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ORIGINAL ARTICLE

Glc8, a regulator of protein phosphatase type 1, mediates oxidation tolerance, asexual development and virulence in *Beauveria bassiana*, a filamentous entomopathogenic fungus

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Abstract

Protein phosphatase type 1 (PP1) plays an important role in cellular metabolism and development in yeast. In PP1 enzyme complex, Glc8 protein is a global regulatory subunit and regulates many physiological processes. However, its biological roles are unexplored in filamentous fungi. In this study, we characterized a yeast ortholog of Glc8 in *Beauveria bassiana*, a filamentous entomopathogenic fungus. Gene disruption of *BbGlc8* had no significant effect on vegetative growth, but resulted in a significant reduction in conidiation (51%) and blastospore yield (55%) in the mutant. The Δ*BbGlc8* mutant displayed an enhanced sensitivity to oxidative stress and a weakened virulence as indicated by cuticle infection and intrahemocoel injection assays. Transcriptomic analysis indicated that the genes regulated by *BbGlc8* during conidiation were primarily associated with metabolism, cell rescue and cell wall formation. Notably, as a down-regulated gene in Δ*BbGlc8* mutant, *BbOsmC2* (a member of OsmC protein family) contributes to fungal resistance to salt stress, spore differentiation and virulence. Thus, *BbOsmC2* functions as a down-stream target of *BbGlc8* during spore differentiation, but not in stress response. Our findings indicate that *BbGlc8* contributes to the biocontrol potential of *B. bassiana* by mediating comprehensive genetic pathways.

Keywords Protein phosphatase type 1 · Oxidation tolerance · Fungal differentiation · Virulence · *Beauveria bassiana*

Introduction

Protein phosphatase type 1 (PP1), belonging to the PPP family of protein phosphatase, regulates a plethora of physiological processes in yeast cells (e.g., budding yeast and fission yeast), including ion homeostasis, glycogen metabolism, transcription, sporulation and so on (Williams-Hart et al. 2002; Cannon 2010; Martín and Lopez-Aviles 2018).

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In yeast, PP1 enzyme functions as a heterotrimeric complex containing a catalytic subunit (Glc7) and its distinctive substrate specificities are dependent on variable regulatory subunits (e.g., Glc8, Pan1 and Pex31) (Cannon 2010). In fungi, *Glc8* gene was initially characterized in a glycogen-deficient mutant of budding yeast, and encodes a homologue of PP1 inhibitor-2 (I-2) in mammalian cells (Cannon et al. 1994). Inhibitor I-2 regulates the PP1 activity via changing the conformation of the catalytic subunit (Tan et al. 2003). As a global regulator, Glc8 protein functions in vivo as a major activator of Glc7 protein phosphatase activity, but does not change the Glc7 protein level in cells (Cannon 2010). Under certain physiological conditions, the excessive Glc8 protein also acts as an inhibitor of Glc7 acitivity (Tung et al. 1995). In *Saccharomyces cerevisiae*, Glc8 is a heat-stable protein and specific for PP1 enzyme (Li et al. 2007). As a global regulator, Glc8 is involved in many physiological processes in yeast. For example, Glc8 regulates the metabolism of carbohydrates by sensing the changes of glucose content. *Glc8* expression is repressed by extracellular glucose, and Glc7 protein is completely activated in the absence of glucose (Nigavekar et al. 2002). *Glc8* is involved in regulation of

glycogen synthase, and its loss reduces glycogen accumulation in cells (Cannon et al. 1994). Additionally, *Glc8* controls the chromosome segregation in yeast cell cycle (Tung et al. 1995). To date, the *Glc8* roles in yeasts have been well-studied, but its roles in filamentous fungi are not completely revealed.

Filamentous fungi are characterized with extensive hyphal networks and have the comprehensive impacts on ecosystems (Klein and Paschke 2004). *Beauveria bassiana*, a filamentous fungus, is a ubiquitous insect pathogen with broad host spectrum and has been widely used for the biocontrol of insect pests (De la Cruz Quiroz et al. 2015). In nature, fungal conidia attach to the body surface of host and invade into host by breaching the cuticle (Lewis et al. 2009; Wanchoo et al. 2009). After reaching the hemocoel, invasive hyphae undergo morphological transition to generate yeast-like hyphal bodies (in vivo blastospore) which utilize various nutrients in hemolymph (Lewis et al. 2009). After killing the host, hyphae penetrate through the cuticle again and grow on the host cadaver followed by generation of numerous conidia (He et al. 2015, 2016). In *B. bassiana*, several virulence-related pathways have been characterized to be associated with autophagy (e.g., *BbATG1*), cell cycle (e.g., *BbCdc14*), metabolism (e.g., *BbSNF1*), cytoskeleton (e.g., *BbGEL1*) and so on (Ying et al. 2016; Wang et al. 2013, 2014; He et al. 2016), but none was associated with the protein dephosphorylation pathway regulated by PP1.

In this report, we used *B. bassiana* as a representative of filamentous fungus to explore the roles of *Glc8* in fungal development and pathogenicity. The results indicate that *B. bassiana Glc8* (*BbGlc8*) significantly contributes to fungal sporulation (including conidial and blastospore development) and virulence. Comparative transcriptomics between the wild-type and Δ*BbGlc8* mutant strains indicated that *BbGlc8* gene mediated sets of genes during conidial development. Among the differentially repressed genes, *BbOsmC2* (a member of osmotically inducible protein C family) was verified to be involved in spore development and virulence. This study suggests that *Glc8* gene functions as an important role in fungal asexual development associated with lifecycle of *B. bassiana*.

Materials and methods

Microbial strains and cultivation

Microbial strains were cultivated as previously described (Ying et al. 2014). The wild type of *B. bassiana* ARSEF2860 (Bb2860) (US Plant, Soil and Nutrition Laboratory, Tower Road, Ithaca, NY, USA) is the first isolate whose genome has been sequenced, and it is used as model strain of *B. bassiana* (Xiao et al. 2012). Fungal strain was maintained on Sabouraud dextrose agar (SDAY: 4% glucose, 1% peptone and 1.5% agar plus 1% yeast extract). *Escherichia coli* DH5α (Invitrogen, Carlsbad, CA, USA) was cultured in Luria–Bertani (LB) medium with required antibiotics for plasmid proliferation. *Agrobacterium tumefaciens* AGL-1 (a bacterium) was proliferated in YEB broth (w/v: 0.5% sucrose, 1% peptone, 0.1% yeast extract and 0.05% $MgSO_4$) and added as a donor strain in fungal transformation. Czapek-Dox agar (CZA) (3% glucose, 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) was used to screen the transformants.

Phylogenetic analysis of the *B. bassiana* Glc8 protein

The sequence of yeast Glc8 protein (GenBank No.: AAA53673) was used as a query to search the potential homologs in the Bb2860 genome (Xiao et al. 2012), and then the *B. bassiana* homolog (Locus tag: BBA_06202) was identified and named as BbGlc8. Then, the cDNA sequence of BbGlc8 protein was mapped onto the genome sequence of *B. bassiana*, and its whole open reading frame (ORF) was obtained.

The Glc8 orthologs in fungi were obtained from the NCBI protein database using the online BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/). The protein sequences of all orthologs were aligned with ClustalW, and their phylogenetic relationships were constructed with MEGA version 5 (Tamura et al. 2011).

Construction of *BbGlc8* gene disruption and complemented strains

The *BbGlc8* gene disruption vector was constructed as previously described (Gao et al. 2018b), and all the required primers are listed in Supporting Information Table S1. In brief, upstream (1.04 kb) and downstream (1.35 kb) flanking sequences of *BbGlc8* ORF were prepared by PCR reaction with the primer pair P_G1/P_G2 and P_G3/P_G4, respectively. The resultant PCR fragments were ligated into the *Eco*RI/*Bam*HI and *Xba*I/*Hpa*I sites of p0380-bar (Xie et al. 2012), respectively, using ClonExpress II One Step Cloning Kit (Vazyme Biotech, Nanjing, China). The resulting vector was named p0380-bar-Glc8 and used for gene disruption. To complement the gene disruption mutant, the *BbGlc8* ORF plus 2.13 kb of upstream and 0.48 kb of downstream sequences was amplified with primers P_G7/P_G8. The resultant DNA fragment was cloned into the vector p0380-sur-gateway as previously described (Xie et al. 2012), generating the plasmid p0380-sur-Glc8 with sulfonylurea resistance marker. The disruption and complementation plasmids were introduced into the wild-type and gene disruption mutant strains, respectively, using *Agrobacterium*-mediated transformation procedure (Fang et al. 2004). Putative disruption mutants

were screened on Czapek-Dox agar (CZA) supplemented with 200 μg/ml phosphinothricin, and the complemented strains were screened on CZA plates with 15 μg/ml chlorimuron ethyl. To confirm the correct recombination events, PCR reaction was performed in candidate transformants with primer P_G5 and P_G6 (Supplementary Table S1). Recombination events were further confirmed by Southern blot analysis, using DIG High Prime DNA Labeling and Detection Starter Kit II (Roche, Penzberg, Germany). The probes were labeled with digoxin, using DNA fragment (416 bp) amplified with primers P_G9/$_GP10$ (Supporting Information Table S1) as template. The target fragments were hybridized and visualized according to the manufacturer's instruction.

Phenotypic analyses of fungal strains

Phenotypic assays, including conidial germination, radial growth, spore production, stress response and virulence, were performed as previously described (Ying et al. 2016). All assays were repeated three times.

Radial growth on plates

Aliquots of 1 μl conidial suspension (1×10^6 conidia/ml) were spotted on SDAY or CZA modified with different carbon and nitrogen sources. In carbon-modified CZA, carbon sources included (final concentration, 3%) glucose, sucrose, trehalose, glycerol, mannitol, fructose and maltose. In nitrogen-modified CZA, nitrogen sources (final concentration) included urea (0.3%), NH_4Cl (0.3%), N-acetylglucosamine (0.5%), chitin (0.3%), proline (0.5%) and gelatin (0.3%). After 7 days of cultivation at 25 °C, the colony diameters were measured.

Responses to chemical stress

Chemical stress was initiated by adding different chemical reagents (final concentration) into CZA plates, including hypertonic reagents (0.5 M NaCl and 1M sorbitol), oxidation reagents (2 mM hydrogen peroxide and 0.02 mM menadione) and cell wall stressor (3 μg/ml Congo red). Aliquots of 1 μl suspensions (1×10^6 conidia/ml) were placed on plates and cultured at 25 °C for a week. Colony diameters were examined, using CZA plates without stress chemicals as a control.

Spore development and production

To assess conidial yield, aliquots of 100 μl of conidia suspension (10^7 conidia/ml) were smeared evenly on SDAY agar plates and incubated for 7 days at 25 °C. Mycelial disks (Ø 5 mm) were rinsed with 0.02% Tween 80 solution by vigorous vortex, and the conidial concentration was used to calculate conidial yield on mycelia as the number of conidia/cm^2.

For blastospore production, conidia were inoculated into SDB broth at a final concentration of 10^6 conidia/ml. After an incubation of 3 days at 25 °C with constant shaking (150 rpm), spore concentration was measured and calculated as the number of cells per ml.

Conidial virulence

The conidial virulence was determined using *Galleria mellonella* larvae as bioassay insects via two infection routes. The 7-day old conidia were grown on SDAY plates. In cuticle infection bioassay, the larvae were submerged in the conidial suspension (1×10^7 conidia/ml) for 10 s. In intrahemocoel injection bioassay, 5 μl of conidial suspension (1×10^5 conidia/ml) was injected into the host hemocoel. Tween 80 solution (0.02%) was used as a blank control. The treated insects (30–35 larvae per treatment) were reared at 25 °C for 7 days. The median lethal time (LT_{50}) was estimated by Probit analysis from the trend of mortality recorded daily.

Unraveling the *BbGlc8*-mediated transcriptome

To probe the effects of *BbGlc8* on global gene expression during conidial development, a comparative transcriptomic analysis was performed between the wild-type and Δ*BbGlc8* mutant strains as previously described (Ying et al. 2014).

The wild-type and Δ*BbGlc8* mutant strains were cultured on SDAY plates for 2 days, and total RNA was extracted from mycelia of indicated strains. RNA samples were constructed into two libraries which were sequenced on Illumina Hiseq X Ten platform at Vazyme Biotech Co., Ltd (Nanjing, China). Sequence data have been deposited in NCBI's Gene Expression Ominibus under the accession No. GSE116102. Each library was replicated two times in the independent experiments.

All clean reads from each library were mapped onto the genome sequence of Bb2860 (Xiao et al. 2012), using the HISAT program (Kim et al. 2015). All mapped genes were quantified in terms of the expected number of fragments per kilobase of transcript sequence per millions base pairs sequenced (FPKM) with Cufflinks software (Trapnell et al. 2010). The differentially expressed genes (DEGs) between two libraries were screened with the Cuffdiff method (Trapnell et al. 2013). Genes with a q value of < 0.05 (5% false discovery rate) and the absolute value of log square ratio > 1 were considered significant. To probe the potential function of DEGs, an enrichment analysis was performed with Functional Catalog (FunCat) method (Ruepp et al. 2004), using online portal (http://mips.helmholtz-muenchen.de/funcatDB/) at the threshold of $P < 0.01$.

Validation of the downstream target of *BbOsmC2*

The significantly repressed genes in Δ*BbGlc8* mutant were deemed as the *BbGlc8*-mediated downstream targets. To probe the potential targets involved in conidiation, a member of the OsmC (osmotically inducible protein C) family (locus tag: BBA_08760; named as *BbOsmC2*) was selected as a representative.

The roles of *BbOsmC2* in conidial and blastospore development were elucidated via gene disruption and complementation, using the same strategy applied to functional analysis of *BbGlc8*. All required primers are also listed in Supporting Information Table S2. All phenotypic assays for disruption mutant of *BbOsmC2* were performed with the same methods described in the section "Phenotypic analyses of fungal strains".

Data analysis

Tukey's honest significance test (Tukey's HSD) was used to evaluate the significant difference in the indicated phenotype among the wild-type, disruption mutant and complemented strains.

Results

Bioinformatic features of BbGlc8 protein and construction of its gene disruption and complementation strains

On the basis of BLAST search, a single highly related *B. bassiana* gene, BBA_06202 (Identity: 30%; E-value: 3e-5) was identified and designated as *BbGlc8*. The ORF sequence of *BbGlc8* gene was 923 bp long with one intron in genomic sequence, and coded for a 281-amino acid protein. The deduced BbGlc8 protein contained an IPP-2 domain which could be seen in Glc8 homologues in other fungi (Supporting Information Table S3). Phlyogenetic analysis indicated that the homologues of filamentous fungi and yeasts were sorted into two independent branches (Supporting Information Fig. S1). *B. bassiana* BbGlc8 was sorted into a cluster of entomopathogenic fungi, and is closely related to that of *Cordyceps militaris* than to homologues in two *Metarhizium* species.

To determine the potential roles of *BbGlc8*, its gene disruption mutant was constructed by replacing its partial ORF with the phosphinothricin resistance gene (bar), using a homologous recombination strategy (Supporting Information Fig. S2). The gene disruption mutant was complemented via ectopic integration of the full-length *BbGlc8* ORF plus the upstream promoter sequence, using the sulfonylurea resistance gene (*sur*) as the second selection marker. The correct gene disruption mutant and complementation strains were screened by PCR reaction and further confirmed by Southern blotting.

Disruption of the *BbGlc8* affected fungal oxidation tolerance, spore production and virulence

After 7 days of growth on various plates, the Δ*BbGlc8* mutant did not show significant growth defects when compared with the wild-type and complemented strains (Fig. S3). On SDAY plates, disruption of *BbGlc8* had no significant effect on the vegetative growth; however, fungal development was significantly impaired in the gene disruption mutant strain (Fig. 1a). On the culture surface, the wild-type and complemented strains produced 7.37 ± 0.37 and $6.67 \pm 0.91 \times 10^8$ conidia/cm^2 [mean ± standard deviation (SD)], respectively. However, the disruption strain only yielded $3.58 \pm 0.39 \times 10^8$ conidia/cm^2 at 7 days post incubation (dpi). In the submerged culture, the gene disruption strain also displayed a significantly decreased blastospore yield ($5.47 \pm 0.38 \times 10^7$ spores/ml (mean ± SD), whereas the wild-type and complemented strains produced 12.30 ± 0.61 and $10.50 \pm 0.44 \times 10^7$ spores/ml (Fig. 1b), respectively. Additionally, gene loss enhanced fungal sensitivity to oxidation resistance (Fig. 1c). Mortality was measured over an 8 day period, and all strains tested were able to kill all test insects within 8 days post inoculation (Fig. 1d, f). As for fungal virulence, the loss of *BbGlc8* resulted in a slight but statistically significant delay in LT_{50}s from two kinds of bioassays [20% in topical test (Fig. 1e) and 14% in injection test (Fig. 1g)], indicating that fungal virulence had been slightly weakened.

Ablation of *BbGlc8* significantly affected global transcriptome

To identify the potential *BbGlc8*-mediated gene targets during conidial development, the global expression profiles were compared between the wild-type and Δ*BbGlc8* mutant strains. Ablation of *BbGlc8* resulted in altered expression of 1185 genes, with 414 up-regulated (~4.0% of the genome) and 771 down-regulated (~7.4% of the genome) genes in the mutant compared with the wild type (Table S4). In addition, twenty-six genes were only transcribed in the wild-type strain, and four genes were only transcribed in Δ*BbGlc8* mutant.

Enrichment analysis indicated that the down-regulated DEGs were over-presented in functional catalogs of metabolism, cell transport, cellular defense and so on (Fig. 2 and Table S5). Overall, repressed genes were involved in metabolism, including a large number of genes associated with amino acid and carbonhydrate metabolism (e.g., glycoside hydrolase, glucanosyltransferase, and alcohol dehydrogenase); cell rescue, defense and virulence (e.g., heat shock

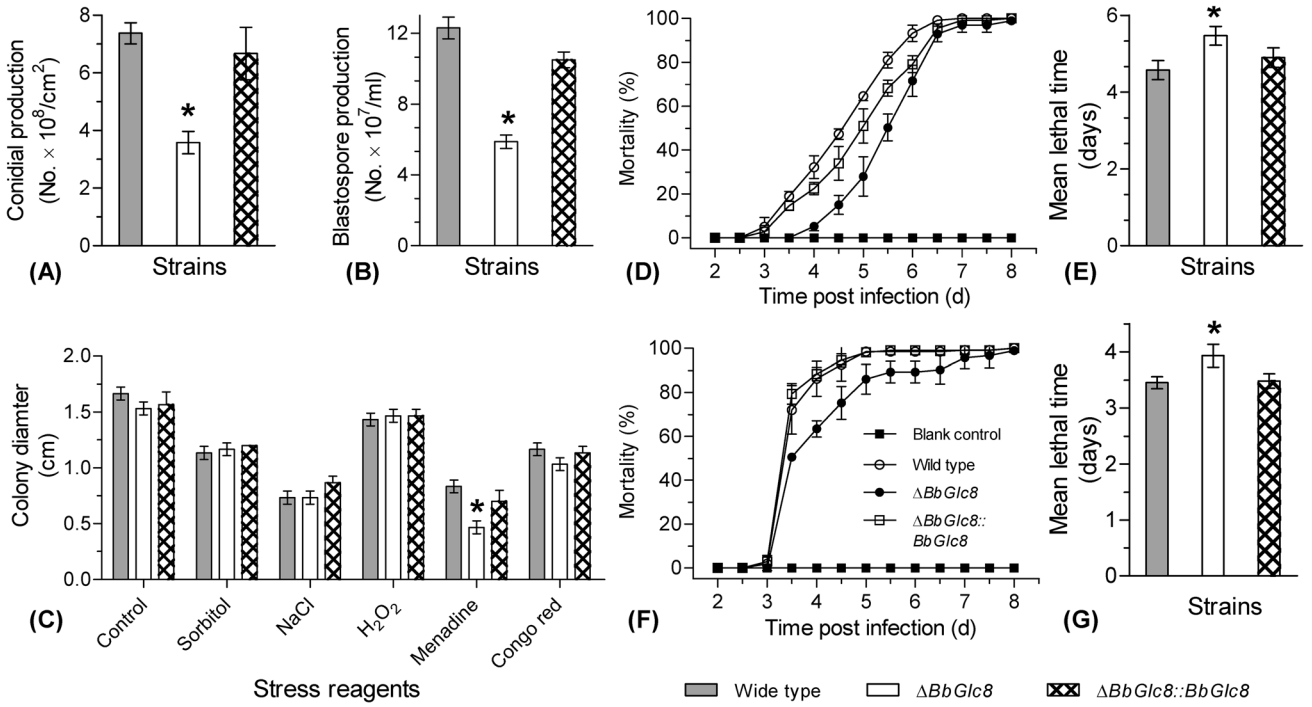

Fig. 1 Effects of the *BbGlc8* loss on phenotypic aspects in *B. bassiana*. **a** Conidial yield. Fungal strains were grown on SDAY for conidiation up to 7 days, and the yield is calculated as the number of conidia per square centimeter. **b** Blastospore production. Fungal stains were inoculated into SDB broth (SDAY without agar) for 3 days, and the yield is presented as the cell number per milliliter of culture broth. **c** Fungal tolerance to chemical stress. Conidia were inoculated on Czapek-Dox agar plates supplemented with either NaCl (0.5 M), sorbitol (1.0 M), H_2O_2 (2 mM), menadione (0.02 mM) or Congo red (3 μg/ml). Fungal virulence was evaluated with two bioassay methods. Mortalities in topical (**d**) and intrahaemoceol injection (**f**) assays were recorded within 8 days post infection. The mean lethal time (LT_{50}) for topical (**e**) and intrahaemoceol injection (**g**) assays was estimated by Probit analysis. Asterisks (*) on columns indicate a significant difference between the gene disruption mutant and the wild-type or complemented strain (Tukey's HSD: $P<0.05$). Error bars: standard deviation for three replicates

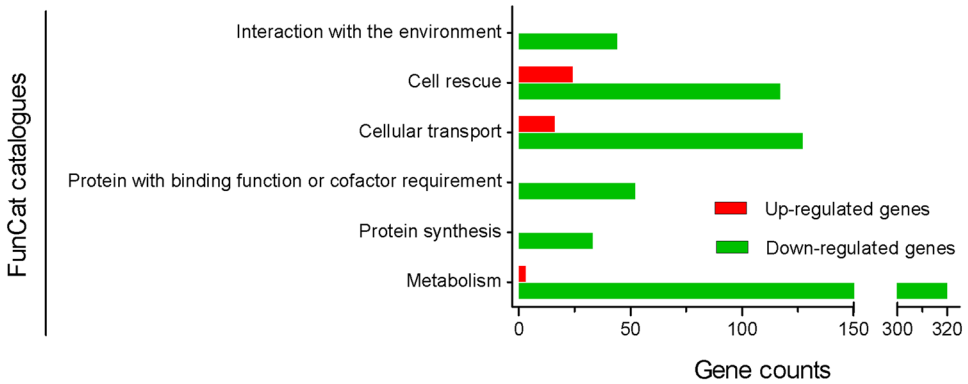

Fig. 2 Functional Catalog (FunCat) analysis of the *BbGLC8*-mediated trancriptome. Differentially expressed genes (DEGs) were determined by comparing the transcriptomes of the wild-type and Δ*BbGLC8* mutant strains. FunCat analysis was used to sort all DEGs, and these DEGs were over-presented in six functional categories

protein (Hsp) 30, laccase 2 and OsmC protein); cellular transport (e.g., major facilitator superfamily transporter and monocarboxylate permease). A set of genes related to cell wall were also found to be repressed in Δ*BbGlc8* mutant strain, including cell surface protein (BBA_09174), hydrophobin (BBA_00530), cell wall glucanosyltransferase Mwg1 (BBA_08214) and so on. In addition, a mitogen-activated protein kinase (sty1) (BBA_09043) was also down-regulated in gene disruption mutant. Up-regulated genes in the *BbGlc8* mutant were only enriched in three functional catalogs, including metabolism, cellular transport as well as cell rescue defense and virulence (Fig. 2 and Table S6). These genes were mainly involved in metabolism of prosthetic groups (e.g., pyruvate decarboxylase: BBA_08386), allantoate transport (e.g., allantoate permease: BBA_03541) and cell defense (e.g., drug resistance protein: BBA_06344).

Functional analyses of *BbOsmC2* gene

The unidentified OsmC-like protein (locus tag: BBA_08760) belongs to the OsmC superfamily and is named as *BbOsmC2*. The *BbOsmC2* ORF was 525 bp long without intron in the genomic sequence and coded a protein with 174 amino acid residues.

The physiological roles of *BbOsmC2* gene in *B. bassiana* were revealed by gene disruption and complementation. PCR reaction and Southern blot indicated that the gene disruption mutant and complementation strains were successfully constructed (Fig. S4).

Disruption of *BbOsmC2* did not significantly affect vegetative growth on various media (Fig. S5). However, *BbOsmC2* significantly contributed to conidial and blastospore production. Conidial yield was significantly decreased in gene disruption mutant, which produced $2.19 \pm 0.08 \times 10^8$ conidia/cm^2 (mean ± SD), whereas the wild-type and the complementation strains yielded 7.37 ± 0.37 and $5.70 \pm 0.43 \times 10^8$ conidia/cm^2, respectively (Fig. 3a). The blastospore yield was shown as the spore number per milliliter of culture media. The loss of *BbOsmC2* resulted in a significant reduction in blastospore yield, with a yield of $3.10 \pm 0.85 \times 10^7$ spores/ml (mean ± SD). However, the wild-type and complemented strains generated 12.30 ± 0.61 and $10.73 \pm 0.67 \times 10^7$ blastospores/ml, respectively, which did not significantly differ with each other (Fig. 3b). In addition, ablation of *BbOsmC2* caused a reduced resistance to hyperosmotic stress (Fig. 3c). During 8 day bioassay, all strains could kill all inoculated insects (Fig. 3d, f). In cuticle infection bioassay (Fig. 3e), the LT_{50} values for the wild-type and Δ*BbOsmC2* strains were 4.57 ± 0.25 days (mean ± SD) and 5.20 ± 0.11 days, respectively, indicating that a increase of 14.0% in time to kill 50% of insect hosts for Δ*BbOsmC2* mutant when compared with the wild-type strain. In intrahaemocoel-infection bioassay (Fig. 3g), the LT_{50} values Δ*BbOsmC2* strains was only elongated by approximately 7.0% as compared with the wild-type strain.

Discussion

As presented above, *Glc8* gene was proven to be involved in spore (conidium and blastospore) differentiation, oxidation resistance and virulence of the filamentous entomopathogenic fungus *B. bassiana*. In yeasts, Glc8 protein contributes to metabolism and chromosome segregation (Tung et al. 1995; Cannon 2010). Thus, Glc8 protein functions as an important regulator in single-cell yeast and filamentous

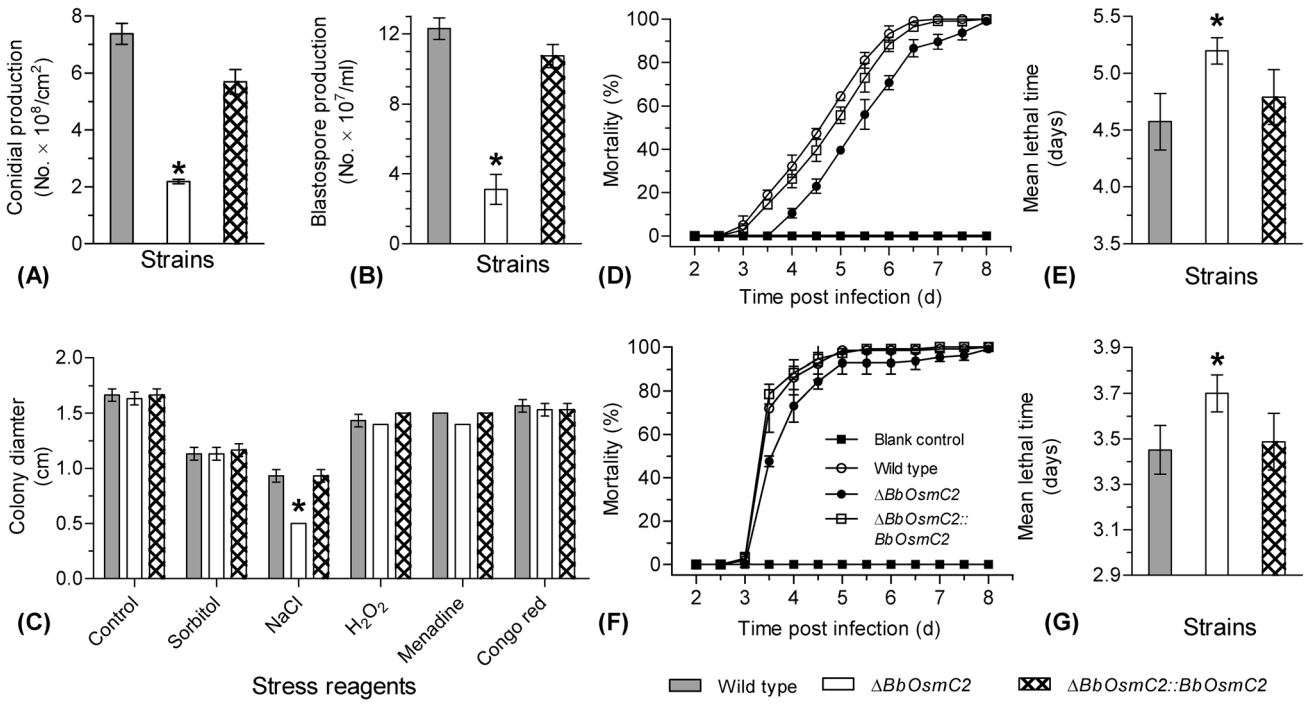

Fig. 3 Phenotypic assays of the wild-type, Δ*BbOsmC2* mutant and complemented strains. A series of phenotypic assays were performed to evaluate the gene disruption on fungal physiologies, including conidial production (**a**), blastospore yield (**b**), stress response (**c**), as well as virulence via cuticle penetration (**d, e**) and intrahaemoceol infection (**f, g**). All experiments were conducted as same as those used in phenotypic evaluation of Δ*BbGlc8* mutant (as described in Fig. 1). Asterisks (*) on columns indicate a significant difference between the Δ*BbOsmC2* mutant and the wild-type or complementation strain (Tukey's HSD: $P < 0.05$). Error bars: standard deviation

fungi. The philological roles for *Glc8* gene in the lifecycle of *B. bassiana* is discussed below.

The virulence of entomopathogenic fungi is crucial for their efficacies as biocontrol agents (St. Leger et al. 2011). The ability to perform dimorphic change between hyphae and yeast-like cells is an important determinant for the virulence of fungal pathogens (Gauthier 2015). In the host hemocoel, *B. bassiana* generates hyphal bodies (in vivo blastospores) via dimorphic transition (Lewis et al. 2009). The *BbGlc8* gene contributes to 55% of blastospore yield in the wild-type strain. In *B. bassiana*, genes involved in dimorphic transition are associated with autophagy (e.g., *BbATG5*) (Zhang et al. 2013), cell cycle (e.g., *BbCdc14*) (Wang et al. 2013), energy sensing (e.g., *BbSNF1*) (Wang et al. 2014) and cytoskeleton (e.g., *BbGEL1*) (He et al. 2016). The current study adds new understanding to fungal dimorphic transition. Additionally, in the host hemocoel, the fungal growth will be inhibited by the oxidative stress caused by the insect's immune reaction (Bergin et al. 2005). Fungal resistance to oxidation is another determinant for *B. bassiana* virulence (Ortiz-Urquiza and Keyhani 2015; Chu et al. 2018). The *BbGlc8* gene is involved in fungal tolerance to oxidative stress caused by menadione. Thus, the weakened virulence of disruption mutants could be due, in part, to their defects in blastospore development and resistance to oxidative stress. *Glc8* has been linked to glycogen accumulation and chromosome segregation in yeast (Cannon et al. 1994; Tung et al. 1995). Our data indicate that *Glc8* links the fungal development and stress tolerance to the virulence of entomopathogenic fungi.

Asexual condiation promotes fungal disperse in nature (Gao et al. 2018a). At the last stage of infection cycle of *B. bassiana*, mycelia spread saprophytically on cadavers and generate plentiful conidia via asexual development (He et al. 2015, 2016). *BbGlc8* contributes to conidiation, and acts as a regulator for asexual development in *B. bassiana*. This is the first report about the *Glc8* roles in the asexual development of filamentous fungi, and no information is available for its mediated pathways. Thus, a comparative transcriptomic analysis was conducted to probe the potential targets of *BbGlc8* during conidiation. *BbGlc8* has a comprehensive influence on the global gene expression during fungal conidiation. First, *BbGlc8* mediates the transcription of genes involved in amino acid and carbohydrate metabolism. *B. bassiana* SNF1 kinase contributes to conidiation by regulating the amino acid metabolism (He et al. 2015). These results suggest that amino acid metabolism during conidiation is regulated by multiple pathways. Second, transcriptomic analysis indicated that *BbGlc8* is required for the expression of genes involved in cell rescue. For instance, several heat shock protein (Hsp) genes are repressed in *BbGlc8* mutant strain, including *Hsp30, Hsp70* and *Hsp90* genes. Previous study indicated that an *Hsp40* gene contributes to conidial development in *B. bassiana* (Wang et al. 2016), but is not found downstream of *BbGlc8*. In *Trichoderma atroviride* (a filamentous fungus), the pathways associated with metabolism and cell rescue are involved in fungal conidiation (Sanchez-Arregui et al. 2012). This suggests that the cellular metabolism is finely regulated during conidiation in filamentous fungi. Third, *BbGlc8* regulates the expression of cell wall protein (e.g., hydrophobin). In *B. bassiana*, hydrophobins form a rodlet layer on conidial surface (Zhang et al. 2011). Although the hydrophobin roles in conidiation have not been examined in *B. bassiana*, a hydrophobin-like protein (*BbHyd3*) contributes to conidiation (He et al. 2016). In addition, the requirement of hydrophobin for conidiation has been established in other filamentous fungi (e.g., *M. brunneum* and *Ma. grisea*) (Kim et al. 2005; Sevim et al. 2012). Finally, *BbGlc8* controls the expression of genes in signal transduction. For example, the mitogen-activated protein kinase (MAPK) cascade is an important signal transduction pathway (Liu et al. 2017). *BbGlc8* is required for the expression of Mpk3 in MAPK pathway. This result suggests that *BbGlc8* has an interaction with signaling transduction pathways, although the detailed mechanisms need to be investigated. In terms of physiological terms, *BbGlc8* contributes to conidiation by mainly mediating cellular metabolism, stress defense, signal transduction and cell wall formation.

To explore more "cell rescue"-related genes involved in conidiation, we identified a member of OsmC family (*BbOsmC2*) as a contributor to conidiation in *B. bassiana. OsmC* gene was initially characterized in bacteria and induced by hyperosmosis caused by salt shock (Atichartpongkul et al. 2001; Shin et al. 2004), but it also plays a significant role in cellular defense against oxidative stress (Lesniak et al. 2003). In *B. bassiana, BbOsmC2* contributes to fungal resistance to salt stress (an expected role), spore differentiation (including conidium and blastospore) and virulence, but not to fungal tolerance to oxidative stress. Dimorphic transition is crucial for fungal pathogenicity (Gauthier 2015). *BbOsmC2* is involved in fungal virulence, which might be due to its significant role in blastospore production. These findings suggest that the OsmC proteins play divergent roles in prokaryotic and eukaryotic organisms. This is the first report about the biological roles of OsmC gene in fungi, including filamentous fungi and yeasts, although the members of OsmC family are prevalent in fungi (Meireles et al. 2017). As mentioned above, *BbGlc8* significantly contributes to conidiation and blastospore production in *B. bassiana*. These results indicate that *BbOsmC2* functions as a down-stream gene of *BbGlc8* during spore development, but they are not in same pathway during fungal tolerance to environmental stresses. This finding suggests that *BbOsmC2* is controlled by different upstream regulators as cell needs, although it entails much work to elucidate the detailed mechanisms.

Taken together, *BbGlc8* plays an important role in oxidation tolerance, spore differentiation and virulence in *B. bassiana*. Transciptomic analysis unraveled that *BbGlc8* has the comprehensive effects on genes involved in various cellular processes during fungal conidation. More importantly, we characterized *BbOsmC2*, a member of OsmC family, as a down-stream target of *BbGlc8* during conidial formation. The present study links the PP1 pathway to fungal differentiation, and develops an initial framework to elucidate the *BbGlc8*-mediated pathways in conidiation. Future work, identifying more downstream genes and actual PP1 phosphatase regulated by Glc8 protein in *B. bassiana*, may enhance our understanding of the physiological processes in the infection cycle of entomopathogenic fungi.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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