Fungal Genetics and Biology 118 (2018) 1–9

Contents lists available at ScienceDirect

Fungal Genetics and Biology

journal homepage: www.elsevier.com/locate/yfgbi

Regular Articles

A Myb transcription factor represses conidiation and cephalosporin C production in *Acremonium chrysogenum*

Ying Wang[a,1], Pengjie Hu[a,1], Honghua Li[a,b,1], Yanling Wang[a], Liang-kun Long[a], Kuan Li[a], Xiaoling Zhang[a], Yuanyuan Pan[a], Gang Liu[a,b,*]

[a] State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Keywords:
Acremonium chrysogenum
AcmybA
Regulation
Conidiation
Cephalosporin C production

ABSTRACT

Acremonium chrysogenum is the industrial producer of cephalosporin C (CPC). We isolated a mutant (AC554) from a T-DNA inserted mutant library of *A. chrysogenum*. AC554 exhibited a reduced conidiation and lack of CPC production. In consistent with it, the transcription of cephalosporin biosynthetic genes *pcbC* and *cefEF* was significantly decreased in AC554. Thermal asymmetric interlaced polymerase chain reaction (TAIL-PCR) was performed and sequence analysis indicated that a T-DNA was inserted upstream of an open reading frame (ORF) which was designated *AcmybA*. On the basis of sequence analysis, *AcmybA* encodes a Myb domain containing transcriptional factor. Observation of red fluorescent protein (RFP) tagged AcMybA showed that AcMybA is naturally located in the nucleus of *A. chrysogenum*. Transcriptional analysis demonstrated that the *AcmybA* transcription was increased in AC554. In contrast, the *AcmybA* deleted mutant (ΔAcmybA) overproduced conidia and CPC. To screen the targets of *AcmybA*, we sequenced and compared the transcriptome of ΔAcmybA, AC554 and the wild-type strain at different developmental stages. Twelve differentially expressed regulatory genes were identified. Taken together, our results indicate that AcMybA negatively regulates conidiation and CPC production in *A. chrysogenum*.

1. Introduction

Acremonium chrysogenum is an important industrial fungus for producing the pharmaceutically relevant *β*-lactam antibiotic cephalosporin C (CPC). Like most fungi in which secondary metabolite productions are related with cellular morphological differentiation and development (Calvo et al., 2002; Keller et al., 2005), cephalosporin biosynthesis is also closely associated with the morphogenesis of *A. chrysogenum* (Sándor et al., 2001). During the developmental process, three morphological types are generally observed in *A. chrysogenum*. The formation of conidia from vegetative mycelium and the formation of arthrospores at the late stage of fungal development are associated with the primary metabolism and CPC production. The arthrospore chains (also called “yeast-like” cell) are the phenotype of metabolically active cells enriched with intracellular organelles and lipid-containing vacuoles. The formation of arthrospore chains coincides with the high production of CPC (Bartoshevich et al., 1990). DL-methionine (Met) used for increasing CPC production in industry could significantly stimulate the formation of arthrospore chains in *A. chrysogenum* (Demain and Zhang, 1998). A similar stimulatory phenomenon was also found when glycerol was added during the fermentation of *A. chrysogenum* producer strain M35 (Shin et al., 2010). Some regulators of cephalosporin biosynthesis are also involved in fungal morphogenesis, such as CPCR1 (Hoff et al., 2005). Conversely, the regulators of morphological differentiation are also involved in antibiotic biosynthesis, such as the APSES transcription regulator AcStuA, the septation-related AcSepH and AcVeA (Hu et al., 2015; Long et al., 2013; Dreyer et al., 2007). Disruption of *AcstuA* blocked the conidiation and drastically reduced CPC production. Disruption of *AcvelA* led to earlier onset of hyphal fragmentation, but reduced CPC production. However, deficiency of the axial budding pattern protein AcAxl2 and overexpression of the Sterile 20-like kinase gene *Acmst1* only affect arthrospore development (Kluge and Kück, 2017). The conserved autophagic process involved in conidiation was also affect CPC production (Wang et al., 2014). Although no obligate relationship between cephalosporin biosynthesis and morphogenesis is elucidated, analysis of the morphogenesis related genes is important for a good understanding of cephalosporin biosynthesis in *A. chrysogenum*.

* Corresponding author at: State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China.
E-mail address: liug@im.ac.cn (G. Liu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.fgb.2018.05.006
Received 31 January 2018; Received in revised form 25 May 2018; Accepted 28 May 2018
Available online 02 June 2018
1087-1845/

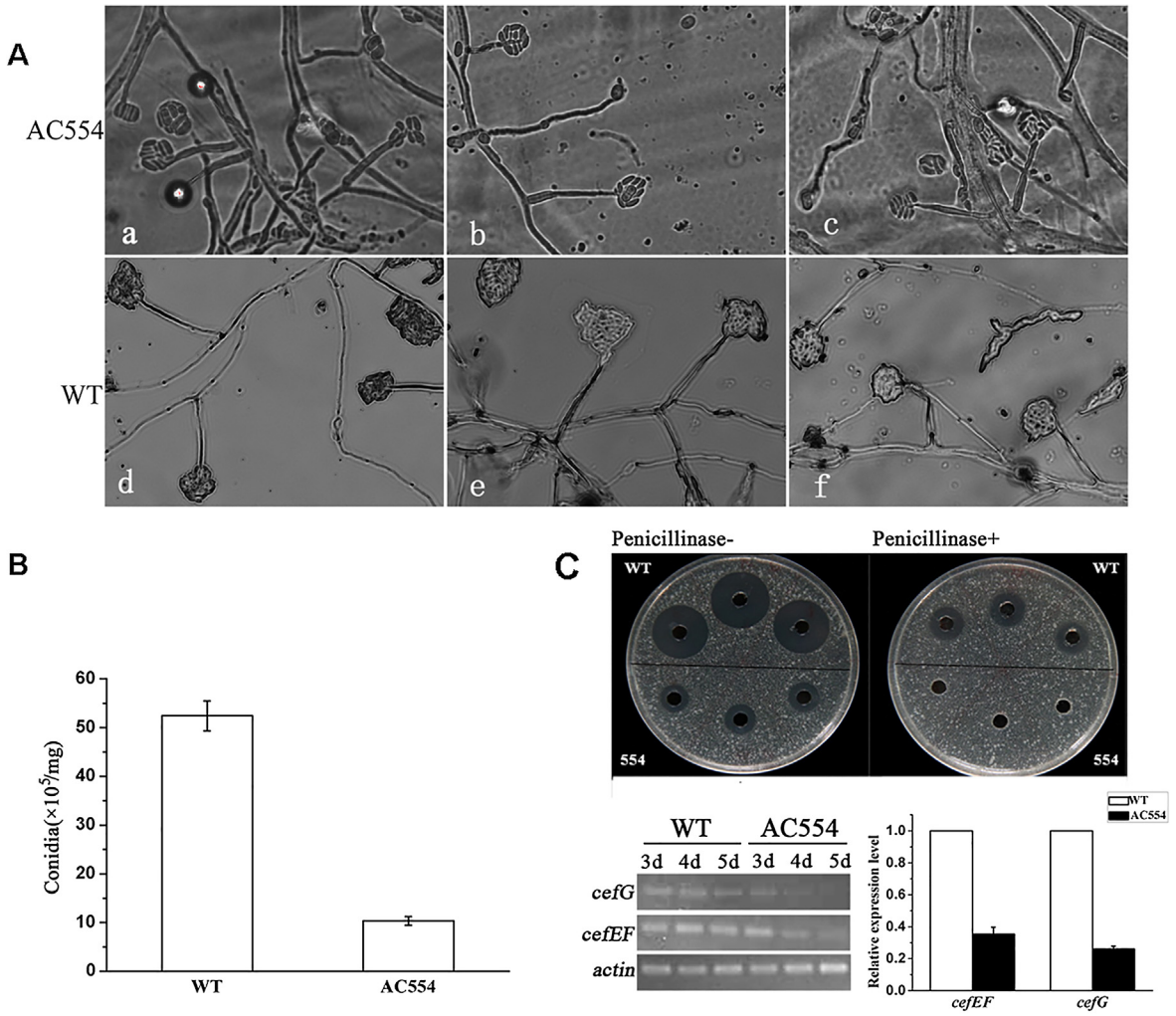

Fig. 1. Comparison of conidia formation and CPC production in WT and AC554. (A) The formation of conidiophores and conidia in WT and AC554 under microscope. a and d, WT and AC554 grew for 3 days on the LPE plates; b and e, WT and AC554 grew for 5 days on the LPE plates; c and f, WT and AC554 grew for 7 days on the LPE plates. (B) Conidia formation in WT and AC554. 1×10^6 spores from WT or AC554 were spread onto a LPE plate covered with a cellophane paper. After incubated at 28 °C for 7 days, the conidia were harvested and counted. Error bars represent standard deviations from three independent experiments. (C) CPC production was determined by bioassays against *B. subtilis CGMCC 1.1630*. 20 μl of culture filtrates from 4 days fermentation was added to the plate without penicillinase to detect the penicillin and CPC production, and 40 μl of the culture filtrates was added to the plate supplemented with 50,000 units of penicillinase to detect the CPC production. WT, the *A. chrysogenum* wild-type strain; AC554, the T-DNA inserted mutant; CPC, cephalosporin C.

The v-Myb gene of avian myeloblastosis virus (AMV) was the first identified Myb gene (Klempnauer et al., 1982). After that, three v-Myb-related genes (*a-Myb*, *b-Myb*, and *c-Myb*) were found in many vertebrates and these genes play important roles in the regulation of cell proliferation, differentiation, and apoptosis (Rosinski and Atchley, 1998; Weston, 1998). Homologs of *Myb* genes were subsequently found in plant, insects, slime molds, and fungi (Lipsick, 1996). In *Saccharomyces cerevisiae*, Myb-related Reb1 is involved in termination of rRNA transcription (Reeder et al., 1999). A study in *Schizosaccharomyces pombe* showed that Reb1 regulates transcription of *ste9* + under nitrogen starvation (Rodríguez-Sánchez et al., 2011). Myb-related Cdc5p is required for G2/M progression in *S. pombe*, and is essential for pre-mRNA splicing (McDonald et al., 1999). In filamentous fungi, the Myb domain containing protein FlbD was reported to control conidiophore development of *Aspergillus nidulans* (Wieser and Adams, 1995). But its homolog of FlbD in *Neurospora crassa* has no identifiable role (Shen et al., 1998). A recent publication showed that *mybA* is responsible for conidiation, spore viability, trehalose accumulation, cell wall integrity and protection against reactive oxygen species in *Aspergillus fumigatus* (Valsecchi et al., 2017). A Myb-like domain containing protein MYT1 was found to play an important role in perithecia development, vegetative growth and toxin production in *Gibberella zeae* (Lin et al., 2011).

Due to lacking a sexual cycle and conventional genetic analysis, the regulation of morphological differentiation and cephalosporin biosynthesis is poorly understood in *A. chrysogenum* (Schmitt et al., 2004). Since isolation of mutant could help us to understand morphological differentiation, we constructed a mutant library of *A. chrysogenum* through T-DNA randomly insertion (Long et al., 2013). In this study, we identified a novel gene *AcmybA* which encodes a Myb domain containing protein from this mutant library, and the function of *AcmybA* in conidiation and cephalosporin biosynthesis was investigated.

2. Materials and methods

2.1. Strains, media and growth conditions

Strains and plasmids used in this study are listed in Supplementary Table S1. LPE medium was used for conidiation and TSA medium was used for fungal growth and the constructed strain screening (Long et al., 2013). For fermentation, the modified MDFA medium was used as described previously (Guan et al., 2017). Minimal medium (MM),