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Adenylyl cyclase is required for cAMP production, growth, conidial germination, and virulence in the citrus green mold pathogen *Penicillium digitatum*

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ABSTRACT

Penicillium digitatum is the causative agent of green mold decay on citrus fruit. The cAMP-mediated signaling pathway plays an important role in the transduction of extracellular signals and has been shown to regulate a wide range of developmental processes and pathogenicity in fungal pathogens. We cloned and characterized a *Pdac1* gene of *P. digitatum*, which encodes a polypeptide similar to fungal adenylyl cyclases. Using a loss-of-function mutation in the *Pdac1* gene we demonstrated a critical requirement for hyphal growth and conidial germination. Deletion of *Pdac1* resulted in decreased accumulation of cAMP and down-regulation of genes encoding a G protein α subunit, both catalytic and regulatory subunits of PKA, and two transcriptional regulators StuA and Som1. Fungal mutants lacking *Pdac1* produced abundant conidia, which failed to germinate effectively and displayed an elevated sensitivity to heat treatment. *Pdac1* mutant failed to utilize carbohydrates effectively and thus displayed severe growth retardation on rich and synthetic media. Slow growth seen in the *Pdac1* mutants could be due to a defect in nutrient sensing and acquisition. Quantitative RT-PCR analysis revealed that *Pdac1* was primarily expressed at the early stage of infection. Fungal pathogenicity assayed on citrus fruit revealed that *P. digitatum* strains impaired for Pdac1 delayed lesion formation. Our results highlight important regulatory roles of adenylyl cyclase-mediated cAMP production in *P. digitatum* and provide insights into the critical role of cAMP in fungal growth, development and virulence.

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1. Introduction

The ascomycetous fungi belonging to the *Penicillium* genus contain more than 300 species that are commonly found in the air, soil, and plant debris. Many of them are saprophytic and are capable of decomposing dead or decaying organic materials; some are important in industry, producing antibiotics and enzymes. Others are capable of causing diseases in animals and plants (van den Berg et al., 2008). *Penicillium expansum*, *P. italicum*, and *P. digitatum* cause postharvest decay of many fruit. Citrus green mold caused by *P. digitatum* Sacc. is one of the most destructive postharvest diseases in citrus, resulting in major economic losses to citrus growers and packers worldwide (Janisiewicz and Korsten, 2002; Palou, 2014). Although it is very rare, *P. digitatum* has been reported to cause fatal pneumonia in an immunocompromised patient in Japan (Oshikata et al., 2013).

It has been well-known that cAMP-mediated signaling regulates numerous biological processes in cells (Daniel et al., 1998; McDonough and Rodriguez, 2011). Adenylyl cyclase converts ATP to form cAMP and pyrophosphate, providing the primary source of intracellular cAMP. The cAMP produced by adenylyl cyclase serves as an important regulatory signal by activating downstream protein kinases (e.g., cAMP-dependent protein kinase A; PKA) or transcription factors (Daniel et al., 1998). The level of cAMP is primarily regulated by its production and degradation via a cAMP-specific phosphdiesterase to form 5′ adenosine monophosphate (5′ AMP)

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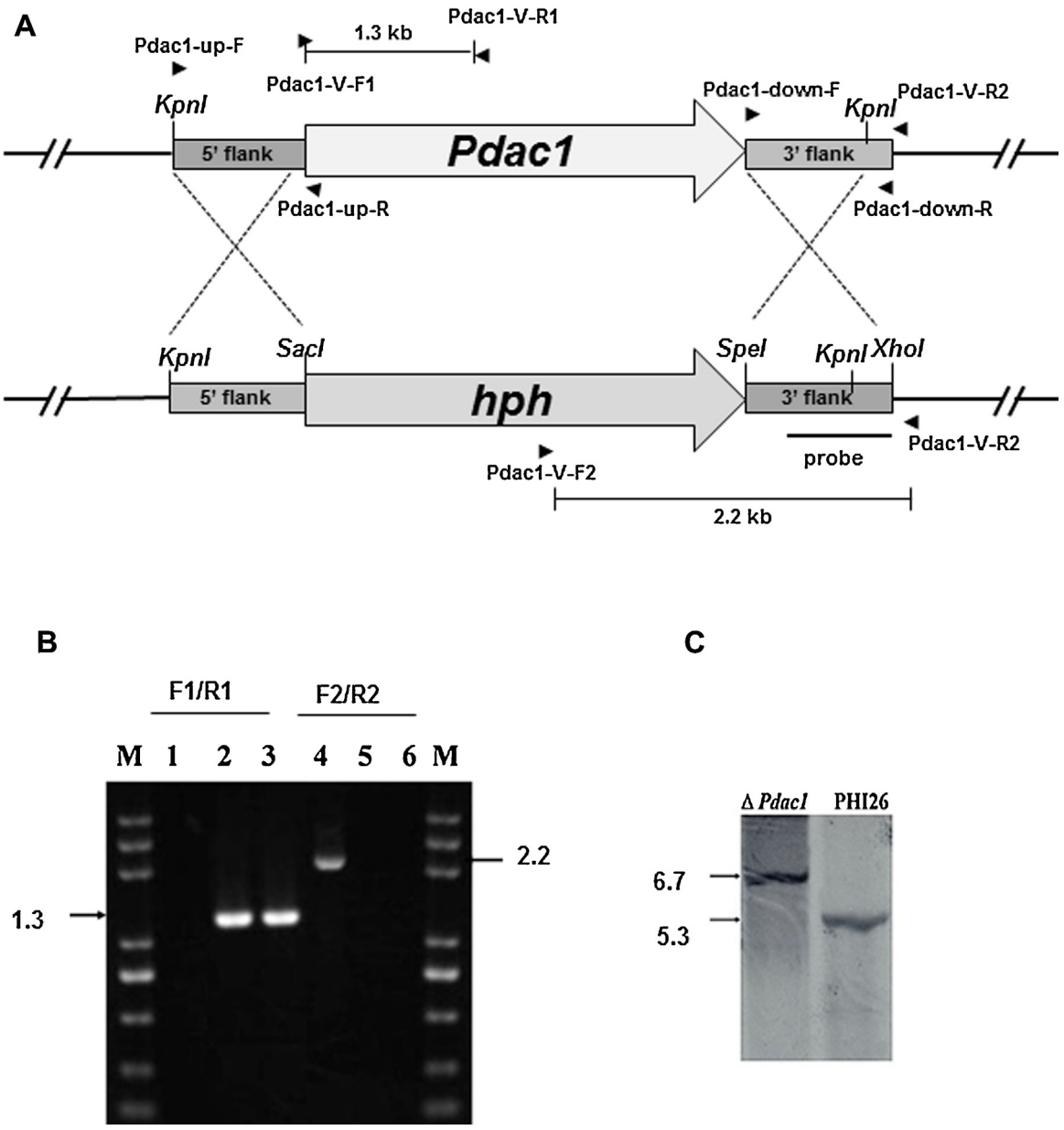

Fig. 1. Mutational inactivation of *Pdac1* encoding an adenylyl cyclase in the genome of *P. digitatum*. (A) Integration of a hygromycin-resistance gene cassette (*hph*) within *Pdac1* of *P. digitatum* via double crossover recombination. (B) Image of DNA fragments amplified by PCR from genomic DNA of wild-type (lanes 3 and 6), *ΔPdac1* (lanes 1 and 4) and ectopic transformant (lanes 2 and 5) with the primers indicated, revealing that *hph* is integrated within *Pdac1* in the genome of *ΔPdac1*. (C) Autographic image of a DNA gel blot of *Kpn*I genomic digests of *P. digitatum* wild type (PHI26) and *ΔPdac1* deletion strain hybridized with a *Pdac1* probe. Hybridizing patterns indicate successful disruption at the *Pdac1* locus. Of 30 transformants examined by PCR, eight additional transformants had similar patterns (data not shown) as seen in the *ΔPdac1* (lanes 1 and 4), indicating that they were *Pdac1* disruptants.

(Li et al., 2007). Activating adenylyl cyclase activity or suppressing phosphodiesterase activity could elevate the cellular cAMP level. Adenylyl cyclase and cAMP have been shown to be involved in normal vegetative growth, formation of infectious structures, virulence, production of secondary metabolites and enzymes, sexual and asexual reproduction, and resistance to environmental stress in fungi (Gold et al., 1994; Choi and Dean, 1997; Ivey et al., 2002; Klimpel et al., 2002; Jurick and Rollins, 2007; Liu et al., 2012; Schuster et al., 2012; Studt et al., 2013; Bormann et al., 2014; Hu et al., 2014).

Although *P. digitatum* is a devastating pathogen of citrus, there have been relatively few studies of pathogenic mechanisms in this fungal species. Several genes required for virulence have recently been characterized in *P. digitatum* (Zhang et al., 2013a,b,c; Wang et al., 2014; Zhu et al., 2014). The genome sequence of *P. digitatum* also identified numerous genes that are potentially associated with virulence and host specificity (van den Berg et al., 2008; Marcet-Houben et al., 2012). Although cAMP-mediated signalling pathway is well conserved in fungi, the regulatory functions may vary considerably in different fungal species. Little is known about the roles of adenylyl cyclase and cAMP in the postharvest pathogen *P. digitatum*. In the present study, we created an adenylyl cyclase loss-of-function mutant by deleting a *Pdac1* gene encoding an adenylyl cyclase from the genome of *P. digitatum*. We provide evidence for a crucial role of adenylyl cyclase and cAMP-mediated signaling on vegetative growth, formation and germination of conidia, and virulence in this important postharvest pathogen of citrus.

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