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Lysyl-tRNA synthetase (Krs) acts a virulence factor of *Beauveria bassiana* by its vital role in conidial germination and dimorphic transition

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ABSTRACT

Krs is a class II lysyl-tRNA synthetase (KRS) that is involved in cytosolic protein synthesis in budding yeast but functionally has not been explored in filamentous fungi. Previous transcriptomic analysis has revealed that a Krs-coding gene is likely involved in pathogenesis of *Beauveria bassiana*, a classic insect pathogen as a global source of fungal insecticides. Here, we show that Krs is localized in the cytoplasm of hyphal cells and acts as a substantial virulence factor in *B. bassiana*. Deletion of *krs* resulted in 10-h delayed germination, decreased (15 %) thermotolerance, and lowered (46 %) UV-B resistance of aerial conidia despite limited impact on conidiation capacity and slight or inconspicuous influence on radial growth on rich and minimal media with different carbon (10 sugars/polyols) and nitrogen (17 amino acids) sources. The deletion mutant suffered 58 % reduction in submerged blastospore yield (an index of *in vitro* dimorphic transition rate) in a minimal medium, and the reduction increased to 71 % in another trehalose-based medium mimic to insect haemolymph. In standardized bioassays, median lethal actions of Δ*krs* against *Galleria mellonella* larvae through the infections passing and bypassing the insect cuticle were prolonged to 192 and 153 h from wild-type median lethal time (LT_{50}) estimates of 119 and 109 h, respectively. Microscopic examination revealed 2-d delayed presence of *in vivo* formed hyphal bodies in the haemolymph of the larvae infected by Δ*krs* in either mode. These findings unveil a vital role of Krs in conidial germination and dimorphic transition and its contribution to the fungal potential against arthropod pests.

Introduction

Aminoacyl-tRNA synthetases (ARSs) are a family of enzymes that catalyse the esterification of specific amino acids to cognate tRNAs in the process of protein synthesis (Guo & Yang 2014). There exist 20 ARSs fallen into two classes based on sequence motifs and topology in their catalytic domains (Eriani *et al.* 1990). Class I comprises predominantly monomeric enzymes that catalyse the coupling of aminoacyl group to 2′-hydroxyl group of tRNA and are featured with the ATP binding motifs HIGH and KMSKS (Rossmann fold) while class II consists of polymer enzymes to target 3′-hydroxyl site (Goerlich *et al.* 1982). Most class II enzymes are lysyl-tRNA synthetases (KRSs) (Oka *et al.* 2015) and characterized by a smaller N-terminal tRNA anticodon binding domain and a larger C-terminal catalytic domain comprising three conserved sequence motifs

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(Desogus *et al.* 2000). Motif 1 helps to form an interface between two catalytic domains during enzyme dimerization while motifs 2 and 3 bind to ATP and stabilize a bent conformation of ATP (Onesti *et al.* 1995).

Krs is a class II KRS that is localized in cytosol and acts as a component of multisubunit tRNA synthetase complex (MSC). The MSC comprises nine catalytic ARSs and three non-catalytic or auxiliary proteins, namely AIMP1–3 (Lee *et al.* 2004; Han *et al.* 2006). The Krs homodimer binds to the N-terminal peptide of AIMP2 for formation of Krs homodimer–AIMP2 monomer complex in the MSC, and hence is considered to be a tetramer in comparison to other MSC components as monomers or dimmers (Guo *et al.* 2008). Msk1 and Krs1 are two KRSs in *Saccharomyces cerevisiae*. Msk1 originates from bacteria and functions in mitochondrial protein synthesis whereas Krs1 is evolved in eukaryotes and functions in cytosolic protein synthesis (Diaz-Lazcoz *et al.* 1998; Woese *et al.* 2000). Aside from the basic function of protein translation in yeast, many studies have shown diverse functions of Krs associated with human health and diseases (Motzik *et al.* 2013), such as signalling in immune response (Yannay-Cohen *et al.* 2009), death signalling of stressed cells (Kepp *et al.* 2010), cytokine-like immune signalling (Park *et al.* 2005), transcriptional mediation during viral replication (Saadatmand *et al.* 2008), targeting of Cu,Zn-superoxide dismutase (SOD) involved in amyotrophic lateral sclerosis (Kawamata *et al.* 2008). A recent genome-wide analysis has revealed a linkage of Krs (KARS) with mitochondrial respiratory chain complex deficiencies (Kohda *et al.* 2016). The Krs homologues of the parasites *Loa loa*, *Schistosoma mansoni*, and *Plasmodium falciparum* have been found to be a new target for drug development against causative agents of Loiasis, Schistosomiasis, and malaria (Hoepfner *et al.* 2012; Sharma *et al.* 2016). Human Krs can complement the growth defect caused by loss-of-function mutation of Msk1 in yeast (Sepuri *et al.* 2012).

However, functions of KRSs have been generally unexplored in fungal pathogens of plants, insects, and animals. Our recent transcriptomic analysis revealed that a Krs-coding gene could likely participate in the host infection of *Beauveria bassiana* or act as a virulence factor due to its transcriptional upregulation during the fungal infection against a lepidopteran pest (Chu *et al.* 2016). As a filamentous fungal insect pathogen, *B. bassiana* has been widely applied in biological control programs of insect pests (de Faria & Wraight 2007; Ortiz-Urquiza *et al.* 2015). The present study sought to elucidate possible functions of Krs in the insect pathogen through the analyses of phenotypic changes attributed to its knockout mutation. We found that Krs was localized in cytosol, sustained conidial germination and dimorphic transition *in vivo* or *in vitro*, and hence acted as an important virulence factor in *B. bassiana*.

Materials and methods

Bioinformatic analysis of Krs in Beauveria bassiana

The open reading frame (ORF) of the Krs-coding gene [tag locus: BBA_03037 under the NCBI accession NZ_ADAH00000000 (Xiao *et al.* 2012)] upregulated in the host infection of *B. bassiana* was amplified from the cDNA of the wild-type strain *B. bassiana* ARSEF2860 (named WT hereafter) with paired primers (Table S1) and verified by sequencing. Possible domains and signal peptide were predicted from the Krs sequence deduced from the verified ORF using the online programs SMART at http://smart.embl-heidelberg.de, SignalP 4.1 at http://www.cbs.dtu.dk/services/SignalP, and TMHMM 2.0 at http://www.cbs.dtu.dk/services/TMHMM-2.0/TMHMM2.0.guide.html. The deduced Krs sequence was phylogenetically compared with the sequences of Krs homologues found in the NCBI protein database of human and fungal representatives using a neighbour-joining method in MEGA7 software at http://www.megasoftware.net.

Subcellular localization of Krs

The verified ORF was digested with XbaI/SpeI and fused to the N-terminus of enhanced green fluorescence protein gene (*eGFP*) in pGMB-eGFP-bar with multiple restriction enzyme sites (Guan *et al.* 2016). The resultant plasmid pGMB-krs-eGFP-bar was transformed into the WT strain using a method of blastospore transformation (Ying & Feng 2006). Putative transformant colonies were screened by *bar* resistance to phosphinothricin (200 μg ml^{-1}). A colony showing a maximal eGFP signal under a laser scanning confocal microscope (LSCM) was chosen for normal cultivation on Sabouraud dextrose agar (4 % glucose, 1 % peptone, and 1.5 % agar) plus 1 % yeast extract (SDAY) until full conidiation. Conidia from the culture were suspended in SDB (i.e., agar-free SDAY), followed by 2 d shaking (150 rpm) incubation at 25 °C. LSCM images for the hyphal cells expressing eGFP-tagged Krs fusion protein were collected and merged with an image browser to judge subcellular localization of Krs in *Beauveria bassiana*.

Generation of krs *mutants*

The *krs* gene was deleted from the WT strain by homogeneous recombination of its 5′ and 3′ coding/flanking fragments (~1500 bp each) separated by *bar* marker and rescued in Δ*krs* by ectopic integration of a cassette comprising its full-length coding sequence with flank regions and *sur* marker using the backbone plasmids p0380-bar and p0380-sur-gateway and protocols as described previously (Xie *et al.* 2013). Putative deletion and complementary mutant colonies were screened by the *bar* resistance to phosphinothricin (200 μg ml^{-1}) and the *sur* resistance to chlorimuron ethyl (10 μg ml^{-1}) in a selective medium. The recombination events were examined *via* PCR and Southern blotting analyses with paired primers and amplified probe (Table S1). Positive Δ*krs* and Δ*krs::krs* mutants (Fig S1) were evaluated in parallel with WT in the following phenotypic experiments of three replicates.

Phenotypic experiments

Aliquots of 1 μl × 10^6 conidia ml^{-1} suspension were spotted centrally onto the plates (9-cm diameter) of SDAY, 1/4 SDAY (amended with 1/4 nutrients of SDAY), Czapek agar (CZA; 3 % sucrose, 0.3 % $NaNO_3$, 0.1 % K_2HPO_4, 0.05 % KCl, 0.05 % $MgSO_4$, and 0.001 % $FeSO_4$ plus 1.5 % agar), and CZA modified with one of nine carbon or 17 nitrogen (amino acids) sources