Zhang[1,2,3] · **Qinglv Wei**[1,2,3] · **Yuxian Xia**[1,2,3] · **Kai Jin**[1,2,3]

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Abstract

PacC is a pH-responsive transcription factor gene highly expressed at alkaline pH and plays distinct roles in environmental fitness, conidiation and virulence of different fungi. Here, we show biological functions of orthologous MaPacC in the locust-specific fungal pathogen *Metarhizium acridum*. Disruption of *MapacC* slowed down the fungal growth only under alkaline conditions. Intriguingly, the fungal thermotolerance was enhanced by the *MapacC* deletion, accompanied by transcriptional upregulation of some heat shock-responsive genes. The disruptant suffered a reduction in conidial yield and a change in conidial surface structure, but showed little change in cell wall integrity. The virulence of the disruptant against a locust species was markedly attenuated due to delayed appressorium formation, repressed expression of some insect cuticle hydrolases and slowed growth in locust hemolymph. The phenoloxidase activity and nodules of the locusts infected by the disruptant were also boosted. All of these phenotypic changes were restored by targeted gene complementation. Our results indicate that MaPacC acts a negative regulator of thermotolerance and contributes to the virulence of *M. acridum* by an involvement in hyphal penetration through insect cuticle and evasion from insect immunity.

Keywords *Metarhizium acridum* · PacC · Thermotolerance · Virulence · Insect immune evasion · Conidial surface structures

Introduction

Entomopathogenic fungi can infect host through cuticular penetration (Clarkson and Charnley 1996) and serve as biological control agents of arthropod pests (Lord 2005; Peng et al. 2008). Wide application of mycoinsecticides is restrained due to their sensitivity to environmental stresses and slower killing action (Rangel et al. 2005; Fernandes et al. 2012, 2015). Advanced fungal biotechnology has promoted genetic improvement of fungal virulence and/or stress tolerance (Lovett and St Leger 2018). Thus, elucidation of the signaling network involved in stress tolerance and virulence will facilitate fungal improvement and commercial development.

As a zinc finger transcription factor, PacC/Rim101 plays critical roles in fungal adaptation to broad ambient pH conditions and hosts (Peñalva et al. 2008; Cornet and Gaillardin 2014). Besides typical zinc finger C_2H_2 domains, PacC protein also features a nuclear localization signal and three conserved domains (region A, B and C) (Arst and Peñalva 2003). OH^- can activate the Pal pH signaling pathway to

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Maoge Zhang and Qinglv Wei contributed equally to this work.

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✉ Yuxian Xia
yuxianxia@cqu.edu.cn

✉ Kai Jin
jinkai@cqu.edu.cn

[1] Genetic Engineering Research Center, School of Life Sciences, Chongqing University, Chongqing 401331, People's Republic of China

[2] Chongqing Engineering Research Center for Fungal Insecticide, Chongqing 401331, People's Republic of China

[3] Key Laboratory of Gene Function and Regulation Technologies Under Chongqing Municipal Education Commission, Chongqing 401331, People's Republic of China

of *B. bassiana* (Zhu et al. 2016). In this study, conidial tolerance to high temperature was significantly enhanced by the disruption of *MapacC* in *M. acridum*, accompanied by up-regulated expression of several heat-shock responsive genes, which encode heat shock proteins (HSPs) and catalase or are involved in ubiquitin accumulation or ESCRT complexes and hence function in protecting fungal cell from heat stress (Parsell and Lindquist 1993; Loser and Weltring 1998; Noventa-Jordão et al. 1999; Wang et al. 2014; Brune et al. 2019). Therefore, our results indicate that MaPacC serves as a negative regulator of conidial thermotolerance in *M. acridum*.

In *M. robertsii*, virulence was attenuated by the deletion of *pacC*, which impaired the fungal capability of insect cuticle penetration and host immunity evasion (Huang et al. 2015). Our Δ*MapacC* mutant also showed attenuated virulence due to delayed appressorium formation and reduced expression levels of *Pr1A* and *Chit1* critical for insect cuticle hydrolysis (St Leger et al. 1996; Fang et al. 2005). Moreover, development of hyphal bodies in locust hemolymph was delayed in Δ*MapacC* than in control strains likely due to suppression by host immune defense, which fungal cells must overcome to propagate by yeast-like budding in host hemocoel (Cerenius and Söderhäll 2004; Wang and St Leger 2006). In this study, the locusts infected by Δ*MapacC* displayed increased PO activity and formed more nodules implicating an involvement of *MapacC* in the fungal evasion from locust immunity defense. Some fungal cell-wall components are often targeted by host immune system (Gow et al. 2012). In this study, reduced conidial hydrophobicity and altered distributions of carbohydrates on conidial surface resulted from the *MapacC* deletion. Therefore, locust immune response triggered by the infection of Δ*MapacC* could be attributable to altered cell-wall composition, particularly the increased distribution of β-1,3-glucan a cell-wall component readily recognized by insect host immune system (Ochiai and Ashida 2000).

Altogether, *MapacC* contributes to conidiation capacity and virulence, but negatively regulates thermotolerance of *M. acridum*. Conidial yield, thermotolerance and virulence are important phenotypes associated with fungal biocontrol potential. Therefore, *MapacC* may act as a candidate gene for genetic improvement of the fungal potential against locusts.

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ORIGINAL ARTICLE

The phosphatase gene *MaCdc14* negatively regulates UV-B tolerance by mediating the transcription of melanin synthesis-related genes and contributes to conidiation in *Metarhizium acridum*

Pingping Gao[1,2,3] · **Kai Jin**[1,2,3] · **Yuxian Xia**[1,2,3]

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Abstract

Reversible phosphorylation of proteins regulated by protein kinases and phosphatases mediate multiple biological events in eukaryotes. In this study, a dual-specificity cell division cycle 14 phosphatase, MaCdc14, was functionally characterized in *Metarhizium acridum*. Deletion of *MaCdc14* decreased branch numbers, affected septum formation and resulted in multiple nuclei in each hyphal compartment, indicating nuclear division and cytokinesis defects. The spore production capacity was severely impaired with decreased conidial yield and delayed conidiation in *MaCdc14*-deletion mutant (Δ*MaCdc14*). The transcription levels of conidiation-related genes were significantly changed after *MaCdc14* inactivation. The morphology of conidia was uneven in size and the germination rate of conidia was increased in Δ*MaCdc14*. In addition, Δ*MaCdc14* displayed significantly enhanced conidial tolerance to ultraviolet (UV) irradiation but had no significant effect on the thermotolerance, the sensitivities to cell wall damage reagents, osmotic and oxidative stresses, and virulence compared to the wild-type strain and complementary transformant. Furthermore, the pigmentation of Δ*MaCdc14* was increased by the upregulated expression of melanin synthesis-related genes, which may result in the enhanced UV-B tolerance of Δ*MaCdc14*. In summary, MaCdc14 negatively regulated UV-B tolerance by mediating the transcription of melanin synthesis-related genes, contributed to conidiation by regulating the expression levels of conidiation-related genes and also played important roles in cytokinesis and morphogenesis in *Metarhizium acridum.*

Keywords *Metarhizium acridum* · Cdc14 phosphatase · Conidiation · UV-B tolerance · Melanin

Introduction

Metarhizium spp. as entomopathogenic fungi have been extensively studied because of their safety and environmental friendliness (Aw and Hue 2017). *Metarhizium acridum,* a locust-specific pathogen, has been used as a model fungus to study issues in ecology, evolution and the mechanisms of speciation, and has shown great potential in biological control (Roberts and St Leger 2004; Aw and Hue 2017). Such biological agents have been widely used in Australia (Hunter et al. 1999), Africa (Niassy et al. 2011) and Asia (Peng et al. 2008). However, ultraviolet (UV) irradiation (UV-A and UV-B) from sunlight usually leads to DNA damage, mutation and protein denaturation, and it also damages RNA, ribosome, biofilms and membrane lipids (Griffiths et al. 1998; Yao et al. 2010; Braga et al. 2015). Vulnerability to environmental disturbances and poor efficacy are grave impediments for the application of biological agents in farmland (Rangel et al. 2015; Fernandes et al. 2015). Thus,

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✉ Kai Jin
jinkai@cqu.edu.cn

✉ Yuxian Xia
yuxianxia@cqu.edu.cn

[1] Genetic Engineering Research Center, School of Life Sciences, Chongqing University, Chongqing 401331, People's Republic of China

[2] Chongqing Engineering Research Center for Fungal Insecticide, Chongqing 401331, People's Republic of China

[3] Key Laboratory of Gene Function and Regulation Technologies Under Chongqing Municipal Education Commission, Chongqing 401331, People's Republic of China

◂**Fig. 5** Assays for stress tolerance and virulence. **a** Conidial germination after UV-B irradiation (1350 mW/m^2) treatment for 1.5, 3, 4.5, and 6 h. **b** The half-inhibition time (IT_{50}) after UV-B irradiation. **c** Conidial germination after exposure to 45 °C wet-heat stress for 3, 6, 9 and 12 h. **d** IT_{50} after heat shock. **e** Fungal virulence to fifth-instar nymphs of *L. migratoria*. Paraffin oil was used as controls. **f** The mean 50% lethality time (LT_{50}) for the virulence assays. a, b: significant difference, $P<0.05$; A, B: significant difference, $P<0.01$. Error bars: standard deviation. WT, the wild-type strain; Δ*MaCdc14*, *MaCdc14*-deletion transformant; CP, complementary transformant

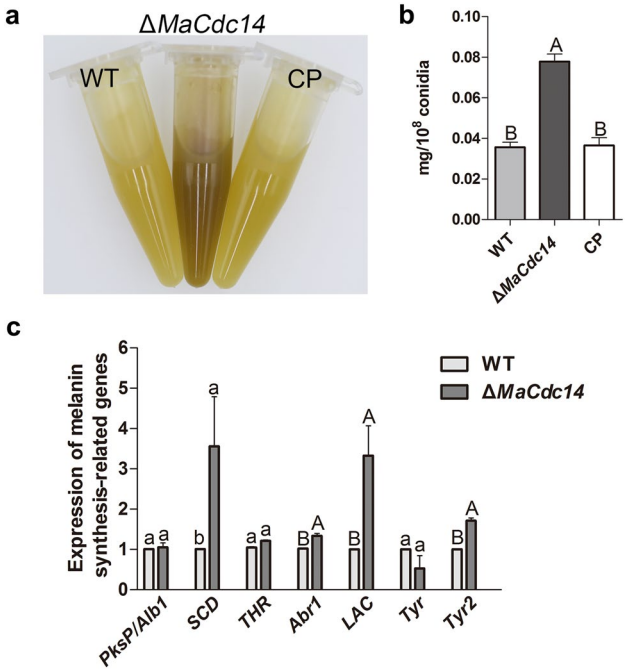

Fig. 6 Melanin pigment assays. **a** Conidial suspensions (1×10^8 conidia/mL) of each strain were photographed. **b** Conidial melanin pigment assays. **c** qRT-PCR of melanin synthesis-related genes. *PksP/Alb1*: MAC_09310; *SCD*: MAC_09308; *THR*: MAC_09308; *Abr1*: MAC_07244; *LAC*: MAC_01518; *Tyr*: MAC_02956; *Tyr2*: MAC_02717. a, b: significant difference, $P<0.05$; A, B: significant difference, $P<0.01$. Error bars: standard deviation. WT, the wild-type strain; Δ*MaCdc14*, *MaCdc14*-deletion transformant; CP, complementary transformant

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ORIGINAL ARTICLE

MaPmt4, a protein *O*-mannosyltransferase, contributes to cell wall integrity, stress tolerance and virulence in *Metarhizium acridum*

Tingting Zhao[1,2,3] · **Huiting Tian**[1,2,3] · **Yuxian Xia**[1,2,3] · **Kai Jin**[1,2,3]

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Abstract

In eukaryotic cells, protein *O*-glycosylation is an essential protein modification. Analysis of the *Metarhizium acridum* genome database revealed a total of three *O*-glycoside mannosyltransferase homologs (Pmt1, Pmt2 and Pmt4), closely related to *Saccharomyces cerevisiae* Pmt1, Pmt2, and Pmt4. In this study, the functions of *MaPmt4*, encoding a protein *O*-mannosyltransferase in *M. acridum*, were characterized using disruption and complementation strategies. Disruption of *MaPmt4* delayed the conidial germination and reduced the fungal tolerances to heat shock and UV-B irradiation, but did not affect conidial yield. Inactivation of *MaPmt4* displayed increased sensitivity to cell wall-perturbing agents, formed thinner cell walls, and changed composition of fungal cell wall, demonstrating that *MaPmt4* was also important to maintain fungal cell wall integrity. Bioassays by topical inoculation and intrahemocoel injection showed that the *MaPmt4* deletion mutant exhibited greatly reduced virulence. The subsequent examination revealed that the inactivation of *MaPmt4* impaired appressorium formation, decreased fungal growth in locust hemolymph in vitro, and boosted insect immune responses, the latter in part potentially owing to the changes in conidial surface structures, and thus attenuated the virulence of *MaPmt4* deletion mutant. Furthermore, the results of comparative proteomics showed that *MaPmt4* played important roles in fungal cell wall integrity, stress tolerances, and virulence via broad genetic pathways.

Keywords *Metarhizium acridum* · Protein *O*-mannosyltransferase (PMT) · MaPmt4 · Virulence · Cell wall · Proteomic analysis

Introduction

O-mannosylation is an important post-translational modification of protein and plays important roles in improving the stability and solubility of various proteins (Goto 2007). In eukaryotes, the first step of *O*-mannosylation is that the addition of a mannose residue on secreted acceptor proteins at serine/threonine residues is catalyzed by protein *O*-mannosyltransferases (PMTs) on the membrane of the endoplasmic reticulum (Lommel and Strahl 2009; Loibl and Strahl 2013). *O*-mannosylation is conserved from bacteria to humans, but not found in algae, plants and protozoa. The physiological roles of *O*-mannosylation have been characterized in certain bacterial genera (Liu et al. 2013; Katja et al. 2017), and some fungal species (Gentzsch and Tanner 1997; Strahl-Bolsinger et al. 1999; Oka et al. 2004; Wang et al. 2014; Shimizu et al. 2014; Pan et al. 2018; Le et al. 2018).

In *Saccharomyces cerevisiae*, seven Pmt family members (*Sc*Pmt1p–*Sc*Pmt7p) are present and subdivided into the Pmt1, Pmt2, and Pmt4 subfamilies by phylogenetic

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Tingting Zhao and Huiting Tian contributed equally to this work.

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✉ Yuxian Xia
yuxianxia@cqu.edu.cn

✉ Kai Jin
jinkai@cqu.edu.cn

[1] Genetic Engineering Research Center, School of Life Sciences, Chongqing University, Chongqing 401331, People's Republic of China

[2] Chongqing Engineering Research Center for Fungal Insecticide, Chongqing 401331, People's Republic of China

[3] Key Laboratory of Gene Function and Regulation Technologies Under Chongqing Municipal Education Commission, Chongqing 401331, People's Republic of China

Thus, the weakened cell wall and the changes of conidial surface structure in Δ*MaPmt4* would make the fungus susceptible to host defenses. The disruption of *MaPmt4* also leads to the reduction of fungal virulence because of the decline in appressorium formation, the decrease of fungal growth in hemolymph, and the impairments of fungal evasion from insect immune responses owing to the alteration in conidial surface structures.

In summary, *MaPmt4* is crucial for *M. acridum* cell wall integrity, stress tolerances and virulence. Stress tolerances and virulence are important considerations in entomopathogenic fungi to control insect pests effectively. Thus, MaPmt4 and differential proteins may be some potential candidates for the improvement of mycopesticides.

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PLOS PATHOGENS

RESEARCH ARTICLE

Members of chitin synthase family in *Metarhizium acridum* differentially affect fungal growth, stress tolerances, cell wall integrity and virulence

Junjie Zhang[1,2,3☯], **Hui Jiang**[1,2,3☯], **Yanru Du**[1,2,3☯], **Nemat O. Keyhani**[1,4], **Yuxian Xia**[1,2,3*], **Kai Jin**[1,2,3*]

1 School of Life Sciences, Chongqing University, Chongqing, People's Republic of China, **2** Chongqing Engineering Research Center for Fungal Insecticide, Chongqing, People's Republic of China, **3** Key Laboratory of Gene Function and Regulation Technologies under Chongqing Municipal Education Commission, Chongqing, PR China, **4** Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida, United States of America

☯ These authors contributed equally to this work.
* yuxianxia@cqu.edu.cn (YX); jinkai@cqu.edu.cn (KJ)

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Abstract

Chitin is an important component of the fungal cell wall with a family of chitin synthases mediating its synthesis. Here, we report on the genetic characterization of the full suite of seven chitin synthases (*MaChsI-VII*) identified in the insect pathogenic fungus, *Metarhizium acridum*. Aberrant distribution of chitin was most evident in targeted gene knockouts of *MaChsV* and *MaChsVII*. Mutants of *MaChsI*, *MaChsIII*, *MaChsIV* showed delayed conidial germination, whereas Δ*MaChsII* and Δ*MaChsV* mutants germinated more rapidly when compared to the wild-type parent. All *MaChs* genes impacted conidial yield, but differentially affected stress tolerances. Inactivation of *MaChsIII*, *MaChsV*, *MaChsVII* resulted in cell wall fragility, and Δ*MaChsV* and Δ*MaChsVII* mutants showed high sensitivity to Congo red and calcofluor white, suggesting that the three genes are required for cell wall integrity. In addition, Δ*MaChsIII* and Δ*MaChsVII* mutants showed the highest sensitivities to heat and UV-B stress. Three of seven chitin synthase genes, *MaChsIII*, *MaChsV*, *MaChsVII*, were found to contribute to fungal virulence. Compared with the wild-type strain, Δ*MaChsIII* and Δ*MaChsV* mutants were reduced in virulence by topical inoculation, while the Δ*MaChsVII* mutant showed more severe virulence defects. Inactivation of *MaChsIII*, *MaChsV*, or *MaChsVII* impaired appressorium formation, affected growth of *in insecta* produced hyphal bodies, and altered the surface properties of conidia and hyphal bodies, resulting in defects in the ability of the mutant strains to evade insect immune responses. These data provide important links between the physiology of the cell wall and the ability of the fungus to parasitize insects and reveal differential functional consequences of the chitin synthase family in *M. acridum* growth, stress tolerances, cell wall integrity and virulence.

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APPLIED GENETICS AND MOLECULAR BIOTECHNOLOGY

The homeobox gene *MaH1* governs microcycle conidiation for increased conidial yield by mediating transcription of conidiation pattern shift-related genes in *Metarhizium acridum*

Pingping Gao [1,2,3] · Muchun Li [1,2,3] · Kai Jin [1,2,3] · Yuxian Xia [1,2,3]

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Abstract

Conidiation capacity and conidial quality are very important for the production and application of mycopesticides. Most filamentous ascomycetous fungi have two distinct patterns of conidiation. Conidiation through microcycle conidiation proceeds to more rapidly achieve a maximum of conidial yield than normal conidiation and hence is of greater merit for exploitation in mass production of fungal insect pathogens, such as *Metarhizium acridum*. In this study, the mechanism underlying the conidiation pattern shift in *M. acridum* was explored by characterization of the fungal homeobox gene *MaH1*. MaH1 was evidently localized to the nuclei of hyphae and transcriptionally expressed at a maximal level when conidiation began. Intriguingly, deletion of *MaH1* in *M. acridum* resulted in a shift of normal conidiation to microcycle conidiation on one-quarter strength Sabouraud's dextrose agar medium, and hence accelerated conidiation and increased conidial yield. In the deletion mutant, moreover, conidia became larger in size and hyphae cells were shorter in length while conidial virulence and stress tolerance were not altered. As revealed by digital gene expression profiling, *MaH1* controlled the shift of conidiation patterns by mediating transcription of a set of genes related to hyphal growth, cell differentiation, conidiation, and some important signaling pathways. These findings indicate that *MaH1* and its downstream genes can be exploited to increase the conidial yield for more efficient production of mycopesticides.

Keywords *Metarhizium acridum* · Homeobox gene · Transcription factor · Microcycle conidiation · Conidiation pattern shift

Introduction

Entomopathogenic fungi are biological control agents of insect pests (Charnley and Collins 2007). These fungi infect hosts by directly penetrating their external cuticle, including those of insects and other arthropods such as ticks and mites (Federici et al. 2008; Lacey et al. 2015). *Metarhizium* spp. are insect mycopathogens that have a high potential for pest control and are safe for humans and other non-target organisms (St Leger et al. 1996; Lacey et al. 2001). However, poor efficacy and high production cost have retarded their widespread application as chemical insecticides (Lacey et al. 2001; Hajek et al. 2007). Formulated conidia are infective cells often applied for insect pest control (Nielsen 1992; Papagianni 2004). Thus, conidiation capacity and conidial quality are important properties that determine the efficiency of mass production and the application of mycopesticides.

Most filamentous ascomycetous fungi undergo normal conidiation and microcycle conidiation (Hanlin 1994). Normal conidiation is a common reproductive mode (Park

Pingping Gao and Muchun Li contributed equally to this work.

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✉ Kai Jin
jinkai@cqu.edu.cn

✉ Yuxian Xia
yuxianxia@cqu.edu.cn

[1] Genetic Engineering Research Center, School of Life Sciences, Chongqing University, Chongqing 401331, People's Republic of China

[2] Chongqing Engineering Research Center for Fungal Insecticide, Chongqing 401331, People's Republic of China

[3] Key Laboratory of Gene Function and Regulation Technologies under Chongqing Municipal Education Commission, Chongqing 401331, People's Republic of China

which are all related to conidiation and hyphal development (Traag et al. 2004; Leuthner et al. 2005; El-Ganiny et al. 2010; Ulrych et al. 2013). The negative regulation of microcycle conidiation by *MaH1* observed in the WT is likely achieved through expression of these genes which are found to be repressed when *MaH1* lost function in *M. acridum*. In addition, KEGG analysis indicated that the WD-repeat containing protein slp1 is involved in meiosis and the cell cycle, and putative cephalosporin C regulator 1 is involved in cell cycle. We speculate that *MaH1* may regulate genes related to meiosis, cell cycle, conidial formation, and hyphal growth-related genes to affect conidial size and length of hyphal cells.

In *Schizosaccharomyces pombe*, cell membrane proteins as well as diaphragm wall and actin proteins are important factors in cell division (Sethi et al. 2016; Green et al. 2017). In the present study, these protein-coding genes were differentially expressed in the absence of *MaH1*, including those encoding DUF914 domain membrane protein, inner membrane magnesium transporter MRS2 precursor, and cell integrity proteins. Changes in cell polarity and morphology may be also important for the shift of conidiation pattern in *M. acridum*, although the mechanism underlying the shift remains poorly understood. In *M. acridum*, genes involved in the conidiation pattern shift have been identified by RNA-seq (Wang et al. 2016), including those downregulated during normal conidiation of Δ*MaH1*. These genes encode UDP-glucose 4-epimerase, which is involved in hyphal morphogenesis and conidiation (El-Ganiny et al. 2010), GABA permease, which is essential for normal carbon metabolism (Michaeli et al. 2011), putative hydantoinase/oxoprolinase, and hypothetical proteins (MAC_03248 and MAC_06604).

Taken together, our results demonstrate that disruption of *MaH1* accelerated conidiation and increased conidial yield through a shift to the microcycle conidiation pattern as a result of reduced expression of genes which exhibit high expression levels during normal conidiation. *MaH1* and associated genes may be candidate genes to be exploited for improved production efficiency of mycopesticides. Further elucidation of the functions of the DEGs identified in this study may be helpful for uncovering the molecular mechanisms underlying the conidiation pattern shift in *M. acridum* and other entomopathogenic fungi.

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APPLIED GENETICS AND MOLECULAR BIOTECHNOLOGY

The protein phosphatase gene *MaPpt1* acts as a programmer of microcycle conidiation and a negative regulator of UV-B tolerance in *Metarhizium acridum*

Jie Zhang [1,2,3] · Zhenglong Wang [1,2,3] · Nemat O. Keyhani [1,4] · Guoxiong Peng [1,2,3] · Kai Jin [1,2,3] · Yuxian Xia [1,2,3]

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Abstract

The Ser/Thr protein phosphatase Ppt1 (yeast)/PP5 (humans) has been implicated in signal transduction–mediated growth and differentiation, DNA damage/repair, cell cycle progression, and heat shock responses. Little, however, is known concerning the functions of Ppt1/PP5 in filamentous fungi. In this study, the Ppt1 gene *MaPpt1* was characterized in the insect pathogenic fungus, *Metarhizium acridum*. The MaPpt1 protein features a three-tandem tetratricopeptide repeat (TPR) domain and a peptidyl-prolyl cis-trans isomerase-like (PP2Ac) domain. Subcellular localization using an MaPpt1::eGFP fusion protein revealed that MaPpt1 was localized in the cytoplasm of spores, but gathered at the septa in growing hyphae. Targeted gene inactivation of *MaPpt1* in *M. acridum* resulted in unexpected reprogramming of normal aerial conidiation to microcycle conidiation. Although overall vegetative growth was unaffected, a significant increase in conidial yield was noted in Δ*MaPpt1*. Stress-responsive phenotypes and virulence were largely unaffected in Δ*MaPpt1*. Exceptionally, Δ*MaPpt1* displayed increased UV tolerance compared to wild type. Digital gene expression data revealed that *MaPpt1* mediates transcription of sets of genes involved in conidiation, polarized growth, cell cycle, cell proliferation, DNA replication and repair, and some important signaling pathways. These data indicate a unique role for Ppt1 in filamentous fungal development and differentiation.

Keywords Protein phosphatase Ppt1 · *Metarhizium acridum* · Microcycle conidiation · UV tolerance

Introduction

The competing states of phosphorylation/dephosphorylation of serine and threonine residues in a wide range of proteins mediate diverse and sometimes essential cellular processes and rely upon the activities of protein kinases and phosphatases. In eukaryotes, the phosphoprotein phosphatases (PPP) family members are evolutionarily highly conserved proteins and fall into several subfamilies, including PP1, PP2A, PP2B, PP4, PP5, PP6, and PP7 (Barford 1996; Kennelly 2001). Among those, the PP5 (Ppt1 in yeast) subfamily contains as a single member throughout the *Eukaryota* (de la Fuente van Bentem et al. 2003; Shi 2009) and structurally features two domains not found in other PPP family: one is a peptidyl-prolyl cis-trans isomerase-like (PP2Ac) domain and another is a three-tandem tetratricopeptide repeat (TPR) domain which acts as a protein-protein interaction motif at the N-terminus (Das et al. 1998). PP5 first characterized in mammalian

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✉ Kai Jin
jinkai@cqu.edu.cn

✉ Yuxian Xia
yuxianxia@cqu.edu.cn

[1] Genetic Engineering Research Center, School of Life Sciences, Chongqing University, Chongqing 401331, People's Republic of China

[2] Chongqing Engineering Research Center for Fungal Insecticide, Chongqing 401331, People's Republic of China

[3] Key Laboratory of Gene Function and Regulation Technologies under Chongqing Municipal Education Commission, Chongqing 401331, People's Republic of China

[4] Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611, USA

negative molecular feedback loop involving the rhythmic levels of frequency clock protein (Guo et al. 2010; Diernfellner and Schafmeier 2011). Mutation of the PKA-dependent phosphorylation sites on the phosphoprotein RCM-1 results in WC-independent transcription of frequency clock protein and impaired clock function in *Neurospora* (Liu et al. 2015). Frequency clock protein phosphorylations occur in the majority of the proteins involved and phosphorylation status influences their stability, activity, and subcellular localization (Diernfellner and Schafmeier 2011). DNA replication licensing factors, including mcm2, mcm5, and mcm7, were all upregulated in Δ*MaPpt1*-6 days. The minichromosome maintenance (MCM) complex is a putative DNA helicase complex that facilitates the initiation of DNA replication (Yoshida and Inoue 2003). MCM plays a critical role in DNA replication initiation and cell proliferation of eukaryotic cells (Wei et al. 2013). OxdC was present in the periplasm and remained firmly bound to cell-wall materials in *Collybia velutipes* (Azam et al. 2001). As demonstrated in *S. cerevisiae*, incorporation of cell wall protein in fungi can be completely determined by the timing of transcription during the cell cycle (Smits et al. 2006). Covalently linked cell wall protein gene (MAC_03347) was downregulated in the Δ*MaPpt1* strain, suggesting that MaPpt1 is also involved in fungal cell wall organization. In summary, these results indicated that the differential expression of genes involved in conidiation, cell cycle, cell proliferation, and cell wall organization potentially contributed to the enhanced formation of microcycle conidia in the Δ*MaPpt1* strain.

Besides increasing the ability of microcycle conidiation, deletion of *MaPpt1* also promoted the ability of the fungus to counteract UV irradiation which resulted in DNA damage. A number of repair or tolerant strategies were developed to counteract the DNA damage caused by UV in cells, such as photoreactivation, excision repair, and conidial pigmentation (Sinha and Häder 2002). Previous studies have reported that PP5 interacts with other proteins and influences cell cycle after DNA damage (Ali et al. 2004; Zhang et al. 2005; Yong et al. 2007). In mammalian systems and some lower eukaryotes including *Trypanosoma brucei* and *Toxocara canis*, loss or reduction of PP5/Ppt1 resulted in decreased ability to respond to DNA damage (Chaudhuri 2001; Ma et al. 2014). However, our data indicate that in *M. acridum*, Ppt1 acts as a negative regulator of DNA damage pathways, as the mutant strain was more resistant to UV exposure than the wild type and complementary strains. These results suggest that participation of PP5/Ppt1 in this pathway may conserve the outcome of its activity in DNA damage response has diverged. From DGE data, we found that 21 DNA damage repair-related genes were upregulated in Δ*MaPpt1*. Of these genes, 4 genes are involved in base excision repair, including the genes for G-specific adenine glycosylase (Hašplová et al. 2012), DNA-3-methyladenine glycosylase (Troll et al. 2014), ADP-ribosyltransferase (Eberle et al. 2015), and Rad7 (Venkannagari et al. 2016). Four genes are related to nucleotide excision repair, including the genes for ATP-dependent DNA ligase domain protein (Doherty and Wigley 1999), mating-type switching protein swi10 (Rödel et al. 1999), and two DNA glycosylases (D'Errico et al. 2017; Lee and Wallace 2016). Two mismatch repair genes, encoding proliferating cell nuclear antigen (Emptage et al. 2008) and replication factor-A protein 1 (Emptage et al. 2008), are also included. In addition, the genes for DNA repair protein UVS6, which is known to be involved in UVB resistance and efficient DNA damage repair (Schroeder 1975), and DNA polymerase ε, which plays important roles in DNA damage tolerance repair (Fumasoni et al. 2015), were both upregulated in Δ*MaPpt1*. Thus, the enhanced UV tolerances of Δ*MaPpt1* are likely due to upregulation of these DNA damage repair-related genes in *M. acridum*.

In summary, our results demonstrated that deletion of *MaPpt1* induced microcycle conidiation and conidia with higher UV tolerance, providing significant advantages to develop as control agents against insect pests. The deletion of *MaPpt1* increased conidial yield which is of benefit for industrial fermentation of biological control agents. This study provides new insight into dephosphorylation by the MaPpt1 regulator on microcycle conidiation and counteracting the DNA damage caused by UV. However, further work is needed to elucidate the molecular mechanisms of the process, with the genetic manipulation of MaPpt1 related to microcycle conidiation, more UV tolerance, being essential for the utilization of those biological control agents.

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Compliance with ethical standards

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