



BCB1, a member of the acyl-coenzyme A synthetase family, regulates the morphogenesis and pathogenicity of *Botrytis cinerea*

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Abstract

Botrytis cinerea is a non-host-specific phytopathogenic fungus capable of infecting numerous cash crops. Here, we analyzed the functions of the *Bcb1* gene in *B. cinerea*, which encodes a membrane protein belonging to the acyl-coenzyme A synthetase family. Compared to the wild type, *Bcb1*-deletion mutants exhibited obvious morphological abnormalities, including slower vegetative growth and reduced melanin production. The absence of *Bcb1* causes *B. cinerea* to form only small and incompletely developed infection cushions and fail to produce spores. The *Bcb1* mutants displayed hypersensitivity to the membrane stressor SDS, the cell wall stressor Congo red, and the oxidative stressor H₂O₂ and increased resistance to intracellular osmotic stress caused by KCl compared to the wild-type strain. However, there were no differences in tolerance to extracellular osmotic stress caused by NaCl. The deletion of *Bcb1* also caused a reduction in pathogenicity. The qRT-PCR results showed that the genes *Bcpcs12* and *Bcpcs13*, which are related to melanin biosynthesis, and *Bcpg2*, *BcBOT2*, and *cutA*, which are related to virulence, were downregulated in $\Delta Bcb1$. These data suggest that BCB1 is important for conidial morphogenesis, and pathogenesis in *B. cinerea*.

Keywords *Botrytis cinerea* · ACSs · Infection cushions · Conidiation · Pathogenicity

Introduction

Botrytis cinerea is an aggressive plant pathogen that causes gray mold disease in over 500 plant species, including fruits, vegetables, flowers, medicinal herbs, and other critical economic crops (Schumacher et al. 2017; Zhang et al. 2014;

Yu et al. 2014; Moretti et al. 2015; Jin et al. 2021; Wang et al. 2011). It is difficult to control because the pathogen is characterized by abundant conidial production, high field adaptability, and diverse virulence factors and is also prone to chemical resistance (Petrasch et al. 2019). Hence, there is an urgent need to study the sporulation and pathogenesis mechanism of *B. cinerea*.

Membrane proteins are the bridge between the external environment and intracellular signal transduction, and play a key role as receptors and transporters in organisms. Many membrane proteins are potential drug targets. In this study, we compared the specificity of 12 pairs of primers reported for *B. cinerea* detection. The results showed that the primer pair UTAS-F/UTAS-R had the best specificity (Fig. S1, Table S1). Bioinformatics analysis indicated that the gene marked by the primer was a new membrane protein of the acyl-CoA synthetase (ACS) family. ACSs belong to the second subclass (Ib) of the first class of the adenylate forming enzyme superfamily, which is characterized by a variety of AMP-binding domains (Wang et al. 2016; Schmelz and Naismith 2009). The leading role of ACSs is to catalyze the thioesterification reaction of fatty acids with coenzyme A to form activated intermediates, a critical step for fatty acid

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activation and plays a vital role in the dynamic balance of energy supply, lipid metabolism, and lipid-related processes (Black and DiRusso 2007). In the maize pathogen *Cochliobolus heterostrophus*, knocking out of the gene encoding the ACS protein *CPS1* decreased pathogenicity by almost 60% in leaves (Lu et al. 2003). The deletion of *MoCPS1*, a homologous gene of *CPS1* in *Magnaporthe oryzae*, leads to multiple phenotypic changes, such as a reduction in conidial production, alteration in conidial morphology, defects in penetration pegs formation and a significant reduction in the pathogenicity (Wang et al. 2016). It was demonstrated in a recent report that the ACS protein BbFaa1 in *Beauveria bassiana* was associated with cell membrane function, conidial production and pathogenicity in a recent report (Li et al. 2022).

To date, there are no reports on functional studies of ACSs in *B. cinerea*. In this study, we identified and functionally characterized a gene in *B. cinerea* marked by primer pair UTAS-F/UTAS-R and named *Bcb1*. Bioinformatic analysis of the BCB1 protein suggested that it is a membrane protein with an AMP-binding domain belonging to the ACSs. $\Delta Bcb1$ mutants exhibited defects in mycelial growth, infection cushion formation, melanin production, conidia formation and virulence. The qRT-PCR result showed that genes related to the biosynthesis of melanin and plant toxin BOT biosynthesis were downregulated in $\Delta Bcb1$. These results suggest that the BCB1 protein is involved in the regulation of development and virulence in *B. cinerea*.

Materials and methods

Fungal strains and culture conditions

The wild-type *B. cinerea* strain (ACCC37712) was purchased from the Agricultural Culture Collection of China. All strains were regularly incubated on potato dextrose agar (PDA, 200 g potato, 20 g glucose, 15 g agar, and 1 L water) plates at 20 °C. Regeneration medium (0.7 M sucrose, 0.5 g/l yeast extract, and 15 g/l agar) was used to regenerate *B. cinerea* protoplasts. The derived $\Delta Bcb1-2$ and $\Delta Bcb1-16$ strains were maintained on PDA amended with 100 µg/ml G418. Mycelial growth assays for each strain under different environmental stresses were performed on PDA containing 0.05% Congo red, 0.05% sodium dodecyl sulfate (SDS) or 1.2 M NaCl or 1.2 M KCl or 0.08% H₂O₂. Each plate was inoculated with a 5 mm-diameter mycelial plug taken from the edge of a 4-day-old colony grown on PDA. The percentage of inhibition of mycelial radial growth (PIMG) was calculated using the formula, $PIMG = [(C - N)/(C - 5)] \times 100$, where *C* is the colony diameter of the untreated control and *N* is that of the treatment. Each experiment was repeated three times independently.

Identification of BCB1 protein

SignalP-5.0 (<https://services.healthtech.dtu.dk/service.php?SignalP-5.0>) was used to predict whether the BCB1 protein contains a signal peptide. Interpro (<https://www.ebi.ac.uk/interpro/>) and Pfam (<https://pfam.xfam.org/>) were used to predicted the conserved domain of the protein and the classification of this domain was predict by BLASTP. InterPro, PRED-TMR2 (<http://athina.biol.uoa.gr/PRED-TMR2/>) and TMHMM-2.0 (<http://www.cbs.dtu.dk/services/TMHMM-2.0/>) were used to predict whether the protein contained a transmembrane region.

Construction of Bcb1-deletion strains

The gene *Bcb1* was deleted by split-marker cassette technology based on PCR fusion as summarized in Fig. S2 A (Catlett et al. 2003). All the primers used in this study are listed in Table S1. The 1.4-kb upstream “UP” and the 1.2-kb downstream “DOWN” were amplified from genomic DNA of the *WT* strain by primers UP-F/UP-R and DOWN-F/DOWN-R, respectively. Overlapping marker fragments “NE” and “EO” of the *Neo* cassette were amplified from the PKN plasmid vector using the NE-F/NE-R and EO-F/EO-R primers, respectively. Fragment “UP-NE” and “EO-DOWN” were amplified by primer pairs UP-NE-F/UP-NE-R and EO-DOWN-F/EO-DOWN-R, respectively. The fragments were purified for transformation.

Protoplasts were prepared as follows. Approximately 10⁸ conidia of the *WT* strain were transferred to a flask containing 150 ml of potato glucose liquid medium (PDB) incubated in a rotary shaker (22 °C, 160 rpm) for 24 h. Mycelia were collected by filtration and was washed once with sterile water and twice with KC buffer (0.6 M KCl, 50 mM CaCl₂). The mycelium was suspended in 20 ml of 0.5% Glucanex (Sigma, St. Louis) solution (dissolved in KC buffer) and incubated at 30 °C and 100 rpm for 2–3 h to generate protoplasts. Protoplasts were concentrated by centrifugation at 6000 rpm, and 4 °C for 10 min, washed twice and resuspended in STC buffer to a final concentration of 10⁸ protoplasts per milliliter. Protocols for transformation were performed as described previously (Xue et al. 2016). Finally, the transformation mixture was mixed gently with melted RM agar medium (approximately 45 °C), and poured into 6-cm Petri dishes. After incubating at 20 °C for 20 h, each Petri dish with regenerating protoplasts was overlaid with 10 ml of RM agar medium containing 250 µg/ml G418. After incubating for 3–5 days at 20 °C, transformants were transferred to PDA containing 100 µg/ml G418 for further selection. The purified

transformants were analyzed by PCR, Southern blot (validation strategy, Fig. S2D), and RT–PCR (*GAPDH* gene as positive control) to ensure that the gene *Bcb1* was completely knocked out.

Mycelial growth and conidiation

For vegetative growth assays, a 5-mm-diameter mycelial plug of wild-type or mutant strains was transferred onto the center of the PDA plates incubated in the dark at 20 °C. The colony diameter was measured every 24 h. For the conidial yield assay, the method described by Ma was performed (Ma et al. 2020). Three replicates were set for each strain treatment and the experiment was repeated three times.

Pathogenicity assays

Protocols for the pathogenicity assay were slightly modified from an established procedure (Feng et al. 2017). A 5-mm diameter mycelial plug of *WT* or a mutant was transferred to the surface of strawberry fruits and the underside side of strawberry leaves. Then fruits and leaves were incubated in artificial climate incubator at 20 °C with constant humidity and a 12 h light/12 h dark cycle environment. Three replicates were set for each strain treatment and the experiment was repeated three times.

Observation of infection cushions

Referring to a previous study (Liu et al. 2020), we used an optical microscope to observe the formation of infection cushions (ICs) of $\Delta Bcb1$ mutants and the *WT* strain on microslides and the onion inner epidermis.

Quantitative RT-PCR assay

All the primers for the qRT–PCR assay are listed in Table S1. Total RNA was extracted from the mycelial samples of mutants and *WT* cultured in liquid YEPD (10 mg/ml peptone, 3 mg/ml yeast extract, 20 mg/ml glucose) (Shao et al. 2017), excepted for the samples for detecting the expression of *Bcpg1*, *Bcpg2*, *BcBOT2*, or *CutA* which were cultured with polygalacturonic acid medium (0.2% $(\text{NH}_4)_2\text{SO}_4$, 0.05% MgSO_4 , 0.1% K_2HPO_4 , 0.05% NaCl , 1% polygalacturonic acid) (Liu et al. 2007).

RNA was isolated from mycelia with the RNA-Easy Isolation Regent (Vazyme., Nanjing, China). First-strand cDNA was synthesized with the HiScript II 1st Strand cDNA Synthesis Kit (+ gDNA) (Vazyme., Nanjing, China). The reagents used for real-time PCR were ChamQ SYBR qPCR Master Mix (Vazyme., Nanjing, China). For each sample, the reference sequences were the genes encoding *GAPDH* and β -tubulin in *B. cinerea*. The analysis of relative gene

expression levels using the qRT–PCR data was performed according to the $2^{-\Delta\Delta\text{CT}}$ method described previously (Livak and Schmittgen 2001). Data from three biological replicates were analyzed.

Results

Analysis of *Bcb1* in *B. cinerea*

The gene *Bcb1* (Gene ID: BCIN_13g02260) encodes a protein of 541 aa, which has a transmembrane region (226 aa–246 aa) but no signal peptide. Interpro and Pfam analyses show that it has a 306 aa adenosine monophosphate binding (AMP) domain (42 aa–347 aa) that belongs to ACSs (Fig. S3). It is a number of prokaryotic and eukaryotic enzymes, which appear to act via an ATP-dependent covalent binding of AMP to their substrate, share a region of sequence similarity. This region is a Ser/Thr/Gly-rich domain that is further characterised by a conserved Pro-Lys-Gly triplet (Jackson et al. 2016).

Bcb1 disruption resulted in abnormal colony morphology and a reduction in melanin and conidial formation

To investigate the function of the *Bcb1* gene, we obtained *Bcb1*-deletion mutants by replacing the gene with the neomycin resistance gene (*Neo*). After PCR screening two stable mutants (Fig. S2B), $\Delta Bcb1-2$ and $\Delta Bcb1-16$, were confirmed by RT–PCR (Fig. S2E) and Southern blotting analysis (Fig. S2C) and used for further analysis.

When cultured on PDA medium at 20 °C, the colony morphology of the two independent mutants was significantly different from that of the wild-type. The $\Delta Bcb1$ mutants presented as a flat colony due to the reduced aerial hyphal growth and were whiter in color (Fig. 1A). After incubation on PDA for 25 days, the colonies of the *WT* strain turned gray and lots of melanin deposits appeared at the bottom of plates, while the colonies of mutants remained white and without melanin deposit at the bottom of plates (Fig. 4S). Compared with the *WT* strain, the colony growth rates of mutants $\Delta Bcb1-2$ and $\Delta Bcb1-16$ were significantly reduced by 67.56% and 67.07% at 96 h, respectively (Fig. 1B). After incubation on PDA for 10 days, the conidia of wild-type and mutants on each plate were collected by double-distilled water and filtered. The conidia number was quantitatively compared between wild-type and mutant strains using a hemacytometer. A large number of spores could be observed for the wild-type strain, while no conidia could be observed with the mutants (Fig. 1C). Melanin formation of all strains on sterilized carrots was observed for 4 weeks, and the expression levels of melanin biosynthesis related

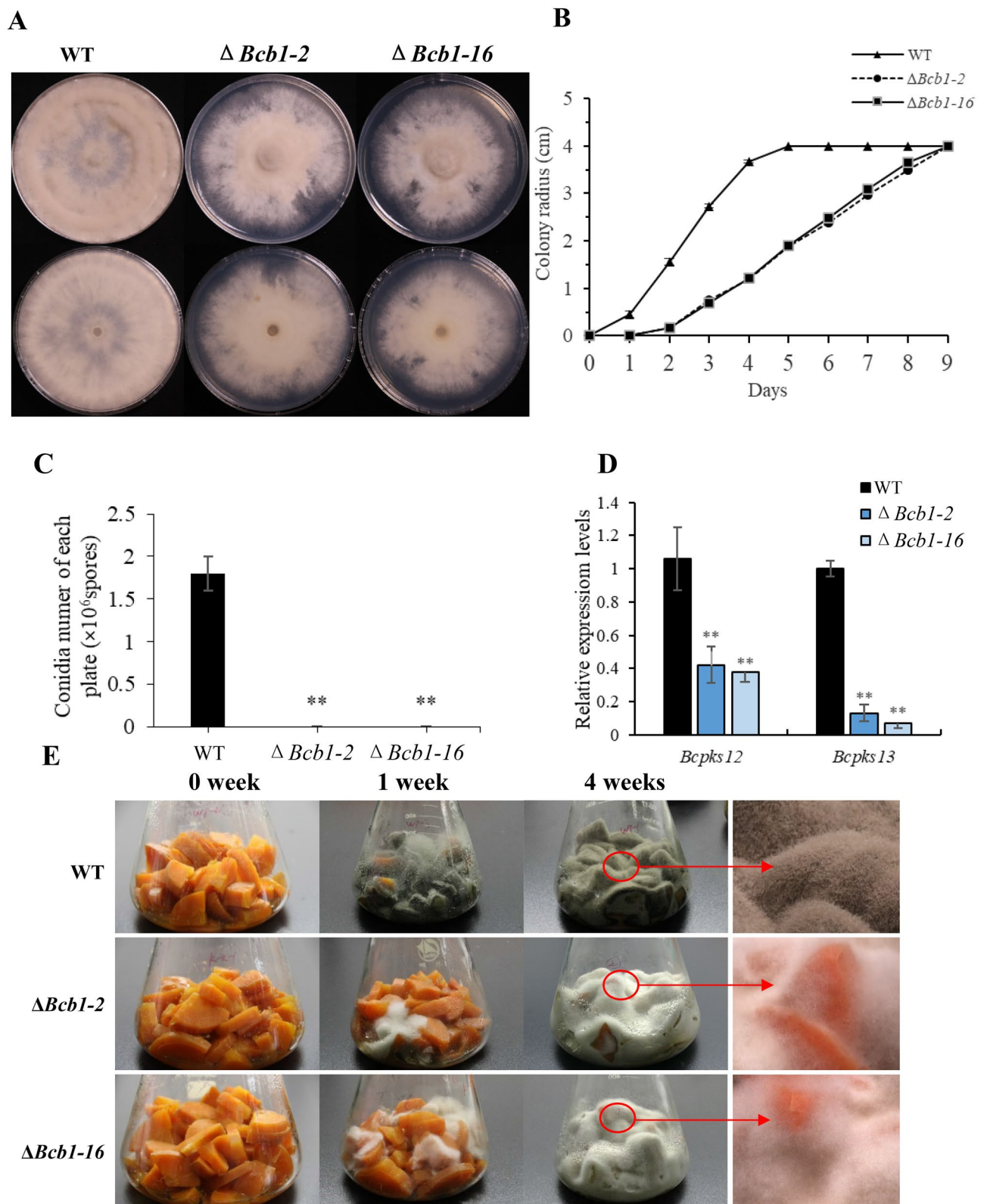


Fig. 1 *Bcb1* disruption resulted in abnormal colony morphology and a reduction in melanin and conidial formation. **A** Phenotypes of the WT strain and $\Delta Bcb1$ mutants after 10 days on PDA. **B** Radial growth rate of each strain. **C** Conidia number of the WT strain and $\Delta Bcb1$ mutants after 10 days on PDA. **D** Relative expression lev-

els of *Bcpks12* and *Bcpks13* in each strain after 10 days on carrots. Bars denote standard deviation from three experiments ($*p < 0.05$; $**p < 0.01$). **E** Processes of melanin accumulation in each strain on fresh carrots for 4 weeks.

genes in wild-type and mutants were measured after 10 days on carrots (Zhang et al. 2015; Schumacher et al. 2016). The qRT-PCR results showed that the expression levels of the melanin biosynthesis-related genes, *Bcpks12* and *Bcpks13* were significantly downregulated in $\Delta Bcb1$ mutants compared to the wild-type strain (Fig. 1D). After cultured on carrots for one week, the colonies of the WT strain began to be gray, while the colonies of $\Delta Bcb1-2$ and $\Delta Bcb1-16$ were white. Even four weeks after cultured on carrots, the colonies of two mutants remained white (Fig. 1E). These results indicate that *Bcb1* is essential for hyphal growth, conidia and melanin formation.

Bcb1 is required for infection cushions and conidial formation

B. cinerea produces multicellular appressoria dedicated to plant penetration, named infection cushions (ICs). ICs develop into a haptophores from which conidiophores differentiate (Backhouse and Willetts 1987). We compared the ability of the WT strain and $\Delta Bcb1$ mutants to form ICs and conidiation. After 3 days of incubation on PDA, visible ICs formed from the tip of the hyphae of the wild type. After 5 days, the ICs of the WT became larger and more complex and some of them developed into haptophores, while the $\Delta Bcb1-2$ and $\Delta Bcb1-16$ strains formed only small and incompletely developed ICs. (Fig. 2A). After 7 days, conidiophores differentiated from haptophores of the wild-type strain and produced large amounts of conidia, and the ICs of the mutants became larger and more complex; however, no conidium was produced. After 45 days, $\Delta Bcb1$ mutants could form few haptophores but still could not form conidiophores and produce conidia. The same result was observed on the inner epidermis of the onion (Fig. 2B). The above results suggest that the *Bcb1* gene is closely related to the development of ICs and conidia in *B. cinerea*.

Bcb1 contributes to the stress response in B. cinerea

Congo red binds to nascent chitin chains, inhibiting the assembly enzymes connecting chitin to 1,3-glucan and 1,6-glucan, resulting in a weakened cell wall (Bulawa 1993; Ram and Klis 2006). SDS acts as an antimicrobial membrane-active compound, that attacks cellular membranes and can therefore be used to determine the integrity of the cell membrane (Jiménez-Munguía et al. 2019). Four days after inoculation, the relative growth inhibition of $\Delta Bcb1$ mutants on PDA containing 0.05% Congo red and 0.05% SDS was significantly higher than that of the wild type ($p < 0.01$) (Fig. 3A, B). These two results suggest that *Bcb1* affects the cell wall and cell membrane integrity of the strain.

Osmotic stress was triggered by both 1.2 M NaCl and 1.2 M KCl. There were no significant growth differences between the WT strain and $\Delta Bcb1$ mutants on PDA supplemented with 1.2 M NaCl which caused extracellular osmotic stress. However, the relative inhibition of $\Delta Bcb1$ mutants by KCl-generated intracellular osmotic stress was lower than that of the WT strain ($p < 0.01$) (Fig. 3A, B). Additionally, $\Delta Bcb1-2$ and $\Delta Bcb1-16$ exhibited significantly increased sensitivity to oxidative stress generated by 0.08% H₂O₂. The relative growth inhibition of $\Delta Bcb1$ mutants on this medium was substantially higher than that of the WT strain ($p < 0.01$) (Fig. 3A, B). These results indicate that *Bcb1* affects the resistance of *B. cinerea* to intracellular osmotic stress and oxidative stress.

BCB1 is essential for the full pathogenicity of B. cinerea

Apparent disease lesions could be observed on the fruits and leaves inoculated with WT or mutant strains. However, lesions caused by the WT strain expanded much more rapidly (Fig. 4A, B). To explore whether there were other reasons for decreased pathogenicity in mutants addition the defects in vegetable growth and conidia production, at 6 days after inoculation on leaves, we examined the expression of genes associated with pathogenicity in *B. cinerea*, including two genes encoding the polygalacturonases *Bcpg1* and *Bcpg2* (Kars et al. 2005); a gene related to the production of botrydial, *BcBOT2* (Pinedo et al. 2008); and a gene encoding cutinase, *cutA* (Wang et al. 2013). The expression of all genes was downregulated, except for the *Bcpg1* gene. The upregulation of *Bcpg1* gene expression may be related to the functional complementarity between *Bcpg1* and *Bcpg2*. (Fig. 4C). All results indicated that the *Bcb1* gene plays a significant role in the pathogenicity of *B. cinerea*.

Discussion

In this study, we identified and functionally characterized the gene *Bcb1*, which encodes a membrane protein that contains an AMP-binding domain. BCB1 belongs to the ACSs, which play important roles in fatty acid activation and lipid-related metabolism processes.

Loss of *Bcb1* in *B. cinerea* affected the cell integrity. The *Bcb1*-mutants displayed decreased resistance to membrane (SDS) stress and cell wall (CR) stress and increased resistance to intracellular osmotic (KCl) stress compared to the wild-type strain. However, no differences in tolerance to extracellular osmotic (NaCl) stress were identified. The results were slightly different from the deletion of the ACS protein CPS1 in *M. oryzae*: *Cps1* mutants were inhibited

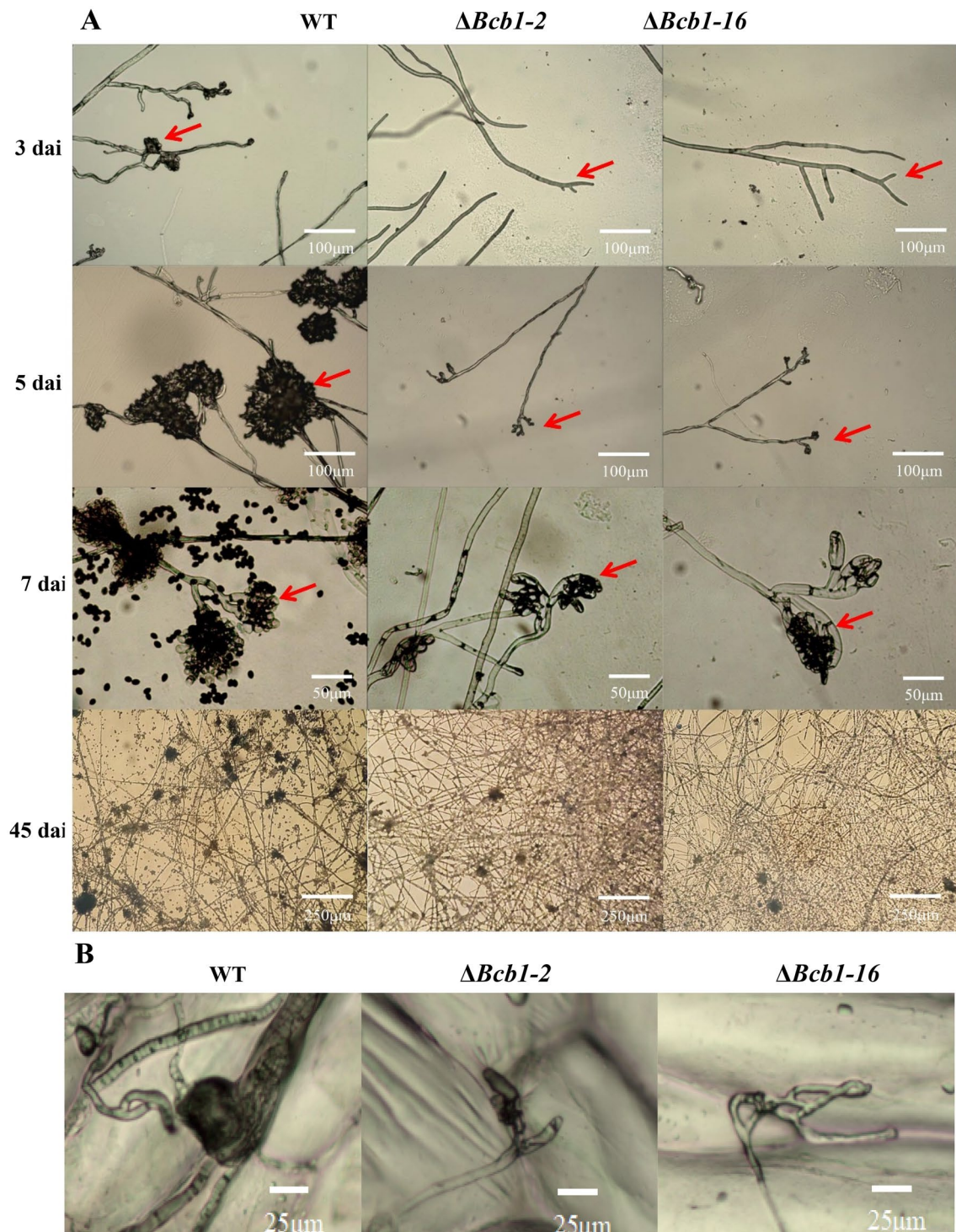


Fig. 2 ICs and conidia formation of each strain. **A** Formation of the ICs of each strain on microslides at 20 °C for one and a half months. **B** Formation of ICs on the inner onion epidermis

under extracellular osmotic (NaCl) stress but were unaffected by membrane (SDS) stress (Wang et al. 2016). In *B. bassiana*, the deletion of the ACS protein Faa1 also impaired cytomembrane integrity (Li et al. 2022).

Lacking *Bcb1* seriously delayed the growth and morphogenetic development of *B. cinerea*. $\Delta Bcb1$ -mutants could only form small and incompletely developed ICs and failed to produce spores. This result agrees with what

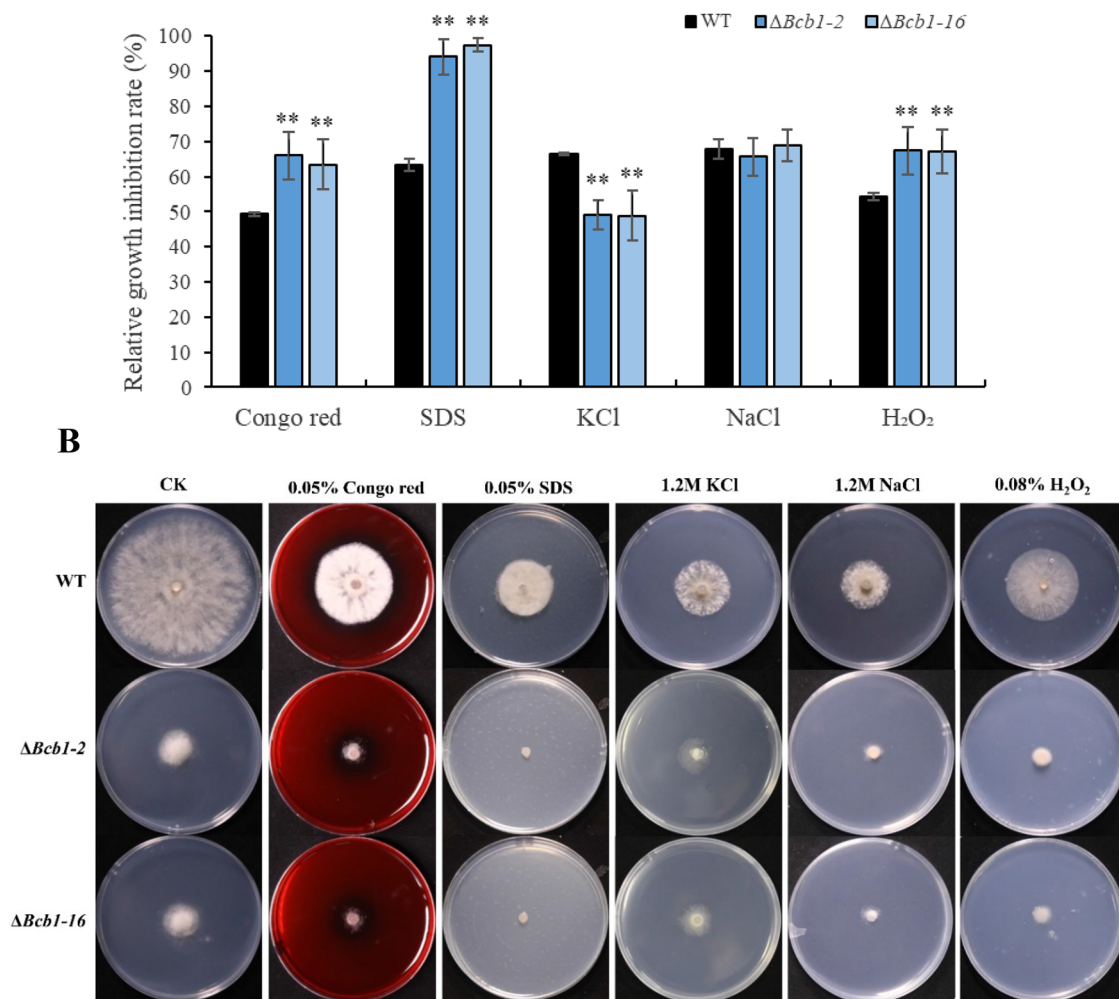


Fig. 3 Growth and relative growth inhibition of each strain under different abiotic stresses. **A** Relative growth inhibition rates of wild-type and mutants under different abiotic stresses. **B** Phenotypes of the

wild-type strain and $\Delta Bcb1$ mutants after 4 days on PDA subjected to different abiotic stresses. Bars denote the standard deviation from three experiments (* $p < 0.05$; ** $p < 0.01$)

has been observed with the mutants of *Cps1* genes encoding ACSs protein in *M. oryzae*. The deletion of MoCPS1 leads to a decrease in conidial numbers and impaired conidial morphology as well as infection hyphal development (Wang et al. 2016). At present, many genes related to sporulation have been studied in *B. cinerea*, such as two genes encoding hypothetical proteins, *BC1G_12707.1* (Wang et al. 2013) and *BcPDR1* (Zheng 2013); a gene encoding a subtilisin-like serine protease, *Bcser2* (Liu et al. 2020); the autophagy-related gene *BcATG14* (Yu 2017); the mitogen-activated protein kinase encoding gene *BcSAK1* (Segmüller et al. 2007); the putative transcriptional regulator encoding gene *Bcreg1* (Michielse et al. 2011), and the aquaporin encoding gene *AQP8* (An et al. 2016). In our study, *B. cinerea* lost sporulation capacity after the deletion of the *Bcb1* gene, which means that BCB1 may function upstream of these signal transduction

pathways. The relationship between BCB1 and the above genes needs further study.

The $\Delta Bcb1$ mutants presented as a white colony due to the reduction in pigment produced by the conidia and sclerotia. The main component of pigment was 1,8-dihydroxynaphthalene (DHN) melanin, which is an important part of the extracellular matrix. DHN imparts environmental tolerance to fungi, and is essential for the normal growth and pathogenicity of some filamentous fungi (Jia et al. 2021; Potisek et al. 2021). Although DHN does not seem necessary for the pathogenicity of *B. cinerea*, it is indispensable for *B. cinerea* to resist adverse external environments. In addition, this pigment may play a role in forming its asexual reproductive structure (Chen et al. 2021). The production of DHN in *B. cinerea* requires two key polyketide synthases, BcPKS12 and BcPKS13 (Schumacher 2016), which are closely related to melanin formation in the sclerotia and

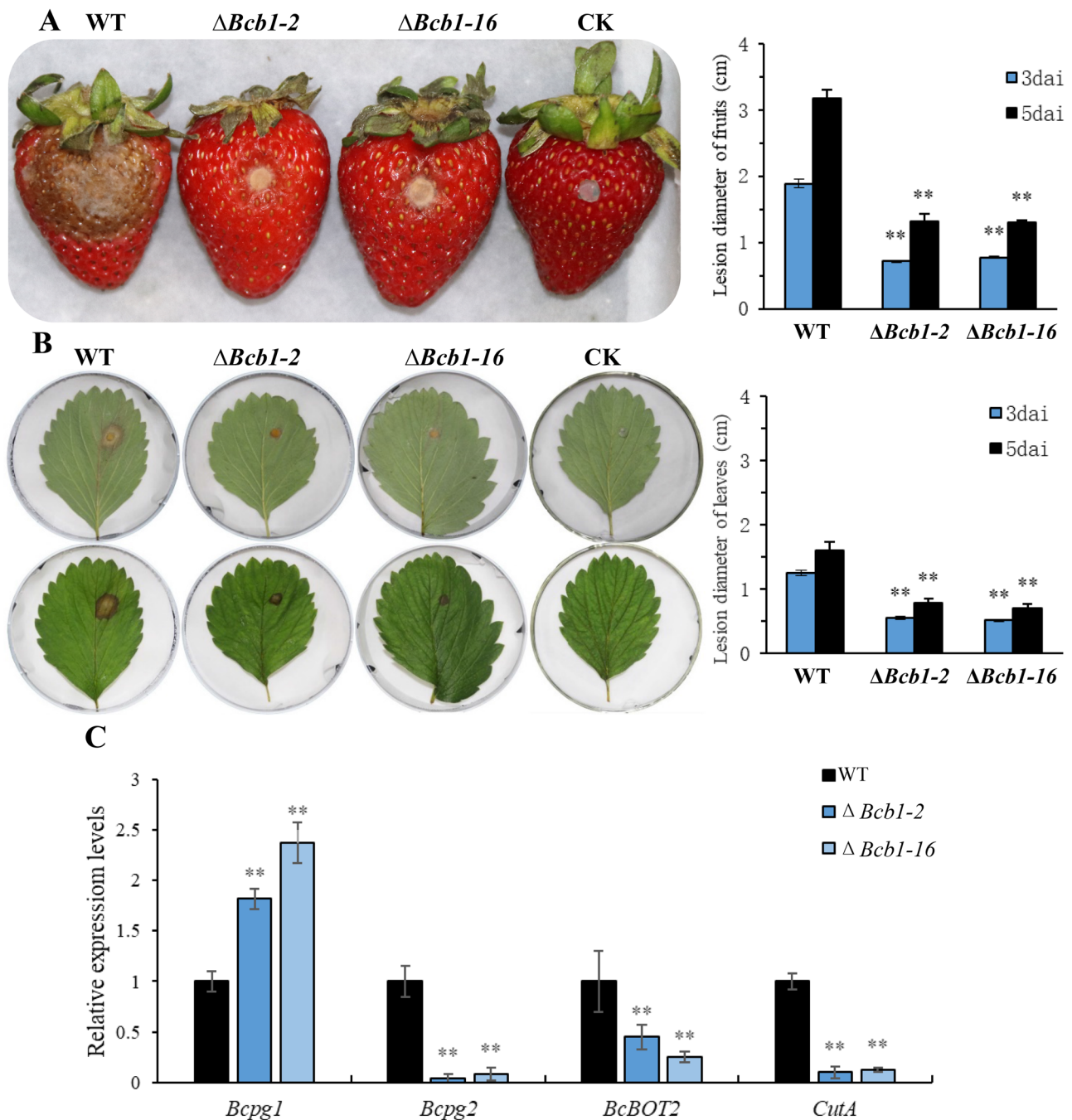


Fig. 4 Knockout of the *Bcb1* gene affects the pathogenicity of *B. cinerea*. **A** Disease symptoms of fruits 5 days after inoculation with a sterile PDA plug as CK and the statistical analysis of disease lesions on intact strawberry fruit 3 days or 5 days after inoculation. **B** Disease symptoms of leaves with a sterile PDA plug as CK and statisti-

cal analysis of disease lesions on intact strawberry leaves 3 days or 5 days after inoculation. **C** Expression levels of selected genes associated with pathogenicity in each strain. Bars denote standard deviation from three experiments (“*” means $p < 0.05$; “**” means $p < 0.01$)

conidia, respectively. In our study, after the deletion of the *Bcb1* gene, the expression of genes encoding these two enzymes was downregulated, especially *Bcpgs13*, which means that BCB1 functions upstream of these signal transduction pathways.

Compared to the WT, the virulence of $\Delta Bcb1$ -mutants decreased dramatically. The affected virulence caused by the deletion of genes encoding ACS proteins has also been reported in the pathogens *C. heterostrophus*, *M. oryzae* and *B. bassiana* (Lu et al. 2003; Wang et al. 2016; Li et al. 2022).

ICs are a special infection structure that is necessary for successful infection of mycelia. ICs of *B. cinerea* also secrete many phytotoxins, ROS, hydrolases, and plant cell death-inducing proteins that facilitate colonization (Choquer et al. 2021). During the infection cycle of *B. cinerea*, maintaining large numbers of conidia is a crucial part of successful primary and secondary infection (Carisse et al. 2018). Spore density was believed to play a major role during the first plant–fungus encounter and to determine whether *B. cinerea* could inhibit the plant's immune response to successfully invade the plant and cause gray mold (Veloso and Kan 2018). The decrease in virulence in the $\Delta Bcb1$ -mutant is most likely due to its IC malformation and failure to sporulate.

In addition to affecting the virulence of *B. cinerea* by affecting the production of ICs and conidia, BCB1 affects the expression of genes directly related to pathogenicity. Polygalacturonase plays a vital role in the infection process of plant pathogens. There are six polygalacturonases (BCPG1–BCPG6) in *B. cinerea*, among which BCPG2 plays a key role in its infection process (Have et al. 1998; Kars et al. 2005). Botrydial is produced by *B. cinerea* during plant infection and induces chlorosis and cell collapse. The BcBOT2 gene encodes a putative sesquiterpene cyclase that is responsible for the committed step of botrydial biosynthesis. *Botrytis cinerea* can infect undamaged plant tissue directly by penetration of the cuticle. Cutinase plays an important role in the infection process (van Kan et al. 1997). The expression levels of *Bcpg2*, *BcBOT2*, and the cutinase-encoding gene *cutA* were significantly downregulated in $\Delta Bcb1$ mutants. In addition, BCB1 is involved in the resistance of *B. cinerea* to H_2O_2 which is produced by plants at the invasion site of pathogens and can kill pathogens or inhibit the growth of pathogens (Wang et al. 2022).

Fatty acids synthesized by plants can be transported to pathogenic fungi and are required for pathogen colonization and successful host infection (Jiang et al. 2017). ACSs are critical enzymes that activate fatty acids before they can be utilized for subsequent metabolism. The ACS protein BCB1 is specific to *B. cinerea* and plays vital roles in the development and pathogenicity of *B. cinerea* and this result suggested that BCB1 has the potential to become a target for developing fungicides for *B. cinerea*.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00203-023-03540-w>.

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Author contributions Jiling Xiao and Ke Yang wrote the main manuscript text and Zhihui Liang performed the data analysis. Yi

Zhang and Lin Wei prepared all the figures. All authors reviewed the manuscript.

Declarations

Conflict of interest The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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