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Improvement of a gene targeting system for genetic manipulation in *Penicillium digitatum*[*#]

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Abstract: *Penicillium digitatum* is the most important pathogen of postharvest citrus. Gene targeting can be done in *P. digitatum* using homologous recombination via *Agrobacterium tumefaciens* mediated transformation (ATMT), but the frequencies are often very low. In the present study, we replaced the *Ku80* homolog (a gene of the non-homologous end-joining (NHEJ) pathway) with the hygromycin resistance cassette (*hph*) by ATMT. No significant change in vegetative growth, conidiation, or pathogenicity was observed in *Ku80*-deficient strain (Δ*PdKu80*) of *P. digitatum*. However, using Δ*PdKu80* as a targeting strain, the gene-targeting frequencies for both genes *PdbrlA* and *PdmpkA* were significantly increased. These results suggest that *Ku80* plays an important role in homologous integration and the created Δ*PdKu80* strain would be a good candidate for rapid gene function analysis in *P. digitatum*.

Key words: *Penicillium digitatum*, Efficiency, Gene targeting, Non-homologous end-joining (NHEJ) pathway, *Ku80*
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1 Introduction

Penicillium digitatum (Pers.:Fr.) Sacc., causing a green mold in citrus, is the most destructive pathogen in the citrus industry and is responsible for the majority of decay losses of postharvest citrus (Eckert and Eaks, 1989; Kanetis *et al*., 2007; Macarisin *et al*., 2007). To date, a limited amount of information regarding the molecular mechanisms of pathogenesis is available for this important pathogen (Wang *et al*., 2012; Zhang *et al*., 2013a; 2013b; 2013c). The availability of genome sequence and genetic transformation has accelerated the progress in molecular biology of *P. digitatum* (Wang and Li, 2008; Marcet-Houben *et al*., 2012; Sun *et al*., 2013).

Targeted gene knockout/replacement is an efficient and essential strategy for the functional characterization of interested genes in filamentous fungi. *P. digitatum* can be genetically transformed with a plasmid containing a selectable marker conferring resistance to antibiotics or herbicides via protoplast transformation of DNA (Hamamoto *et al*., 2000; Nakaune *et al*., 2002) or conidia transformation mediated by *Agrobacterium tumefaciens* (Wang and Li, 2008). However, the transformation via protoplast is usually very difficult for unknown reasons (our unpublished data) and the gene disruption frequency was very low (<4%) or even negligible amongst transformants recovered through *A. tumefaciens* mediated transformation (ATMT) in many cases. This limitation greatly hampered the functional characterization of targeted genes in *P. digitatum*.

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Homologous recombination (HR) of exogenous DNA into the genome occurs easily in the yeast species, but it is not a ready mechanism for filamentous fungi since the non-homologous end-joining (NHEJ) pathway seems to be dominant over the HR pathway for repairing of DNA double-strand breaks (DSBs) in filamentous fungi (Ishibashi *et al*., 2006; Meyer *et al*., 2007; Maruyama and Kitamoto, 2008; Villalba *et al*., 2008). The DNA-dependent protein kinase complexes as well as a Ku70-Ku80 heterodimer are the central components of NHEJ (Haber, 2000; Jones *et al*., 2001). Previous studies indicated that destroying the NHEJ machinery by disrupting the Ku70- or Ku80-coding gene could increase the gene-targeting frequency in a number of filamentous fungi, such as *Aspergillus* spp. (da Silva Ferreira *et al*., 2006; Nayak *et al*., 2006; Meyer *et al*., 2007; Chang, 2008; Maruyama and Kitamoto, 2008), *Botrytis cinerea* (Choquer *et al.*, 2008), *Magnaporthe oryzae* (Villalba *et al*., 2008), *Penicillium decumbens* (Li *et al*., 2010), and *Neurospora crassa* (Ninomiya *et al*., 2004). However, disruption of *Ku70* in *Alternaria alternata*, the pathogenic fungus causing brown spots on citrus, did not apparently improve the efficiency of gene targeting (Wang *et al*., 2011). The objective of this study was to explore the new strategy for increasing the gene-targeting frequency in *P. digitatum*.

2 Materials and methods

2.1 Fungal strains and culture conditions

The wild strain PdKH8 of *P. digitatum* was obtained from infected citrus of Ponkan (*Citrus reticulata* Blanco) in Quzhou, Zhejiang Province, China. The strain was stored as conidial suspensions in 15% glycerol at −80 °C, and recovered on regular potato dextrose agar (PDA). Mycelium was obtained by inoculating 5 μl (1×10^6 conidia/ml) in 150 ml liquid potato dextrose broth (PDB), which was incubated on a rotary shaker at 160×*g* and 25 °C. Conidia were harvested in sterile water from a 7-d-old culture by scrapping the culture with a sterile glass rod, and the desired conidial concentration was adjusted by counting cells using a haematocytometer.

2.2 Cloning and analysis of *Ku80* homolog in *P. digitatum*

All oligonucleotide primers used in this study are listed in Table S1. The *P. digitatum Ku80* ortholog, *PdKu80*, was found by the BLASTp search of the genomic sequence of *P. digitatum* (Sun *et al.*, 2013) using *Ku80* homolog from *Aspergillus oryzae* (*AoKu80*, BAE78503.1). The amplified fragments in this study were cloned into the vector pMD18-T (TaKaRa Biotech. Co., Dalian, China) and the absence of polymerase-derived errors was checked by DNA sequencing. The *PdKu80* sequence was submitted into the GenBank/EMBL/DDBJ database under accession No. KF019685.

2.3 Construction of *PdKu80, PdbrlA*, and *PdmpkA* disruption vectors

The *PdKu80* disruption vector was constructed by inserting the two flanking sequences of *PdKu80* into the up- or downstream side of the hygromycin resistance cassette (*hph*) in the vector pTFCM (Wang and Li, 2008). The primers used to amplify the homologous arms were synthesized according to the genomic sequence of *P. digitatum* (unpublished data). Briefly, a 1.2-kb DNA fragment containing the upstream flanking sequence of *PdKu80* was amplified from the *P. digitatum* genomic DNA by polymerase chain reaction (PCR) using primers Ku80-up-F/Ku80-up-R (Table S1 and Fig. 1a). After digestion with *Kpn*I and *Sac*I restriction enzymes, the PCR fragment was inserted into the *Kpn*I-*Sac*I site of pTFCM to generate the plasmid pTFCM-Ku80-up. Subsequently, a 1-kb fragment representing the downstream flanking sequence of the *PdKu80* gene was obtained using primers Ku80-down-F/Ku80-down-R (Table S1 and Fig. 1a). After digestion with *Spe*I and *Xho*I restriction enzymes, the PCR fragment was inserted into the *Spe*I-*Xho*I site of the plasmid pTFCM-Ku80-up to generate the *PdKu80* disruption plasmid pTFCM-Δ*PdKu80* (Fig. 1a).

Before the construction of the Δ*PdbrlA* and Δ*PdmpkA* vectors, a plasmid pNEO1300 was constructed by removing *hph* and inserting the neomycin resistant gene (*neo*) into the multiple cloning sites (MCSs) in pCAMBIA1300 (CAMBIA, Canberra,

Australia). The *neo* was amplified from plasmid pCA-neo (Jiang *et al.*, 2011) using primer pair Trpc-neo-*Bam*HI/Trpc-neo-*Xba*I (Table S1). The sequences of *brlA* (*PdbrlA*, JX298844) and *mpkA* (*PdmpkA*, KF019686) homologs in *P. digitatum* were obtained from a genomic database (unpublished data). The construction of Δ*PdbrlA* and Δ*PdmpkA* plasmids followed the method described in the construction of a disruption vector of Δ*PdKu80*. Briefly, the up- and downstream flanking sequences (0.7 and 0.9 kb, respectively) of *PdbrlA* were amplified using primers brlA-up-F/brlA-up-R and brlA-down-F/brlA-down-R (Table S1 and Fig. S1a), while the up- and downstream flanking sequences (1.0 and 0.9 kb, respectively) of *PdmpkA* were amplified using primers mpkA-up-F/mpkA-up-R and mpkA-down-F/mpkA-down-R (Table S1 and Fig. S2a). The amplified sequences were then ligated to the *Kpn*I-*Bam*HI and *Xba*I-*Hin*dIII sites of pNEO1300 (Figs. S1a and S2a), respectively.

2.4 Disruptions of *PdKu80*, *PdbrlA*, and *PdmpkA* in *P. digitatum*

The constructed disruption plasmids were transformed into an *A. tumefaciens* strain AGL-1 by the electroporation method described by Wang and Li (2008). To disrupt *PdKu80* in *P. digitatum*, the procedure of ATMT was conducted as the method described previously (Wang and Li, 2008). To disrupt *PdbrlA* and *PdmpkA* in *PdKu80*-disrupted strain (Δ*PdKu80*) of *P. digitatum*, a similar approach was also used, but instead of hygromycin B, 67 μg/ml of neomycin was added into the PDA for the selection of the putative *PdbrlA- or PdmpkA*-disrupted transformants.

The disruptions of *PdKu80*, *PdbrlA*, and *PdmpkA* in *P. digitatum* were identified by PCR and followed by a Southern blot. For identification of Δ*PdKu80*, primer pairs Ku80-up-F/Ku80-i-R, Ku80-up-F/Ku80-i2-R, and Ku80-V-F/hph-R1 (Table S1 and Fig. S3) were used; for Δ*PdbrlA*, primers brlA-i-F/brlA-i-R and brlA-V-F/brlA-V-R (Table S1 and Fig. S1b) were used; for Δ*PdmpkA*, primers mpkA-i-F/mpkA-i-R and mpkA-V-F/mpkA-V-R (Table S1, Figs. S2b and S2c) were used. For Southern blot analysis, genomic DNA (100 μg) was digested with *Xho*I for 24 h, the fragments were separated on a 0.8% (w/v) agarose gel, and transferred to a Nylon membrane (Millipore, Billerica, MA, USA). The probes (Fig. 1a) were labeled by PCR using the primers of hph-F/hph-R2 and Ku80-down-F/Ku80-down-R (Table S1), respectively, with the PCR DIG probe synthesis kit I (Roche, Mannheim, Germany), according to the manufacturer's instruction. Aqueous hybridization was performed at 42 °C overnight and detected using the DIG luminescent detection kit (Roche) according to the manufacturer's instruction.

2.5 Assays of vegetative growth and sporulation

To determine the effect of the deletion of *PdKu80* on vegetative growth and sporulation, mycelial plugs (0.5 cm in diameter) of both wild-type PdKH8 and Δ*PdKu80* strains of *P. digitatum* were prepared and transferred on PDA, Czapek yeast extract agar (CYA), and oat agar (OA) plates as described previously (Zhang *et al.*, 2009). The plates were incubated at 25 °C for 5 d on PDA, and for 8 d on CYA and OA. Sporulation was assayed at the same day as above, following the method described previously (Zhang *et al.*, 2013b). The experiment was conducted twice with at least three replicates.

2.6 Assay of the tolerance to salt stress

To determine whether *PdKu80* is involved in the tolerance to salt stress, mycelial plugs (0.5 cm in diameter) of both wild-type PdKH8 and Δ*PdKu80* strains of *P. digitatum* were prepared and transferred on PDA plates supplemented with 0.5 mol/L NaCl and KCl. The growth on PDA with a non-supplement of either NaCl or KCl was used as control. The plates were incubated at 25 °C for 5 d, and the colony diameter in each plate was measured. Three replicates were used for each strain and the experiments were repeated twice.

2.7 Virulence assay

Mature and un-wounded Ponkan fruits (*C. reticulata*) were wounded with a bunch of five needles (2–3 mm in depth). Conidial suspensions (1×10^6 conidia/ml) of the wild-type PdKH8 and Δ*PdKu80* of *P. digitatum* were collected from 7-d-old PDA plates. Three microliters of the conidial suspensions were added onto each wounded site. The inoculated citruses were incubated at room temperature and disease symptoms were observed daily, and the lesion diameters were recorded at Day 5 after

inoculation. Thirty fruits were used for each strain and the experiment was repeated twice.

3 Results and discussion

3.1 Identification and analysis of the putative *PdKu80* gene in *P. digitatum*

An exhaustive search via the BLASTp program was made to identify the *Ku80* homolog in the *P. digitatum* genome (http://www.ncbi.nlm.nih.gov/) using *AoKu80* gene (BAE78503.1) as a reference sequence. The gene was a bidirectional best hit with *AoKu80*, shared the highest similarity with *AoKu80* (*E*-value 0, identity 68%, similarity 82%, 718 amino acids), and was assumed as the putative *PdKu80* (AGT79985.1) through conserved domain analysis using a Pfam database (Bateman *et al*., 2004). Results showed that the putative *PdKu80* gene was 2673 bp in length, which contained 10 introns and encoded 712 amino acids.

The possible conserved functional domains, Ku70/Ku80 N-teminal α/β domain (positions 6 to 190), Ku70/Ku80 β-barrel domain (positions 274 to 440), and Ku C terminal domain (positions 590 to 710) were presented in this gene, suggesting that it was definitely a *Ku80* gene in *P. digitatum*. Sequence alignment revealed that *PdKu80* had 64%, 46%, 45%, and 41% identity to its homologs identified in *A. oryzae* (BAE78503.1), *Verticillium albo-atrum* (XP_003001068.1), *Fusarium oxysporum* (EGU85850.1), and *Neurospora crassa* (BAD16623.1), respectively.

3.2 Creation of *PdKu80*-disrupted strain (Δ*PdKu80*)

PdKu80 targeting plasmid was constructed by substituting the sequence encoding *PdKu80* with *hph* (Fig. 1a). The constructed targeting plasmid pTFCM-Δ*PdKu80* was transformed into the wild-type strain PdKH8 by the ATMT method. Eighty-three transformants selected from a hygromycin-contained medium were primarily identified by PCR with primer pairs, Ku80-up-F/Ku80-i-R and Ku80-up-F/Ku80-i2-R (Fig. 1a and Table S1). With these primer pairs, the fragments of 1.5 and 1.9 kb were amplified from the PdKH8 and ectopic transformants, while none fragment could be amplified from the *PdKu80*-disrupted mutants (Δ*PdKu80*; Fig. S3a). To get a positive result, primer pair Ku80-V-F/hph-R1 (Fig. 1a and Table S1) was used to amplify a fragment of 2.2 kb from the *PdKu80*-disrupted mutants, while none fragment could be amplified from the PdKH8 or ectopic transformants (Fig. S3b). Based on these PCR results, the transformants Δ*PdKu80*-14, Δ*PdKu80*-22, and Δ*PdKu80*-25 were identified as Δ*PdKu80s*. Southern blot analysis using a probe specific to *hph* verified the insertion of *hph* (Fig. 1b), and the Southern blot analysis using a probe located at the 3' region of *PdKu80* (Fig. 1a) indicated that additional

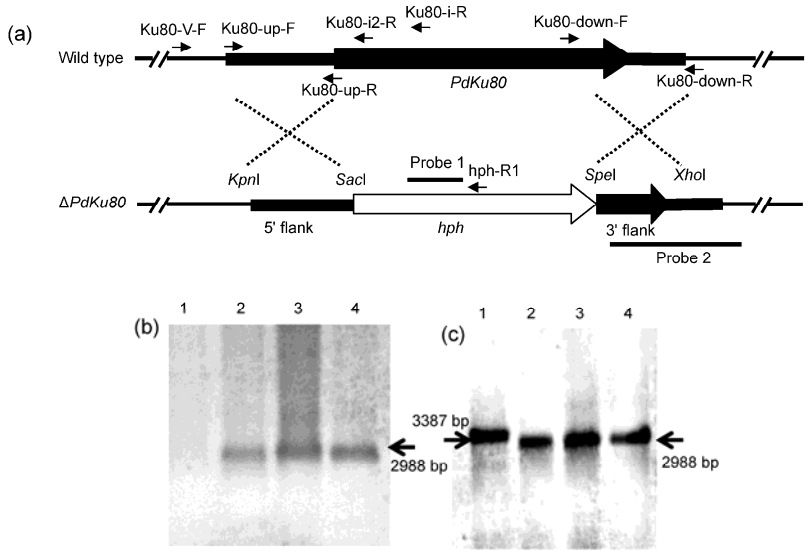

Fig. 1 Schematic diagram showing the construction of *PdKu80* disruption plasmid and molecular identification of Δ*PdKu80* by Southern blot analysis

(a) Construction of *PdKu80* disruption plasmid. Black arrow represented the *PdKu80*, while the white arrow represented a hygromycin resistance cassette (*hph*). The positions of the PCR primers were indicated with small arrows. Probes were indicated with black bars. (b) Identification by Southern blot using a probe specific to *hph*. (c) Identification by Southern blot using a probe specific to the 3' part of the *PdKu80* gene. Lane 1: wild-type strain PdKH8; Lane 2: Δ*PdKu80*-14; Lane 3: Δ*PdKu80*-22; Lane 4: Δ*PdKu80*-25

ectopic integration of the replacement cassette sequence did not occur in these transformants (Fig. 1c). The growth rates of three Δ*PdKu80s* were identical, and the data collected from Δ*PdKu80*-25 were presented hereafter.

3.3 Disruption of *PdKu80* did not affect vegetative growth or sporulation

Plate assay showed that the colony morphology of the Δ*PdKu80*-25 was identical to that of the wild-type strain PdKH8 on PDA, CYA, and OA media (Fig. 2a). The average colony diameter of Δ*PdKu80*-25 was slightly smaller than that of the wild-type strain PdKH8 on PDA; however, no significant difference was observed on CYA or OA (P>0.05; Figs. 2a and 2b). In addition, the sporulation of the Δ*PdKu80*-25 was similar to that of the wild-type strain PdKH8 on PDA, CYA, and OA media (P>0.05; Fig. 2c). These results indicated that disruption of *PdKu80* did not affect vegetative growth or sporulation in *P. digitatum*.

3.4 Disruption of *PdKu80* did not affect the tolerance to salt stress

The radial growths of Δ*PdKu80*-25 and the wild-type strain PdKH8 were compared on NaCl- and KCl-contained PDA (0.5 mol/L) to test the effect of *PdKu80* disruption on the tolerance of *P. digitatum* to salt stress. Compared with non-NaCl- and non-KCl-contained PDA, the mycelial growth for both Δ*PdKu80*-25 and the wild-type strain PdKH8 was severely inhibited (about 60%); however, the inhibition rates were similar for both strains, as indicated that the colony diameters for Δ*PdKu80*-25 and the wild-type strain PdKH8 were not significantly different (P>0.05; Fig. 3). This result indicated that the disruption of *PdKu80* did not affect the tolerance of *P. digitatum* to NaCl or KCl stress.

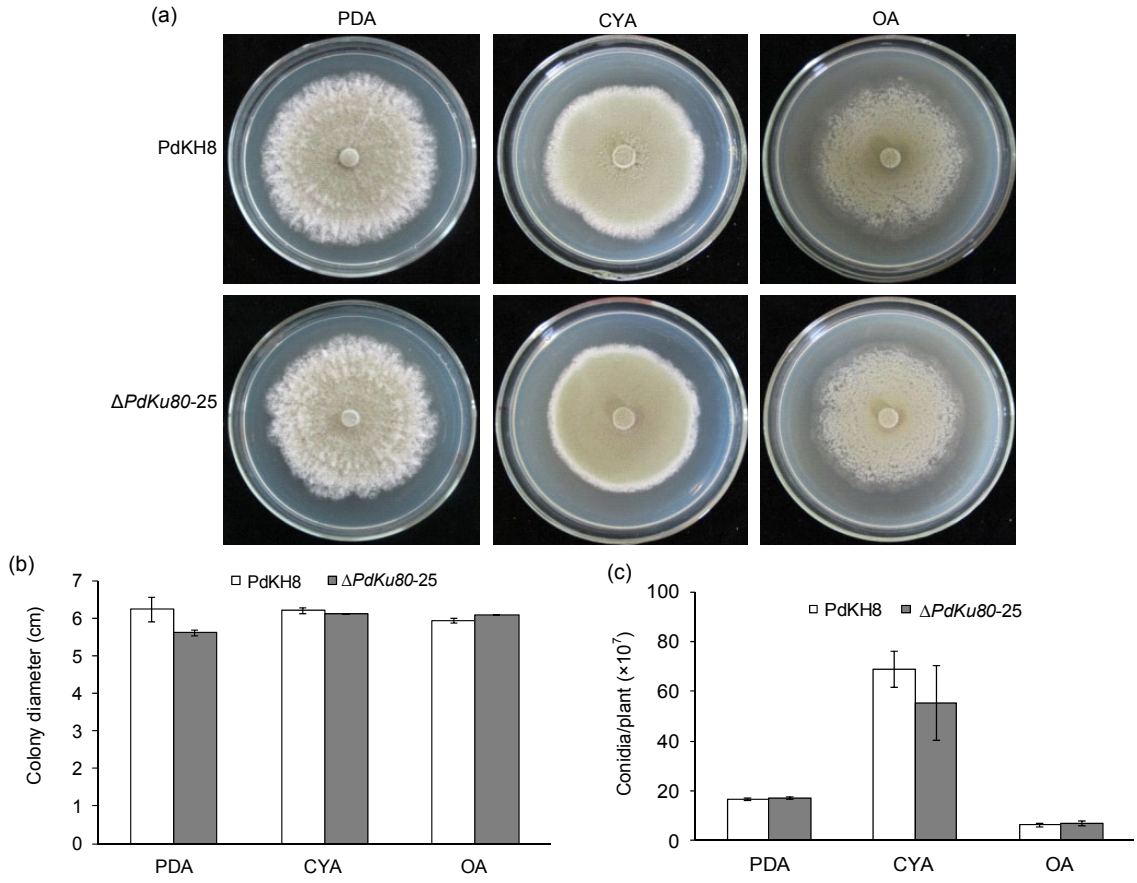

Fig. 2 Effect of disruption of *PdKu80* on the vegetative growth and sporulation

The results showed that disruption of *PdKu80* did not affect vegetative growth or sporulation. (a) The colonies of PdKH8 and Δ*PdKu80*-25 on PDA, CYA, and OA media. (b, c) Columns representing the average colony diameters and number of conidia and bars represented standard errors calculated from three independent colonies. Data were collected 5 d after incubation on PDA and 8 d on CYA and OA at 26 °C

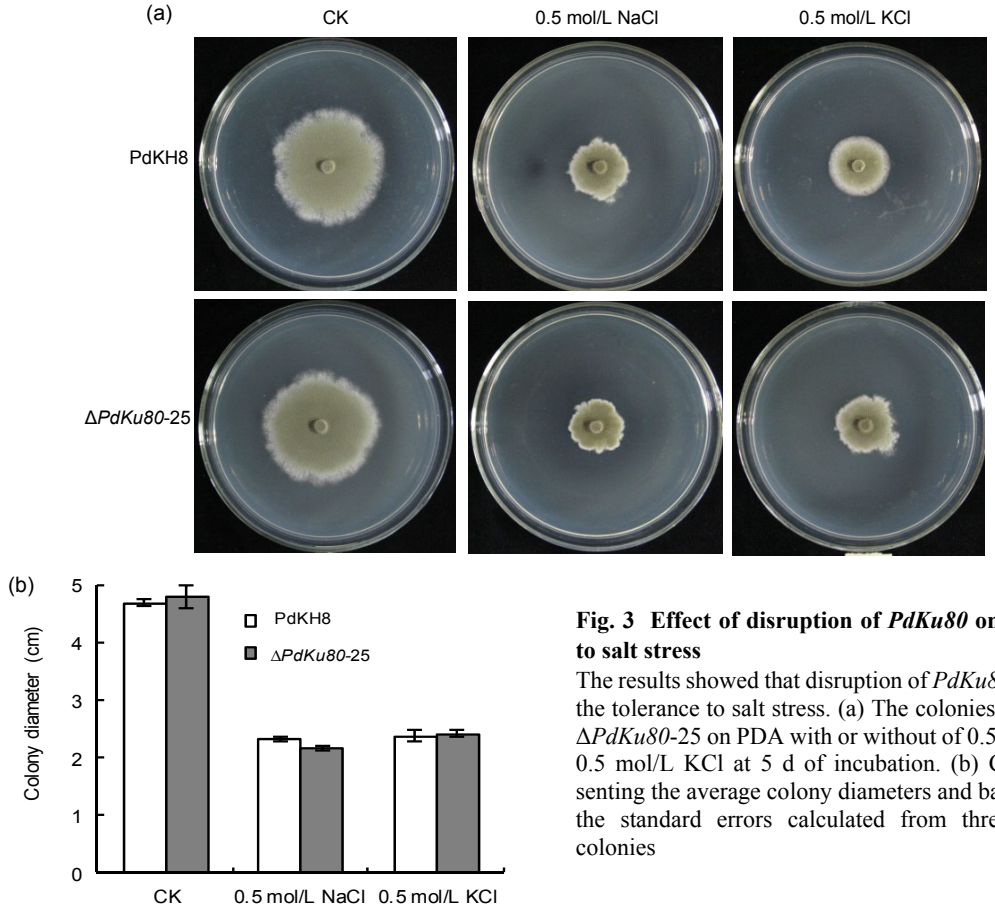

Fig. 3 Effect of disruption of *PdKu80* on the tolerance to salt stress

The results showed that disruption of *PdKu80* did not affect the tolerance to salt stress. (a) The colonies of PdKH8 and Δ*PdKu80*-25 on PDA with or without of 0.5 mol/L NaCl or 0.5 mol/L KCl at 5 d of incubation. (b) Columns representing the average colony diameters and bars representing the standard errors calculated from three independent colonies

3.5 Disruption of *PdKu80* did not affect the virulence

Pathogenicity assays on mature Ponkan fruits by inoculation of conidial suspensions of the Δ*PdKu80*-25 and the wild-type strain PdKH8 revealed that the water-soaked lesion occurred on both strain-inoculated spots. At 5 d post-inoculation, the average lesion diameters caused by Δ*PdKu80*-25 and the wild-type strain PdKH8 were not significantly different (P>0.05) (Fig. 4), indicating that disruption of *PdKu80* did not interfere with the virulence of *P. digitatum* to citrus.

3.6 Disruption of *PdKu80* improved gene targeting efficiency

The effect of the disruption of *PdKu80* on homologous gene targeting was evaluated by disrupting *PdbrlA* (JX298844) and *PdmpkA* (KF019686) genes in both Δ*PdKu80*-25 and the wild-type strain PdKH8 strains, respectively. The strategies for creating the Δ*PdbrlA* and Δ*PdmpkA* targeting cassettes were following the methods described in Section 2, and illustrated in Figs. S1a and S2a. The targeting cassettes of Δ*PdbrlA* and Δ*PdmpkA* were integrated into the strains of Δ*PdKu80*-25 and wild-type PdKH8 by ATMT, respectively. The putative transformants were selected on neomycin-contained media, and analyzed by diagnostic PCR. For Δ*PdbrlA*, a 0.6-kb fragment was able to be amplified from ectopic transformants but was not able to be amplified from Δ*PdbrlA* by primers brlA-i-F/brlA-i-R (Table S1 and Fig. S1b), which were located in the replaced region of *PdbrlA* (Fig. S1a); when using primers brlA-V-F/brlA-V-R (Table S1 and Fig. S1a), two fragments with sizes of 1.0 and 1.2 kb were amplified from ectopic mutants, while only the fragment of 1.2 kb was amplified from Δ*PdbrlA*, and only 1.0 kb fragment was amplified from the wild-type strain of *P. digitatum* (Fig. S1c).

For Δ*PdmpkA*, with primers mpkA-i-F/mpkA-i-R located in the replaced region of *PdmpkA*

(Table S1 and Fig. S2a), a 0.4-kb fragment was able to be amplified from the wild-type and ectopic transformants but could not from Δ*PdmpkA* (Fig. S2b). To get a positive result, primer pair mpkA-V-F/neo-R (Table S1 and Fig. S2a) was used to amplify a fragment of 2.0 kb from the mutants of Δ*PdmpkA*, while no fragments could be amplified from the wild-type and ectopic transformants (Fig. S2c).

For disrupting genes *PdbrlA* and *PdmpkA*, a total of 46 and 50 transformants, respectively, were picked up in the transformation experiment using wild-type PdKH8 as the targeting strain, and only 1 and 2 mutants were demonstrated to be *PdbrlA*- and *PdmpkA*-disrupted mutants, respectively, by diagnostic PCR. The efficiencies of *PdbrlA* and *Pdmpk*A targeting were 2.2% and 4.0%, respectively (Table 1). However, when using Δ*PdKu80*-25 as the targeting strain, the efficiencies of *PdbrlA* and *PdmpkA* targeting were 33.3% and 13.0%, respectively (Table 1).

Targeted gene replacement requires the cellular machinery involved in the repair of DNA DSBs. There are two types of cellular machinery involved in DBS repair, the HR pathway and the NHEJ pathway (Kanaar *et al*., 1998). HR and NHEJ act independently and function competitively (van Dyck *et al*., 1999), thus making it possible to inactivate gene(s) of one pathway to favour another pathway. Ku70-Ku80 heterodimer and a catalytic subunit comprise a DNA-dependent protein kinase complex, which is the central component of NHEJ (Haber, 2000; Jones

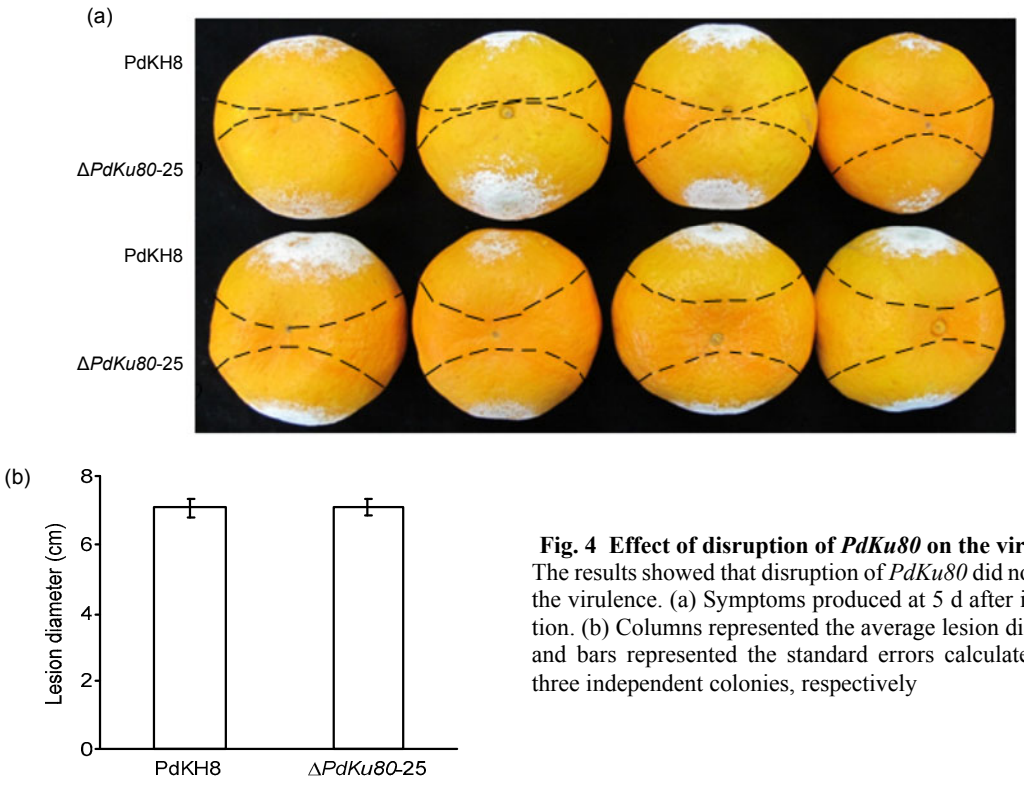

Fig. 4 Effect of disruption of *PdKu80* on the virulence
The results showed that disruption of *PdKu80* did not affect the virulence. (a) Symptoms produced at 5 d after inoculation. (b) Columns represented the average lesion diameters and bars represented the standard errors calculated from three independent colonies, respectively

Table 1 Effect of *Ku80* deletion on the efficiency of gene targeting in *P. digitatum*

Targeting strain	*PdbrlA*			*PdmpkA*		
	No. of transformants	No. of knockout mutants	Targeting frequency (%)	No. of transformants	No. of knockout mutants	Targeting frequency (%)
PdKH8	46	1	2.2	50	2	4.0
Δ*PdKu80*-25	24	8	33.3	23	3	13.0

et al., 2001). Inactivation of the NHEJ machinery by disrupting the Ku70- or Ku80-coding gene has been shown to increase HR in a number of filamentous fungi (Ninomiya *et al*., 2004; da Silva Ferreira *et al*., 2006; Krappmann *et al*., 2006; Nayak *et al*., 2006; Meyer *et al*., 2007; Chang, 2008; Choquer *et al*., 2008; Villalba *et al*., 2008; Li *et al*., 2010).

The frequency of gene targeting in *P. digitatum* via ATMT was always very low (our unpublished data). In the present study, the *Ku80* homolog in *P. digitatum* was identified and its deficient mutant strain was generated. Our experiment showed that the deletion of *PdKu80* did not affect the basic biological characteristics, such as vegetative growth, sporulation, tolerance to salt stress, or pathogenicity, but significantly increased the gene targeting frequency in *P. digitatum*, suggesting that the created Δ*PdKu80* strain can be used as a recipient strain for large-scale gene function characterization in *P. digitatum*. The generation of the Δ*PdKu80* strain will expedite the progress in the functional genomic studies of this fungus.

Compliance with ethics guidelines

Qian XU, Cong-yi ZHU, Ming-shang WANG, Xue-peng SUN, and Hong-ye LI declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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List of electronic supplementary materials

Table S1 PCR primers used in this study

Fig. S1 Schematic diagram showing the construction of *PdbrlA* plasmid and identification of Δ*PdbrlA* transformants by PCR

Fig. S2 Construction of *PdmpkA* disruption plasmid and identification of Δ*PdmpkA* transformants by PCR

Fig. S3 Identification of Δ*PdKu80* by PCR

中文概要：

本文题目： 一种高效的柑橘绿霉菌基因敲除体系的构建

Improvement of a gene targeting system for genetic manipulation in *Penicillium digitatum*

研究目的： 提高柑橘绿霉菌基因敲除效率。

创新要点： 低效的基因敲除与丝状真菌非同源末端链接（NHEJ）的 DNA 双链断裂修复途径有关。为提高柑橘绿霉病菌基因敲除效率，本研究利用农杆菌介导的转化体系，获得 NHEJ 途径中关键因子 *Ku80* 的缺失突变体（Δ*PdKu80*）。

研究方法： 与野生型菌株相比，以 Δ*PdKu80* 作为出发菌株，提高柑橘绿霉病菌 *PdbrlA* 和 *PdmpkA* 的基因敲除效率（见表 1）。

重要结论： Δ*PdKu80* 的营养生长、产孢和致病性与野生型菌株基本一致。Δ*PdKu80* 作为出发菌株，能显著提高柑橘绿霉菌的敲除效率。

关键词组： 指状青霉；基因敲除；非同源末端链接（NHEJ）；*Ku80*