Deletion of Cu/Zn Superoxide Dismutase Gene sodC Reduces Aspergillus niger Virulence on Chinese White Pear

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ADDITIONAL INDEX WORDS. homologous recombination, oxidative stress, fungal sporulation, fruit-pathogen interaction, pathogenicity

ABSTRACT. Aspergillus niger is a common pathogenic fungus causing postharvest rot of fruit and vegetable, whereas the knowledge on virulence factors is very limited. Superoxide dismutase [SOD (EC 1.15.1.10)] is an important metal enzyme in fungal defense against oxidative damage. Thus, we try to study whether Cu/Zn-SOD is a virulence factor in A. niger. Cu/Zn-SOD encoding gene sodC was deleted in A. niger [MA70.15 (wild type)] by homologous recombination. The deletion of sodC led to decreased SOD activity in A. niger, suggesting that sodC did contribute to full enzyme activity. ΔsodC strain showed normal mycelia growth and sporulation compared with wild type. However, sodC deletion markedly increased the cell’s sensitivity to intracellular superoxide anion generator menadione. Besides, spore germination under menadione and H2O2 stresses were significantly retarded in ΔsodC mutant compared with wild type. Further results showed that sodC deletion induced higher superoxide anion production and higher content of H2O2 and malondialdehyde (MDA) compared with wild type, supporting the role of SOD in metabolism of reactive oxygen species (ROS). Furthermore, ΔsodC mutant had a reduced virulence on Chinese white pear (Pyrus bretschneideri) as lesion development by ΔsodC was significantly less than wild type. The determination of superoxide anion, H2O2, and MDA in A. niger-infected pear showed that Chinese white pear infected with ΔsodC accumulated less superoxide anion, H2O2, and MDA compared with that of wild type A. niger, implying that ΔsodC induced an attenuated response in Chinese white pear during fruit–pathogen interaction. Our results indicate that sodC gene contributes to the full virulence of A. niger during infection on fruit. Aspergillus niger is one of the most common species found in fungal communities. It is an important fermentation industrial strain and is also known to cause the most severe symptoms in fruit during long-term storage (Pel et al., 2007). Meanwhile, plants activate their signaling pathways to trigger defense responses to limit pathogen expansion. One of the earliest host responses after pathogen attack is oxidative burst, during which large quantities of ROS are generated by different host enzyme systems, such as glucose oxidase (Govrin and Levine, 2000). ROS such as singlet oxygen, superoxide anion, hydroxyl (OH ·), and H2O2 are released to hinder the advance of pathogens (Gara et al., 2003). ROS can react with and damage cellular molecules, such as DNA, protein, and lipids, which will limit fungal propagation in the host plant (Apel and Hirt, 2004).

Many antioxidant enzymes in fungal pathogens participate in reactive oxygen degradation on plant-released ROS. Among them, SODs are metalloenzymes that form the primary cellular antioxidant defense system by catalyzing the superoxide disproportionation (a type of redox reaction) to produce molecular oxygen and H2O2, which is further catalyzed by peroxidase and catalase (Vallino et al., 2009). SOD activity is usually induced along with overproduction of superoxide anions (Benes et al., 1995; Gupta et al., 1993). Cu/Zn-SOD is a kind of SOD, which is commonly present in the cytoplasm of eukaryotic cells. It is composed of two subunits which contain one Cu2+ and one Zn2+, respectively. Some progress has been made in the research of Cu/Zn-SOD (SOD1, also known as
SodC gene in fungal resistance to oxidative stress was examined (Delmas et al., 2014). In this work, we constructed the deletion knockout, transformation, and dominant selectable markers cpsod1 (2002) found that the Cu/Zn SOD in fungi is not always conserved or the functions of Cu/Zn SOD in fungi on plants (Rolke et al., 2004). However, Moore et al. which proves that this gene is essential for full virulence of A. niger (2017). Activities of A. niger in the necrotrophic fungal pathogen Botrytis cinerea causes smaller lesion on pea (Pisum sativum) leaves, which proves that this gene is essential for full virulence of B. cinerea on plants (Rolke et al., 2004). However, Moore et al. (2002) found that the Cu/Zn SOD cpsod1 gene in Claviceps purpurea is not essential for pathogenicity in rye (Secale cereal), and deletion of cpsod1 caused no increased sensitivity to paraquat, a generator of superoxide, suggesting that the function of Cu/Zn SOD in fungi is not always conserved or the functions of relative genes are redundant. In A. niger, we identified sodC as a homology of SOD1 in S. cerevisiae. However, how A. niger induces responses in fruit and whether sodC is a virulence factor for A. niger infection on fruit are still unclear.

Some molecular biological manipulation techniques for filamentous fungi have been applied in A. niger, such as gene knockout, transformation, and dominant selectable markers (Delmas et al., 2014). In this work, we constructed the deletion mutant ΔsodC by homologous recombination. Then, the role of sodC gene in fungal resistance to oxidative stress was examined, and the infection ability of ΔsodC mutant on chinese white pear fruit was analyzed.

Materials and Methods

Strains, plasmid, and growth conditions. Aspergillus niger MA 70.15 (ΔpyrG, pyrG encodes orotidine-5-phosphate decarboxylase, cell lacking this enzyme cannot grow without exogenous uridine, but can resist toxicity of 5-fluoroorotic acid) was used as wild type strain in this work. Generally, A. niger was grown on potato dextrose agar medium supplemented with uridine (PDA-Uri [200 g L⁻¹ potato (Solanum tuberosum), 20 g L⁻¹ agar, 20 g L⁻¹ glucose, 10 mmol L⁻¹ uridine]) at 28 °C or liquid PDA-Uri medium.

Construction of ΔsodC mutant. The gene sodC deletion mutant was constructed according to the procedure by Delmas et al. (2014). To delete the sodC gene in A. niger, upstream and downstream fragments AB (902 bp) and CD (502 bp) were amplified by polymerase chain reaction (PCR) from A. niger MA70.15 genomic DNA. The upstream and downstream fragments contained a common restriction site HindIII which was used to ligate the two fragments together and restriction sites EcoRI and XhoI, respectively. EcoRI and XhoI restriction sites on the ligated fragment ABCD were used for cloning into the plasmid pC3 (Delmas et al., 2014) to create pC3-ΔsodC integrative plasmid. Primers are shown as following (bp with underline indicates restriction site): sodC-A (5'-CCGGAAATT-CATCCAGTGGTAGACCATGTA-3'); sodC-B (5'-CTCAAAAGGCTTACCTCGAAGATGCTACTTT-3'); sodC-C (5'-GGTGAAGCTTTAGTATGCTCCATTACCC-3'); and sodC-D (5'-CCGGCTAGGGCTTCCGCGATCATATA-3'). Then, the pC3-ΔsodC integrative plasmid was transformed into the A. niger MA70.15 (ΔpyrG) strain by polyethylene glycol-mediated transformation of protoplasts according to Ballance and Turner (1985). The gene pyrG encoding for the orotidine-5-phosphate decarboxylase was chosen as a counter-selection marker (Boeke et al., 1984). Transformed clones were confirmed by PCR of genomic DNA to identify the sodC deletion mutant (Delmas et al., 2014).

DNA extraction and the confirmation of ΔsodC strain. Genomic DNA of A. niger was extracted using Master Pure Yeast DNA Purification Kit (Epicentre, Madison, WI). Primers sodC-A and sodC-D flanking the sodC gene were used as external primers, and primers sodC-F (5'-TCGGTGGAATCTCGTGGT-3') and sodC-R (5'-TCTTGGACCTCCCTTGTCGA-3') designed on the open reading frame (ORF) of sodC gene were used to determine whether the sodC gene was deleted or not.

RNA extraction and quantitative reverse transcription-PCR (q-PCR). Conidia of 0.1 g of the wild type A. niger MA 70.15 and ΔsodC grown on PDA-Uri solid medium for 5 d were harvested. Then, they were ground to powder under liquid nitrogen. Total RNA and cDNA were obtained according to the instructions of RNAiso plus (TaKaRa, Tokyo, Japan) (van Leeuwen et al., 2013) and Prime Script RT Master Mix (TaKaRa), respectively. The procedure of q-PCR was as follows: denaturation at 95 °C for 3 min, followed by cycles of 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 10 s, cycled for 40 times. The primers used for q-PCR were shown below: sodC-S (5'-GAGTTAGTCTGTGCAATTACCC-3') and sodC-A (5'-CTCAGAGCTCTTTCGCGATCATATA-3'). The expression of actin-encoding gene Actn030e03160 was used as a control (Raulo et al., 2016). The primers used for actin-encoding gene were actin-F (5'-ACGTGTGGACGTGCCTC-3') and actin-R (5'-CAATGTTCCGGGTATGTC-3').

Determination of SOD activity. Mycelia of the wild type A. niger MA 70.15 and ΔsodC grown in PDA-Uri liquid medium for 3 d were harvested and washed three times with sterile distilled water. Frozen A. niger mycelia at 1 g was ground to powder under liquid nitrogen using a mortar and pestle and 50 mm ice-cold phosphate buffer (pH 7.8, 1 mM EDTA, 1 mM PMSF) was added to 5 mL. The mixture was centrifuged at 12,000 g, at 4 °C for 10 min to obtain the supernatant which was used for the determination of SOD activity by procedures described by Beauchamp and Fridovich (1971). One SOD unit was defined as the amount of enzyme that inhibits the rate of nitroblue tetrazolium reduction by 50%. Activity was expressed as units per milligram protein. Protein content was measured according to Bradford (1976) using bovine serum albumin as standard.

Growth analysis of A. niger. The conidia of the wild type A. niger MA 70.15 and ΔsodC grown on PDA-Uri solid medium for 5 d were harvested. After removal of mycelia, spore concentration was measured using a haemocytometer and adjusted to the final concentration of 10⁶ spores/mL (Lazar et al., 2008). Then, 5-μL spore suspension of the wild type and ΔsodC were inoculated on PDA-Uri solid medium and medium supplemented with 0.02 μM menadione and were cultured at 28 °C in darkness for 4 d. Colony diameter of A. niger was determined every 24 h.

The spore suspension (10⁶ spores/mL) of wild type A. niger and ΔsodC at 200 μL were inoculated to 20-ml PDA-Uri liquid medium, respectively, and were cultured at 28 °C for 150 rpm in the thermostatic shaker. Dry weight of the mycelia of the A. niger was measured every 12 h according to the method in (Angelova et al., 2005).

Determination of spore germination. Ten microliters of spore suspension (10⁶ spores/mL) for the wild type A. niger and
ΔsodC were placed on a 7-mm-diameter plug of PDA-Uri supplemented with freshly-prepared 0.02 mM menadione and 2 mM H2O2 which were then placed on a glass slide. Menadione and H2O2 were added to PDA-Uri agar medium when the autoclaved medium was cooled down to 60 °C. Each plug-slide was held in a petri dish with moistened filter paper and then was incubated at 28 °C. Conidia germination (germ tube longer than two times the diameter of the corresponding spore) of the wild type A. niger and ΔsodC which were in PDA-Uri culture medium (for 16 h), medium supplemented with 2 mM H2O2 (for 24 h), or medium supplemented with 0.02 mM menadione (for 30 h) were assessed under a compound microscope at 400× total magnification with 40× of the objective lens and 10× of eyepiece lens (Fu et al., 2014).

DETERMINATION OF SUPEROXIDE ANION PRODUCTION AND CONTENT OF H2O2 AND MDA IN A. NIGER AND CHINESE WHITE PEAR SUBJECTED TO A. NIGER INFECTION. A volume of 200-μL spore suspension (10⁶ spores/mL) of wild type and ΔsodC was inoculated to 20-mL PDA-Uri liquid medium and cultured at 28 °C for 3 d. Then, the medium was supplemented with 0.02 mM menadione and sampled at 12 and 24 h. Freshly cultured A. niger mycelia at 1 g was ground to the powder under liquid nitrogen using a mortar and pestle. After A. niger inoculation on Chinese white pear fruit for 3 d, pear flesh 2 cm far from lesion was sampled for assay and uninoculated pear was used as control. Content of superoxide anion, H2O2, and MDA were determined according to the methods described by Hu et al. (2012).

INFECTION ABILITY OF A. NIGER ON CHINESE WHITE PEAR. Chinese white pear fruit were supplied by the Anhui Academy of Agricultural Sciences, Hefei, Anhui, China. Unwounded, healthy fruit of uniform size, color, and weight were selected for experiments. Chinese white pear fruit were washed with tap water and surface-sterilized with 75% ethanol. Then, the surface of the fruit was wounded at 3 sites on each fruit. After air-drying, each of the wounded sites on each fruit was inoculated with 200-μL spore suspension of wild type or ΔsodC.
the wounds (2 mm diameter and 4 mm deep) was injected with 10 μL of spore suspension (10<sup>6</sup> spores/mL) of wild type A. niger and ΔsodC. Each strain was inoculated on three different fruit. Chinese white pear fruit were stored at 25 °C in sealed containers with 200 mL of distilled water on the bottom to maintain the relative humidity which ranged from 85% to 90% (Fu et al., 2014). The lesion diameter caused by A. niger was measured every 24 h.

**Statistical analysis.** The data in the article are based on three replicates. Statistical significance was tested by one-way analysis of variance (ANOVA) using SPSS Statistics (version 20.0; IBM Corp., Armonk, NY), and the results were expressed as the mean ± sd. The least significant difference test was performed on all data after ANOVA tests to test for significant (P < 0.05 or P < 0.01) differences between treatments.

**Results**

**Construction of the ΔsodC deletion mutant.** The plasmid pC3-An_ΔsodC was transformed into A. niger MA 70.15 protoplasts. Then, the genomic DNA of transformants was extracted to identify sodC deletion mutants. As shown in Fig. 1A, the deletion mutant ΔsodC were confirmed by PCR with the primers sodC-A and sodC-D which flanking sodC gene and with primers sodC-F and sodC-R on the ORF of sodC gene. Thus, for the genome of wild type, the PCR product on lane 1 is about 2450 bp, and about 1400 bp for lane 2. Because sodC gene is deleted in ΔsodC mutant, PCR product using external primers sodC-A and sodC-D on lane 3 is about 650 bp and there is no band on lane 4, confirming that sodC was deleted in the mutants. Then, the gene expression of sodC in wild type and sodC deletion mutant was analyzed. As shown in Fig. 1B, sodC gene expression was not detected compared with the high expression in wild type, further confirming that sodC gene was deleted in the mutant. To study whether sodC gene actually contributes to SOD activity in the A. niger, total SOD activity in ΔsodC was determined and compared with that in wild type. As expected, SOD activity in ΔsodC mutant was significantly lower than that of wild type A. niger (Fig. 1C).

**Growth analysis of ΔsodC mutant.** To evaluate whether the lack of sodC would affect fungal growth, we compared growth

![Fig. 3. The effect of menadione on the growth of the wild type Aspergillus niger MA 70.15 (WT) and ΔsodC mutant. (A) The mycelium growth of the wild type A. niger MA 70.15 (WT) and ΔsodC in potato dextrose agar medium supplemented with uridine (PDA-Uri) and PDA-Uri medium supplemented with 0.02 mM menadione for 4 d. (B) The colony diameter of wild type A. niger MA 70.15 (WT) and ΔsodC in A. Values are mean ± sd (n = 9).](image)

![Fig. 4. The effect of menadione and H<sub>2</sub>O<sub>2</sub> on the spore germination of ΔsodC. (A) The spore germination of the wild type Aspergillus niger MA 70.15 (WT) and ΔsodC mutant in potato dextrose agar medium supplemented with uridine (PDA-Uri) solid medium (16 h), PDA-Uri solid medium supplemented with 2 mM H<sub>2</sub>O<sub>2</sub> (24 h), and supplemented with 0.02 mM menadione (30 h); bar = 100 μm. (B) Spore germination percentage of the wild type A. niger MA 70.15 (WT) and ΔsodC in A. Values are mean ± sd (n = 3).](image)
of $\Delta$sodC and $A. \ niger$ MA 70.15 on PDA-Uri medium. As shown in Fig. 2A, within 96 h, there was no difference in the mycelial growth found between the wild type and $\Delta$sodC on PDA-Uri solid medium. Colony diameter in the wild type and the $\Delta$sodC also show that there is no difference in growth (Fig. 2B). Figure 2C shows that $\Delta$sodC mutant grew normally in PDA-Uri liquid medium for 96 h in comparison with wild type.

**sodC in $A. \ niger$ confers resistance to reactive oxygen species.** Menadione, a superoxide generating agent, was supplemented to PDA-Uri medium to evaluate whether the deletion of sodC affects the adaption of cells to oxidative stress. As shown in Fig. 3A, the mycelium growth of $\Delta$sodC was significantly retarded compared with wild type under 0.02 mM menadione. Figure 3B shows that the colony diameter of $\Delta$sodC was significantly smaller than that of wild type $A. \ niger$. However, $\Delta$sodC mutant showed no higher sensitivity to 2 mM H$_2$O$_2$ stress compared with wild type (data not shown).

To further analyze the reduced growth of $\Delta$sodC under menadione stress, spore germination of wild type $A. \ niger$ and $\Delta$sodC was analyzed under 0.02 mM menadione and 2 mM H$_2$O$_2$ stress. As shown in Fig. 4A and B, after 16 h of incubation in control conditions, the spores of the wild type and $\Delta$sodC all germinated. Under 0.02 mM menadione and 2 mM H$_2$O$_2$ stress for 30 and 24 h, respectively, the hyphae were obviously shorter than that in normal conditions, suggesting that 0.02 mM menadione and 2 mM H$_2$O$_2$ effectively influenced hyphal growth of $A. \ niger$. After 30 h of 0.02 mM menadione stress, spore germination percentage of wild type reached 93%, whereas that of $\Delta$sodC was about 27%. Besides, spore germination percentage of $\Delta$sodC with 2 mM H$_2$O$_2$ stress for 24 h was 91%, which was significantly lower than 99% of the wild type $A. \ niger$.

**Deletion of sodC attenuates ROS metabolism in $A. \ niger$.** To verify the enzymatic activity of sodC, superoxide anion production and contents of H$_2$O$_2$ and MDA were determined in menadione-treated $A. \ niger$. As shown in Fig. 5A, superoxide anion production in wild type $A. \ niger$ increased with treatment time of menadione. Meanwhile, menadione induced significantly higher production of superoxide anion in $\Delta$sodC cells during the entire treatment. The significant higher level of superoxide anion was also observed in untreated $\Delta$sodC cells, suggesting that $\Delta$sodC cells may have

![Fig. 5. The effect of menadione on the superoxide anion production (A) and content H$_2$O$_2$ (B) and malondialdehyde (MDA) (C) in Aspergillus niger MA 70.15 (WT) and $\Delta$sodC were subjected to 0.02 mM menadione stress and sampled at 12 and 24 h in potato dextrose agar medium supplemented with uridine (PDA-Uri) liquid medium. Values are mean ± SD ($n$ = 3).](image)

**Fig. 6. Pathogenicity of $\Delta$sodC mutant on Pyrus bretschneideri.** (A) Photographs of $P. \ bretschneideri$ fruit inoculated with the spores of wild type *Aspergillus niger* MA 70.15 (WT) and $\Delta$sodC for 5 d. (B) Lesion diameter caused by wild type *A. \ niger* MA 70.15 (WT) and $\Delta$sodC on $P. \ bretschneideri$ fruit as shown in A. Values are mean ± SD ($n$ = 15).
defect in decomposing background level of superoxide anion (Fig. 5A). Besides, menadione also induced higher level of H$_2$O$_2$ in $\Delta$sodC cells in comparison with wild type cells (Fig. 5B). MDA, which is an index of lipid peroxidation, was determined in menadione-treated cell. As shown in Fig. 5C, the content of MDA in wild type increased slightly along with treatment time; however, MDA accumulated more in $\Delta$sodC cells.

**sodC gene is indispensable for the full virulence of A. niger.** To evaluate whether sodC gene is involved in the virulence of A. niger on fruit, spores were inoculated on chinese white pear fruit. As shown in (Fig. 6A and B), after being infected by A. niger, the lesion diameter caused by A. niger increased gradually. However, lesion diameter caused by $\Delta$sodC mutant was significantly smaller than that of wild type, suggesting that sodC gene is indispensable for the full virulence of A. niger.

**Lack of sodC alters ROS accumulation and MDA in fruit upon A. niger infection.** To study the response of chinese white pear to wild type A. niger and $\Delta$sodC cells, ROS metabolism was analyzed by the determination of superoxide anion production and contents of H$_2$O$_2$ and MDA. Superoxide anion production in chinese white pear fruit increased upon A. niger infection in both wild type and $\Delta$sodC, whereas wild type A. niger induced a stronger response of superoxide anion in comparison with $\Delta$sodC (Fig. 7A). As shown in Fig. 7B, an accumulation of H$_2$O$_2$ occurred in chinese white pear infected with wild type A. niger, while the accumulation was attenuated in $\Delta$sodC-inoculated chinese white pear fruit. Besides, A. niger inoculation increased the content of MDA in chinese white pear fruit subjected to both wild type and $\Delta$sodC. However, MDA content in $\Delta$sodC-inoculated chinese white pear fruit was significantly lower than that of wild type (Fig. 7C).

**Discussion**

Aspergillus rot (black mold rot) is one of the most important postharvest diseases of fruit and vegetable, and A. niger is known to cause the most severe symptoms (Hasan, 2000). Although a lot of work has been done to improve the ability of being a cell factory for a range of products, such as enzymes and organic acids (Archer, 2000), the knowledge on the factors determining its pathogenesis on fruit is very limited.

In this study, we identified sodC gene as a Cu/Zn-SOD coding gene from A. niger. Cu/Zn-SODs are usually located in cytosol and can protect cells from oxidative stress (Holdom et al., 1996). sodC gene was deleted in A. niger by gene deletion via homologous recombination. The decreased SOD activity in $\Delta$sodC mutant compared with wild type confirmed that sodC did contribute to the total enzyme activity. As SOD is responsible for the superoxide disproportionation reaction during oxidative stress (Chary et al., 1994), we test the sensitivity of $\Delta$sodC mutant to oxidative stress. Our results indicate that menadione, a generator of intracellular superoxide anion, could significantly inhibit the mycelium growth of $\Delta$sodC. Further results showed that spore germination of the $\Delta$sodC was significantly inhibited by menadione and H$_2$O$_2$ compared with that of wild type (Fig. 4). All of these indicate that the ability of detoxifying superoxide radicals and even H$_2$O$_2$ was attenuated in $\Delta$sodC mutant and sodC gene did contribute to cell’s resistance to oxidative stress. Consistently, B. cinerea mutants with SOD1 deletion showed increased sensitivity to ROS-generating agent paraquat (Rolke et al., 2004). Besides, Sclerotinia sclerotiorum mutation in SsSOD1 showed growth rate and sclerotial formation similar to that of the wild type but showed increased sensitivity to heavy metal toxicity and oxidative stress (Xu and Chen, 2013). To further study the attenuated tolerance of $\Delta$sodC toward menadione, superoxide anion production and contents of H$_2$O$_2$, and MDA are determined in menadione-treated A. niger. It is found that ROS and MDA increased in A. niger with menadione treatment, while they accumulated more in $\Delta$sodC cells (Fig. 5). Thus, we provide evidence that $\Delta$sodC cells have defect in response to menadione stress because of failure in ROS decomposition and increased lipid peroxidation.

ROS burst occurs as one of the earliest cellular response after successful pathogen recognition (Torres et al., 2006). Accordingly, fungi pathogens need to activate their antioxidant

![Fig. 7. Superoxide anion production (A) and content of H$_2$O$_2$ (B) and malondialdehyde (MDA) (C) in Pyrus bretschneideri inoculated with wild type Aspergillus niger MA 70.15 (WT) and $\Delta$sodC. P. bretschneideri was inoculated with A. niger MA 70.15 (WT) and $\Delta$sodC and were sampled immediately (Control) or on day 3 postinoculation. Values are mean ± SD (n = 3).](image-url)
defenses to cope with the oxidative stress encountered during infection. SODs constitute the primary cellular antioxidant defense system by detoxifying oxygen radicals and we propose that Cu/Zn-SOD might be a virulence factor for A. niger during infection on fruit. In this study, the spores of ΔsodC mutant were inoculated on Chinese white pear fruit. The decreased lesion diameter caused by ΔsodC suggests that Cu/Zn-SOD contributes to the virulence of A. niger (Fig. 6). In normal conditions, antioxidant defense mechanisms are adequate to maintain ROS at basal level and sodC deletion did not make much difference on the growth of A. niger. During infection on fruit, A. niger may need full function of antioxidant system to cope with oxidative stress due to plant response. Cu/Zn-SOD in A. niger is required to detoxify dangerous level of superoxide from host’s response to fungal infection and thus we observed the decreased virulence of ΔsodC mutant. Besides sodC, manganese SOD sodB in A. niger may also contribute to the decomposition of superoxide. There are also other examples of SOD that contribute to virulence in phytopathogenic fungi (Rolke et al., 2004). The deletion of bcsod1 caused smaller lesion and proved that this gene is essential for full virulence of B. cinerea on Arabidopsis thaliana and tomato (Solanum lycopersicum) (López-Cruz et al., 2017; Rolke et al., 2004). Besides, it is also found that SOD1 contributes to the virulence of Fusarium graminearum in wheat (Triticum aestivum) head infection (Yao et al., 2016).

Fruit responses to A. niger infection is still unclear. In this work, ROS and MDA content are determined to evaluate whether similar oxidative burst occurs in Chinese white pear in response to A. niger as observed in other fungi (López-Cruz et al., 2017; Torres et al., 2006). The increased levels of ROS and MDA confirm that A. niger infection induces ROS burst in fruits (Fig. 7). Meanwhile, we found that fruit response toward ΔsodC mutant is attenuated compared with wild type, suggesting the weakened virulence of ΔsodC cells. However, it is also reported that plants infected with Δbcsod1 accumulated less H₂O₂ and more superoxide anion than those infected with wild type (López-Cruz et al., 2017). S. sclerotiorum deletion in SsoF1 showed reduced virulence on tomato and tobacco (Nicotiana tabacum), but the gene deletion induced a host oxidative burst in adjacent uninfected cells in contrast to reduced ROS production during wild-type infection (Veluchamy et al., 2012). These different findings might be due to different plant materials and different fungi used in researches.

In summary, we show that sodC gene in A. niger is necessary for the cells’ tolerance to exogenous ROS. sodC deletion mutant caused reduced virulence on Chinese white pear, which further support the hypothesis that SOD is important for the full virulence of A. niger.

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