The following article appeared in *Applied and Environmental Microbiology, February 2017 vol. 83 no. 3 e02922-16;* and may be found at http://doi.org/10.1128/AEM.02922-16

The histone deacetylase HDA-2 regulates growth, conidiation, blue light perception and oxidative stress responses in *Trichoderma atroviride*

Running title: HDA-2 regulates light perception and oxidative stress

Byline: The chromatin modifications and gene regulation group

Macario Osorio-Concepción[1], Gema Rosa Cristóbal Mondragón[1], Braulio Gutiérrez-Medina[2] and Sergio Casas-Flores[1#]

IPICYT, División de Biología Molecular. Camino a la presa San José No. 2055, Colonia Lomas 4a sección. C.P. 78216. San Luis Potosí, Mexico[1].

IPICYT, División de Materiales Avanzados. Camino a la presa San José No. 2055, Colonia Lomas 4a sección. C.P. 78216. San Luis Potosí, Mexico[2].

Correspondence

Sergio Casas-Flores

IPICYT, División de Biología Molecular. Camino a la presa San José No. 2055, Colonia Lomas 4a sección. C.P. 78216. San Luis Potosí, Mexico.

Phone: +52 (444) 8342046; Fax: +52 (444) 8342010.

ABSTRACT

Fungal blue-light photoreceptors have been proposed as integrators of light and oxidative stress. However, additional elements participating in the integrative pathway remain to be identified. In *Trichoderma atroviride*, the blue-light receptor proteins BLR-1 and -2 are known to regulate gene transcription, mycelial growth and asexual development upon illumination, and recent global transcriptional analysis revealed that the histone deacetylase encoding gene *hda-2* is induced by light. Here, by assessing responses to stimuli in wild-type and Δ*hda-2* backgrounds, we evaluate the role of HDA-2 in the regulation of genes responsive to light and oxidative stress. Δ*hda-2* strains present reduced growth, misregulation of the *con-1* gene and absence of conidia in response to light and mechanical injury. We found that the expression of *hda-2* is BLR-1 dependent and HDA-2 in turn is essential for the transcription of early and late light-responsive genes that include *blr-1*, indicating a regulatory feedback loop. When subject to reactive oxygen species (ROS), Δ*hda-2* mutants display high sensitivity whereas Δ*blr* strains exhibit the opposite phenotype. Consistently, in the presence of ROS, ROS-related genes show high transcription levels in wild-type and Δ*blr* strains but misregulation in Δ*hda-2*. Finally, chromatin immunoprecipitations of histone H3 acetylated at Lys9/Lys14 on *cat-3* and *gst-1* promoters display low accumulation of H3K9K14ac in Δ*blr* and Δ*hda-2* strains, suggesting indirect regulation of ROS-related genes by HDA-2. Our results point to a mutual dependence between HDA-2 and BLR and reveal the role of these proteins in an intricate gene-regulation landscape in response to blue light and ROS.

IMPORTANCE

Trichoderma atroviride is a free-living fungus commonly found in soil or colonizing plant roots, and is widely used as an agent in biocontrol as it parasites other fungi, stimulates plant growth, and induces the plant defense system. To survive in various environments, fungi constantly sense and respond to potentially threatening external factors, such as light. In particular, UV light can damage biomolecules

by producing free-radical reactions, in most cases involving reactive oxygen species (ROS). In *T. atroviride*, conidiation is essential for its survival, which is induced by light and mechanical injury. Notably, conidia are typically used as the inoculum in the field during biocontrol. Therefore, understanding the linkages between responses to light and exposure to ROS in *T. atroviride* is of major basic and practical relevance. Here, the histone deacetylase encoding gene *hda-2* is induced by light and ROS, and its product regulates growth, conidiation, blue-light perception and oxidative stress responses.

***Keywords*:** *Trichoderma*, blue light, oxidative stress, BLR proteins, histone acetylation, histone deacetylases, HDA-2, Hos2p.

INTRODUCTION

Light is one of the most important environmental cues that exert decisive effects on the physiology and behavior of organisms, and fungi are not an exception. In fungi, light controls sexual and asexual development, phototropism, circadian rhythms, and synthesis of pigments among other processes (1–3). One excellent system to study photo-induced responses is *Trichoderma*. The *Trichoderma* genus comprises a number of saprophytic filamentous fungi, which are used in biotechnological applications and biological control of plant-pathogenic fungi and oomycetes (4–6). For decades, these fungi have been used as research models to study intra and extracellular signals that influence their biology. *Trichoderma atroviride* is characterized by the production of conidia in response to environmental stimuli, including light, mycelial injury, carbon, nitrogen, and phosphate deprivation (7–12). Exposure of a dark-grown colony of *T. atroviride* to a brief pulse of blue light results in the formation of a ring of dark green conidia at what had been the perimeter of the colony at the time of illumination (7, 8).

In *T. atroviride*, the Blue Light Regulator-1 (BLR-1) and -2 (BLR-2) proteins, orthologous to White Collar-1 (WC-1) and WC-2 in *Neurospora crassa*, regulate most responses to light (9, 11). Both BLR proteins contain PER-ARNT-SIM (PAS) domains involved in protein-protein interactions, and GATA-type Zn-finger DNA-binding domains. In addition, BLR-1 bears a specialized PAS domain denominated LOV (light, oxygen, and voltage), which harbors a flavin as chromophore. These proteins associate through their PAS domains, forming the BLR complex (BLRC) (9, 11). Strains lacking the *blr-1* or *blr-2* genes show altered phenotypes in photoconidiation, mycelial growth, and gene transcription (9). To account for these facts, BLR-1 has been proposed to act as a blue-light photoreceptor, with an added function as a transcription factor together with BLR-2 (9, 13). Interestingly, *Δblr-1* and *Δblr-2* strains do not produce conidia in response to carbon deprivation in the dark, suggesting a role of the BLR proteins in redox sensing (9). This additional function for the BLR proteins is supported by the presence of PAS and LOV domains, known to act as input modules in proteins that sense oxygen, redox potential, and light, among other stimuli (14).

Organisms produce reactive oxygen species (ROS) as by-products of their metabolism, primarily by aerobic respiration. In cells, ROS are produced by molecular oxygen (O_2) excitation, partial reduction, and the formation of radicals or peroxides with other compounds (15). To counteract ROS-damaging effects, eukaryotic microorganisms have developed sophisticated mechanisms, including the synthesis of antioxidant enzymes [such as superoxide dismutases (SOD), catalases (CAT), and peroxidases] and processes that provide electrochemical reducing power, such as the pentose phosphate pathway and the thioredoxin and glutathione redox systems (15). These observations highlight the importance of ROS in fungal biology (16–19).

The search to identify molecular regulators of transcription in the presence of external stimuli such as light and oxidative stress needs to consider the route that leads to chromatin modifications. In eukaryotic cells, DNA is wrapped around a core of histone proteins, thus limiting transcriptional

activity, where histone tails are subject to posttranslational modifications that contribute to the fine-tuning of gene expression (20). Histone acetylation is one of these modifications, involving a dynamic process controlled by the antagonistic roles of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Deacetylation of histone tails is accomplished by HDACs, and leads to transcriptional inactivation. Consequently, HDACs are frequently found as corepressors in large multiprotein complexes (21). Classical HDACs include class I and II, which share high similarity to yeast Rpd3 (reduced potassium dependency 3) and Hda1 (histone deacetylase 1), respectively. Classical HDACs class IV comprises human HDAC11 and HDA2 of *Arabidopsis thaliana*, absent in fungi (22). The class III HDACs comprises Sirtuins, which are NAD^+-dependent SIR2-type proteins (23). In fungi, transcription of genes encoding for pathogenicity factors, stress response, and secondary metabolism have been related to histone acetylation and deacetylation (24–27). *T. atroviride* contains the classical HDACs class I, II and III (28). A connection between light responses and histone acetylation in filamentous fungi has been demonstrated in *N. crassa*, where both WC-1 and NGF-1 (the orthologous to Gcn5p) physically interact to promote light-induced acetylation of residue K14 of histone H3 associated to the promoter of the *al*-3 gene (29, 30). Recently, it was demonstrated that light-activation of WC-1 and -2 is related to nucleosome removal at their target promoters. Furthermore, *sub1* (a WC-1 and -2 dependent gene that codes for a GATA type transcription factor) is necessary for efficient remodeling of certain nucleosomes (31).

In this work, a global transcriptional analysis revealed that the *hda-2* gene (**JGI ID: 212638**), orthologous to *hda-2* and *HOS2* of *N. crassa* and *Saccharomyces cerevisiae* that encode a classical class I HDAC, was 6-fold induced in response to blue light *in T. atroviride*. This key observation suggests a possible route where responses to light and oxidative stress may be coupled, orchestrated by the BLR and HDA-2 proteins. We investigate the role of HDA-2 in development, blue light and oxidative stress responses of *T. atroviride*, and the relationship with the BLR proteins in these

processes. We show that *hda-2* is transcriptionally activated by light and ROS and that its product is essential for asexual development, mycelial growth, transcription of early and late responsive genes to light, resistance to oxidative stress, and expression and acetylation on the promoters of ROS-related genes. Furthermore, we report that the expression of *hda-2* is BLR-1 dependent, whereas HDA-2 results essential for the transcription of *blr*-1.

MATERIALS AND METHODS

Biological material and growth conditions. *T. atrovirde* IMI 206040 wild-type and Δ*blr-1* and Δ*blr-2* strains (9) were used throughout this study. *T. atroviride* cultures were routinely grown at 28 ºC on PDA plates (potato dextrose agar, Difco) or PDB (potato dextrose broth, Difco); hygromycin B was added at 200 μg/ml when necessary. Experiments to test the effect of pro-oxidants were performed on Vogel's Minimal Medium (VMM) and VMM agar (32). *Escherichia coli* TOP10 F' was used for DNA transformation and grown in Luria-Bertani (LB) broth or on LB agar plates. Carbenicillin (100 μg/ml) was added to LB when necessary (33). The plasmids used were pBHY70, which harbors the hygromycin B phosphotransferase gene under the control of the *Aspergillus nidulans* trpC promoter (34, 35), and pGEMT-easy (Promega).

Generation of the *hda-2* deletion construct. For DNA extraction, *T. atroviride* was grown for 48 h at 28 ºC and the mycelium was scraped and immediately frozen in liquid nitrogen. Total DNA from *T. atroviride* was extracted as described (36). The *hda-2* gene deletion construct was generated as follow: fragments ~1.5 kilobases (kb) long corresponding to each 5'- and 3'-flanking regions for the *hda-2* open reading frame (ORF) were PCR amplified using genomic DNA of *T. atroviride* as template and specific primers bearing restriction enzymes sites (Table S1). *hda-2* 5'- and 3'-flanking regions were

141 cloned in their corresponding restrictions sites into the pBHY70 plasmid, which harbors the *hph* gene
142 into the Eco RV restriction site (35). The *hda-2* deletion construct was PCR amplified using pΔhda-2
143 plasmid as template with hda-2-KpnI-Fw and hda-2-XbaI-Rv primers (Table S1) and used to transform
144 *T. atroviride* protoplasts.

145

146 **Generation of *T. atroviride* protoplasts.** Protoplast generation was performed according to the
147 protocol described in (37) with some modifications: 1×10^6 conidia/ml were inoculated in 100 ml
148 PDYCB medium (24 g/l potato dextrose broth DIFCO^{TM}, 2 g/l yeast extract, 1.2 g/l casamino acids)
149 and incubated for 48 h at 28 °C under shaking at 250 rpm. Mycelium was collected by filtration,
150 washed with water, and 0.5 g was resuspended in osmoticum (50 mM CaCl2, 0.5 M mannitol, 50 mM
151 MES, pH 5.5) with 15 mg/ml lytic enzymes of *Trichoderma harzianum* (Sigma, L1412). The mixture
152 was incubated at 28 °C under gentle shaking for 5 hours. For protoplast regeneration, a selective
153 medium (potato dextrose broth DIFCO^{TM}, 0.8% Agarose (Nara Biotec), and 0.5 M sucrose) containing
154 50 µg/mL hygromycin was used.

155

156 **Genetic transformation of *T. atroviride.*** Protoplasts of *T. atroviride* were transformed with the
157 deletion construct, as described elsewhere (37)**.** Stable transformants were selected by three
158 consecutive transfers of a single colony to PDA medium supplemented with 200 µg/ml hygromycin.
159 For the screening of gene-replacement events, total DNA from hygromycin-resistant colonies was
160 subjected to PCR using primers hda-2-out-Fw and hda-2-out-Rv corresponding to sequences up and
161 down stream of the 5' and 3' regions, respectively, flanking the *hda-2* ORF and that were not present in
162 the construct used to transform *T. atroviride*. For the screening of gene-replacement events, as well as
163 to test for ectopic insertions of *hph* cassette in the *T. atroviride* genome, DNA from the hygromycin-

resistant colonies was subjected to qPCR using specific primers for both genes (Table S1). The copy number of *hph* and *hda-2* in the genome of the transformants was calculated using the $2^{-\Delta\Delta Ct}$ method (38). DNA from the Δ*blr-1* strain was used as a calibrator, since Southern blot analysis has shown that it harbors one copy of the *hph* gene (9).

Photoinduction assays. General growth conditions and manipulations have been described previously (39). Briefly, pre-inoculum of *T. atroviride* was obtained by growing the fungal strains on PDA plates in the dark at 28 ºC for 48 h. Mycelial plugs were inoculated on the center of VMM agar plates layered with a sterile cellophane sheet. For total RNA extraction, colonies were allowed to grow for 48 h in the dark, and exposed to a 1200 µmol m^{-2} pulse of blue light (LEE filter no. 183, fluence rate 5 µmol m^{-2} s^{-1} or 88,800 lux), placed back in the dark at 28 ºC, and collected at the indicated times after the exposure to blue light. Mycelia were collected under red safelight (LEE filter no. 106, fluence rate 0.1 µmol m^{-2} s^{-1}) and immediately frozen in liquid nitrogen for further total RNA extraction. Mycelia grown in darkness were included as control. For photoconidiation assays, the colonies were grown as described above for photoinduction assays, exposed to a blue-light pulse, and incubated 48 h in the dark and photographed. Colonies not exposed to blue light were included as control.

Oxidative stress response assays. Resistance to oxidative stress from different concentrations (0.0 16, 30, and 60 mM) of hydrogen peroxide or menadione (0.0, 0.05, 0.1, 0.2, and 0.3 mM) was determined by measuring the colony radial growth every 24 h and up to 96 h after inoculation on VMM. The autoclaved VMM medium was cooled to 45 ºC before adding the oxidative stress-generating agents. *Trichoderma* cultures were incubated at 28 °C for 96 hours in the dark or subject to 12 h dark/light cycles. Radial growth inhibition percentage was calculated as follows: growth inhibition (%):

[(Control-Treated)/(Control)]×100. Hydrogen peroxide (H_2O_2) 50 wt-(516813) and menadione-(47775) were purchased from Sigma-Aldrich. For gene expression analysis, plugs of actively growing colonies were inoculated on VMM and incubated at 28 °C for 96 h in the dark under agitation at 250 rpm. Mycelium was exposed to 30 mM H_2O_2 or 0.2 mM menadione, and collected 15 and 30 min post treatment. Mycelium grown in medium without the pro-oxidant was included as a control.

RNA extraction and complementary DNA synthesis. Total RNA extraction was performed by the Trizol® method as described by the vendor (Invitrogen). RNA quality was assessed by spectrophotometric methods using an Epoch™ Microplate Spectrophotometer (Bio-Tek instruments), and formaldehyde-agarose gel electrophoresis, taking into account the 28S/18S rRNA ratio. Total RNA (5 µg) was DNase I (RNase-free) (Ambion) treated, followed by cDNA synthesis using SuperScript II Reverse Transcriptase (Invitrogen), following the manufacturer's recommendations. cDNA was used as a template for quantitative real-time PCR reactions with gene-specific primers (Table S1) and the Fast SYBR Green Master Mix (Applied Biosystems) according to the manufacturer's recommendations.

Real Time Quantitative Reverse Transcription-PCR (RT-qPCR). Primers used for RT-qPCR were designed using Primer Express Software version 3.0 (Applied Biosystems). The reaction mixtures were: 10 µL SYBR Green Master Mix (Applied Biosystem), 3 µL cDNA template (100 ng) and 0.3 µL of gene-specific primers (150 nM). The qPCR program consisted of: one cycle at 95 °C for 5 minutes, 40 cycles at (95 °C for 30 s, 65 °C for 30 s, and 72 °C for 40 s). Relative expression was normalized against the level of *tef-1* gene whose mRNA is not affected by light (40) using the $2^{-\Delta\Delta Ct}$ method (41).

Chromatin immunoprecipitations (ChIP) assays. ChIP assays were performed using the Imprint®Chromatin Immunoprecipitation kit (Sigma-Aldrich). Treatment of mycelia with oxidizing agents was performed as described above. For cross-linking chromatin, 40 mg of mycelia per 1 ml of cross-link solution containing 1% formaldehyde were used. The solution was incubated at room temperature on a rocking platform for 15 min and neutralized by adding 1.25 M glycine for 5 min. Crosslinked chromatin was immunoprecipitated using anti-H3 antibody (Abcam, AB1791), anti-H3-Ac (Millipore, 06-599), and 5% of the used chromatin for immunoprecipitation was saved as input fraction. The immunoprecipitated chromatin was analyzed by qPCR using specific primers (Table S1) on the promoter region of *cat-3* and *gst-1* genes. ChIP-qPCR data was analyzed with the $2^{-\Delta\Delta Ct}$ method (41).

Statistical analysis

Results of resistance to oxidative stress were validated with a multiple comparison Tukey's range test ($\alpha = 0.05$), with analysis of variance statistical analysis (Post-hoc analysis) using the SPSS software package (version NCSS 2007).

RESULTS

The *hda-2* gene, encoding a classical class I HDAC, is rapidly induced by light in *Trichoderma atroviride*

The nucleotide sequence of *hda-2* open reading frame (**JGI ID: 212638**) is 1491 bp (base pairs) in length, and codes for a 496 amino acid protein. The predicted amino acid sequence for HDA-2 shows the presence of a histone deacetylase domain and a Zn-binding site, similar to those described in the classical class I HDACs, HDA-2 and Hos2p of *N. crassa* and *S. cerevisiae*, respectively (Fig. S1).

Sequence comparison of HDA-2 with several hypothetical orthologous in the *Trichoderma* genus revealed high degrees of identity, ranging from 90% to 98% (Fig. S1 and S2). The HDA-2 sequences of other Ascomycota including phytopathogenic and entomopathogenic fungi also showed high degrees of identity (~86-77%; Fig. S1 and S2).

A global transcriptional analysis in *T. atroviride* revealed that the *hda-2* was 6-fold induced in response to blue light. To verify such result, we assessed the expression profile of *hda-2* in response to light by RT-qPCR in the wild-type (wt). The *hda-2* gene was 5.5-fold induced 5 min after a blue light stimulus, and after 30 min it decreased to 4-fold (Fig. 1a). This behavior contrasts with that of the class I CPD photolyase encoding gene *phr-1*, the classical marker gene for responses to light in *T. atroviride*, that reaches its highest accumulation 30 min after the light pulse (Fig. 1d), as reported before (13, 39).

Oxidative stress regulates transcription of *hda-2* and *phr-1* in *T. atroviride*

Biological molecules such as flavins, pterins, and porphyrins can absorb blue light, and subsequently transfer energy to molecular oxygen (O_2) in the cell, triggering the production of ROS species like $^1O_2$ and O_2^- (2). To explore a possible link between light and ROS in *T. atroviride*, we tested whether oxidative stress induces the expression of *hda-2* and *phr-1*. Mycelium of *T. atroviride* (wt) was exposed in the dark to two types of ROS-generating sources: hydrogen peroxide (H_2O_2) and menadione. In 30 mM H_2O_2, 30 min after the application of the stimulus, *hda-2* and *phr-1* increased by 1.8- and 4.0-fold, respectively, compared to the control (Fig. 1b and e). In 0.2 mM menadione, *hda-2* was induced 3-fold 30 min after the stimulus (Fig. 1c), whereas *phr-1* levels remained unaltered (Fig. 1f).

The *hda-2* gene is required for proper growth and asexual reproduction in *T. atroviride*

255 To investigate whether the product of *hda-2* is involved in the response to environmental cues such as
256 light and oxidative stress, Δ*hda-2* mutants were generated by double homologous recombination.
257 Several attempts to complement the mutation by introducing the wt gene into a Δ*hda-2* strain failed.
258 Therefore, three independent hygromycin-resistant colonies (Δ*hda-2-1*, Δ*hda-2-2*, and Δ*hda-2-3*) were
259 selected for further experiments. Genomic DNA from Δ*hda-2-1*, Δ*hda-2-2*, and Δ*hda-2-3* and the wt
260 strain was used in qPCR to corroborate the *hda-2* gene replacement as well as to search for ectopic
261 insertions. *hda-2* was detected in the wt but not in Δ*hda-2* mutants (Table 1a). The *hph* cassette was
262 detected in a single copy in Δ*hda-2* mutants, and it was not detected in the wt (Table 1b). DNA from
263 the Δ*blr-1* strain was used to calibrate measurements as southern analysis has shown that it harbors one
264 copy of the *hph* gene (9). The three independent Δ*hda-2* mutants showed slow growth (Fig. 2a and b)
265 and absence of conidia in the dark or when exposed to a blue light pulse (Fig. 2a). When the mycelia
266 corresponding to all three Δ*hda-2* strains were mechanically injured, colonies failed to produce conidia
267 (Fig. 2a). The *con-1* gene (**JGI ID: 237745**) from *T. atroviride* is expressed 12 h after exposure to blue
268 light, which coincides with early stages of conidiophore development (42). Therefore, the expression of
269 *con-1* in response to blue light was analyzed by RT-qPCR in wt and Δ*hda-2* strains. As expected, upon
270 12 h after the application of the stimulus *con-1* transcript levels increased 3-fold in the wt (Fig. 2c). In
271 contrast, all three Δ*hda-2* strains showed low accumulation of *con-1* in the dark and a minimal increase
272 after blue light exposure (Fig. 2c).

273

274 **The expression of blue light responsive genes is dependent on HDA-2**

275 To investigate whether the observed upregulation of *hda-2* in response to light depends on BLR-1,
276 expression analysis of *hda-2* was assessed in the wt and Δ*blr*-1 backgrounds under different light
277 conditions. Indeed, the expression of *hda-2* after a light pulse was found to be BLR-1 dependent (Fig.
278 3a). Taking into account that *hda-2* encodes a putative histone deacetylase and that its transcript is

rapidly induced by light (5 min), we reasoned that HDA-2 could play a role in light perception. Transcript levels of *blr-1* were measured in a Δ*hda-2* background, showing that, contrary to wt, *blr-1* was not induced within 30 min after exposure to a blue light pulse, and its basal levels were lower than its parental strain at all conditions (Fig. 3d). These results show mutual dependence of HDA-2 and BLR-1 at the transcription level, and prompted us to investigate if the expression of other blue light responsive genes was affected in Δ*hda-2* as it occurs in a Δ*blr-1* mutant (9, 13). Expression levels of three well-known blue light upregulated (*blu*) genes were assessed: *phr-1* (Fig. 3b), *grg-2* (Fig. 3e) and *env1* (Fig. 3c) were found to be downregulated in Δ*hda-2*, although the mutant still displays blue-light induction in the case of *phr-1* and *grg-2*. Additionally, *bld-2*, a blue light downregulated (*bld*) gene, was found to be upregulated in the mutant compared to the wt, resembling the Δ*blr-1* phenotype, but showed downregulation upon exposure to blue light as in wt (Fig. 3f).

The *Δhda-2, Δblr-1 and Δblr-2* strains respond differentially to H_2O_2 and menadione

Taking into account that *hda-2* was induced by light and oxidative stress, we decided to evaluate its role to contend against different sources of oxidative stress, as well as a possible role of the *blr-1* and *-2* products in response to such stimuli. The wt, Δ*blr-1*, Δ*blr-2*, and Δ*hda-2* strains were grown in VMM agar amended with 16, 30, 60 mM H_2O_2 or 0.05, 0.1, 0.2, 0.3 mM menadione, in the absence or presence of light. In the dark, Δ*blr*'s and wt showed no remarkable differences in growth inhibition to different concentrations of H_2O_2 (the largest difference was ~3%) (Fig. 4a and b). In contrast, all three Δ*hda-2* mutants were considerably more sensitive to all tested H_2O_2 concentrations, in some conditions reaching 30% less growth compared to the wt and Δ*blr*'s (Fig. 4a and b). In the presence of light, addition of 16 and 60 mM did not show statistically differences in growth between wt and Δ*blr*'s, whereas applications of 30 mM H_2O_2 diminished the growth of Δ*blr-1* and Δ*blr-2* by ~8% as compared to the wt, showing statistical differences (Fig. 4c and d). In contrast, the growth of the Δ*hda-2* mutants

303 was remarkably affected in the presence of both light and H_2O_2 as compared to wt and Δ*blr*'s (Fig. 4c
304 and d).
305 Addition of different concentrations of menadione to the growth medium under dark conditions
306 negatively affected the growth at different levels of the tested strains (Fig. 5a and b). For instance, the
307 Δ*blr-1* and Δ*blr-2* strains were more resistant than wt in 0.05 and 0.1 mM menadione (Fig. 5a and b).
308 However, addition of 0.2 and 0.3 mM menadione affected all Δ*blr-1*, Δ*blr-2* and wt strains at similar
309 rates (Fig. 5a and b). As shown in Fig. 5a and 5b, growth of the Δ*hda-2* mutants was severely affected
310 by all tested menadione concentrations, compared to the wt and Δ*blr*'s. In presence of light, different
311 concentrations of menadione gradually inhibited the growth of the different strains (Fig. 5c and d).
312 Growth was noticeably less affected in Δ*blr-1* and Δ*blr-2* compared to wt at 0.05 and 0.1 mM
313 menadione; however, all three strains were similarly affected in growth at 0.2 and 0.3 mM (Fig. 5c and
314 d). Contrastingly, the Δ*hda-2* strains were considerably affected at all tested conditions compared to the
315 wt and Δ*blr*'s strains (Fig. 5c and d). Interestingly, in medium amended with 0.1 and 0.2 mM
316 menadione Δ*hda-2* mutants grew much better under illumination compared to dark conditions (Fig. 5a-
317 d).
318
319 **Expression of *hda-2*, *blr-1 and blr-2* genes in response to oxidative stress**
320 To evaluate the mutual dependence between HDA-1 and the BLRs at the transcriptional level in
321 response to H_2O_2 and menadione, the expression levels of the *hda-2* and *blr*'s genes were analyzed in
322 Δ*blr-1* and Δ*blr-2*, and Δ*hda-2* backgrounds, respectively. Exposure of wt to 30 mM H_2O_2 caused a
323 0.8-fold decrease of *blr-1* after 15 min; however, transcript amount returned to the basal level after 30
324 min (Fig. 6a). In Δ*hda-2*, H_2O_2 provoked significant accumulation of *blr-1* transcript 30 min after
325 application (Fig. 6a). In wt, transcription of *blr-2* was downregulated after 15 and 30 min of exposure
326 to 30 mM H_2O_2 (Fig. 6b); nevertheless, Δ*hda-2* presented low accumulation of *blr-2* under non-
327 stressful conditions and did not show significant changes after the addition of H_2O_2 (Fig. 6b). As

328 shown in Fig. 6c, upon 15 and 30 min of H_2O_2 application, *hda-2* accumulated at higher levels in Δ*blr-*
329 *1*, followed by Δ*blr-2* and wt (Fig. 6c).
330 Menadione at 0.2 mM caused *blr-1* accumulation in wt after 30 min of application, whereas in Δ*hda-2*
331 the transcript levels of *blr-1* did not change significantly (Fig. 6d). Exposure of wt and Δ*hda-2* to
332 menadione provoked a downregulation of *blr-2* in both strains, being more drastic in the latter (Fig.
333 6e). Intriguingly, in Δ*hda-2*, *blr-2* basal expression levels were lower compared to wt (Fig. 6b and e).
334 In the wt, the *hda-2* transcript was upregulated in response to menadione (30 min), whereas both Δ*blr*
335 showed no considerable changes at all tested conditions (Fig. 6f).
336
337 **HDA-2 and the BLR complex regulate the transcription of ROS-related genes**
338 As Δ*hda-2*, Δ*blr-1* and Δ*blr-2* showed varied degrees of sensitivity to H_2O_2 and menadione, we
339 decided to analyze in mutant backgrounds and in the presence of ROS the expression profile of several
340 ROS-related genes: *gst-1* (glutathione s-transferase; **JGI ID: 93766**), *sod-1* (superoxide dismutase 1;
341 **JGI ID: 298583**), and *cat-3* (catalase 3; **JGI ID: 283309**) and *gpx* (glutathione peroxidase; **JGI ID:**
342 **94401**), which code for antioxidant enzymes to contend against organic hydroperoxide (ROOH), O_2^-,
343 and H_2O_2, respectively. Application of 30 mM H_2O_2 to the wt (at time 0 min) provoked an initial
344 downregulation of *sod-1*, *gst-1*, and *gpx* at 15 min; however, all four genes reached high expression
345 levels at 30 min (Fig. 7a-d and S3). In the case of Δ*blr-1* and Δ*blr-2*, accumulation of *cat-3* and *sod-1*
346 was induced at 15 and 30 min. In Δ*blr-1*, *gst-1* and *gpx* transcript abundance did not change at 15 min,
347 but at 30 min it reached higher levels as compared to wt (Fig. 7c and d). Exposure of Δ*blr-2* to H_2O_2
348 for 15 min led *gst-1* to reach its highest expression levels as compared to all tested strains, but it
349 returned to basal levels at 30 min (Fig. 7c). Interestingly, for Δ*blr-2* at 15 and 30 min *gpx* showed the
350 highest accumulation of transcript as compared to all tested strains (Fig. 7d). As shown in Fig. 7a and
351 7b, expression levels of *cat-3* and *sod-1* were downregulated in Δ*hda-2* at all tested conditions, whereas
352 *gst-1* suffered no considerable changes at 15 min after the application of H_2O_2; but at 30 min *gst-1*

accumulation reached similar levels compared to wt and Δ*blr-1* (Fig. 7c). Intriguingly, *gpx* mRNA levels in Δ*hda-2* showed high accumulation (13-fold) under non-stressful conditions, but depletion to low levels followed by accumulation 15 min and 30 min after the medium was supplemented with H_2O_2, respectively (Fig. 7d).

Addition of 0.2 mM menadione (at time 0 min) to wt did not induce remarkable changes in *cat-3* accumulation in all tested conditions (Fig. 7e). Absence of *cat-3* expression in the wt strain in the presence of menadione is expected, because superoxide is not a substrate for CAT-3. However, Δ*blr-1*, *-2* and Δ*hda-2* showed marginally lower levels of *cat-3* mRNA compared to the wt (Fig. 7e). As shown in Fig. 7f and S3, *sod-1* was induced in wt, Δ*blr-1* and Δ*blr-2*, reaching highest levels 30 min after the application of the stimulus in all three strains, whereas in Δ*hda-2* *sod-1* showed low levels with no significant changes in all tested conditions. Menadione provoked high accumulation of *gst-1* in all tested strains, showing a maximum of expression at 30 min, with Δ*blr-2* showing the highest accumulation, followed by wt, Δ*hda-2* and Δ*blr-1* (Fig. 7g). In wt, *gpx* was slightly downregulated 15 min after menadione addition; nevertheless it reached its highest levels at 30 min (Fig. 7h). Furthermore, in Δ*blr-1* and Δ*blr-2*, *gpx* showed low expression levels under control conditions, and its transcript barely overcame the wt basal levels at all tested times of exposure (Fig. 7h). In Δ*hda-2*, *gpx* surpassed the wt accumulation under non-stressful conditions; nonetheless, this gene was downregulated 15 min after the addition of menadione, and showed recovery at 30 min (Fig. 7h).

HDA-2 and the BLR complex are required for histone H3 acetylation in the promoter of ROS-related genes under oxidative stress

In the Δ*hda-2* strain in the presence of ROS, we observed that transcription of *cat-3* and *gst-1* was downregulated and upregulated, respectively, compared to the wt. To determine whether oxidative stress induces histone H3 acetylation in the promoter region of *cat-3* and *gst-1*, we performed a Chromatin Immunoprecipitation (ChIP) assay analysis. Mycelia of wt, Δ*hda-2-2*, Δ*blr-1* and Δ*blr-2*

strains were grown in darkness for 96 h and treated with 30 mM H_2O_2. Afterwards, the chromatin was cross-linked, sonicated and immunoprecipitated using specific antibodies against H3 and H3K9K14Ac. In wt, 30 min after addition of 30 mM H_2O_2, the H3K9K14Ac signal increased 3-fold respect to the baseline in the untreated condition (Fig. 8a). In the Δ*hda-2*, Δ*blr-1* and Δ*blr-2* strains, the promoter region of *cat-3* showed similar acetylation levels (1.0 fold) as the wt in the control condition, and showed no enrichment of H3K9K14Ac 30 min after H_2O_2 application (Fig. 8a). For the case of the acetylation profile of the *gst-1* promoter, we found 5-fold enrichment in the wt after 30 min of mycelia exposure to H_2O_2 (Fig. 8b), whereas the Δ*hda-2*, Δ*blr-1* and Δ*blr-2* strains showed no enrichment when compared to the untreated control (Fig. 8b).

DISCUSSION

In living organisms, light is one of the main ROS-generating sources ($^1O_2$, O_2^-) (43). Here, blue light and oxidative stress induce the expression of *hda-2*, suggesting a link between these two cues through chromatin modifications and the ensuing regulation of gene transcription (Fig. 9). The early photoinduction of *hda-2* and the fact that it putatively encodes a regulatory protein suggest a role for HDA-2 in early photoresponses (13, 39). Supporting this conclusion, the Δ*hda-2* mutants grow slow, do not conidiate in response to both light and mechanical injury, and barely induce *con-1*. On the contrary, the Δ*blr* strains grow faster than the wt and conidiate in response to mechanical injury, but not in response to light (9). These differences indicate opposite roles for the BLR's and HDA-2 in mycelial growth, but also that HDA-2 regulates an important step in signal transduction that leads *T. atroviride* to condiate, and probably to photoconidiate through the expression of early *blu* genes, including *blr-1* (Fig. 9). Although it is possible that the affected growth of Δ*hda-2* may be altering conidiation and the corresponding expression of genes involved in this process, our results point to HDA-2 and the BLR proteins as key elements in the complex network that integrates responses to blue light and stress-induced ROS in *T. atroviride*.

403 In the particular case of responses to light, the mutual dependence between HDA-2 and BLR-1
404 indicates participation of these elements in a feedback loop that regulates blue light perception and
405 signal transduction (Fig. 9). It is noteworthy that the transcription of *blr-1*, a master regulator of blue
406 light responses (9), showed low expression in Δ*hda-2*, which could explain the weak response of *phr-1*
407 and *grg-2* to light and the null expression of *env1*. At the chromatin level, a surprising result is that
408 HDA-2 had a positive role on the transcription of *blu* genes, even though our analyses suggest that *hda-*
409 *2* codes for an HDAC, which putatively deacetylates histone tails leading to heterochromatin
410 formation. We do not discard the involvement of more regulators. In this regard, similar results for
411 light-regulated genes were published for *T. atroviride* and *A. nidulans* mutants in the *tmk3* and *sakA*
412 genes that code for a MAPK kinase orthologous to Hog1p of *S. cerevisiae*. Importantly, Tmk3 and
413 SakA integrate several stress factors, such as light sensing, stress-sensing and osmosensing (44, 45). It
414 is worth noting that in yeast under hyperosmotic stress the repressor Sko1 is phosphorylated by Hog1
415 and converted to an activator that recruits Hog1 itself as well as the SAGA histone acetyltransferase
416 complex (coactivator) at the promoter of target genes to activate transcription (46). In *T. atroviride*, it is
417 possible that MAP kinases like Tmk3 could be recruiting HDA-2 to the promoters of *blu* genes to avoid
418 repressor binding, thus promoting histone acetylation, the recruitment of activators, and the induction
419 of gene expression (47).
420 Light is absorbed by biological molecules, such as flavins and porphyrins, to subsequently transfer
421 energy to molecular oxygen, triggering the production of ROS (2, 43). Here, we observed that the
422 addition of pro-oxidants plus the presence of light exerts an additive (but marginal) effect in the growth
423 of wt, consistent with the accumulation of externally applied and blue-light-generated ROS (48, 49) to
424 levels that may exceed the antioxidant capacity of the cell, thus affecting mycelial growth (16, 50).
425 Supporting this view, light exacerbates sensitiveness to oxidative stress in *N. crassa cat-3*-lacking
426 strains (51). Our results indicate that HDA-2 regulates positively the resistance to both menadione and
427 H_2O_2, whereas BLR-1 and BLR-2 seem to play negative roles at low concentrations of the pro-

oxidants, probably modifying heterochromatin, thus influencing the regulation of ROS-related genes (Fig. 9). In agreement with the role of HDACs to deal with ROS, deletion of the HdaA encoding gene in *A. nidulans* causes growth defects in the presence of ROS (52), which highlights the importance of acetylation to regulate gene expression under oxidative stress (53).

Motivated by our results for the case of blue light, we attempted to reveal a mutual regulatory dependence between HDA-2 and the BLR proteins at the transcriptional level under oxidative stress. This mutual dependence was not obvious in this case, since the expression of the genes did not show a systematic behavior among different backgrounds and stressful conditions (Fig. 6 and 9). A main factor and immediate conclusion is that the two used pro-oxidants did not provoke the same effects in the wt. In this regard it has been proposed that H_2O_2 and $OH^{\cdot}$ are the main toxic species derived from menadione (54), which may lead to think that the responses to H_2O_2 and menadione must be similar. However, experimental data revealed that several pro-oxidants differentially influence the genome of *S. cerevisiae* with a short range of overlap (55). Therefore, *T. atroviride* may have different ways to regulate resistance to different pro-oxidants trough BLR and HDA-2 proteins, leading to an intricate landscape compared to light responses.

Our hypothesis on the role of HDA-2 in regulating gene transcription to contend against ROS is supported by the transcriptional analysis of ROS-related genes, which suggests a positive role of HDA-2 on the transcription of *cat-3* and *sod-1* in presence of H_2O_2 and *sod-1* in presence of menadione, possibly by modifying heterochromatin on promoters of negative regulators (Fig. 9). The absence of *sod-1* and *cat-3* in Δ*hda-2*, whose products putatively play a role in the dismutation of superoxide and in the removal of H_2O_2 and lipid hydroperoxides, respectively, may explain the sensitivity showed by this null mutant, as demonstrated for other fungi (51, 56–58) (Fig. 9). A surprising observation is that *sod-1* is expressed in presence of H_2O_2, although its product does not remove H_2O_2. Recently, it was described that H_2O_2 is sufficient to promote Sod1p nuclear localization for the activation of ROS-related genes. Therefore, Sod1 is responding to ROS in general, rather than only to its superoxide

substrate, to function as a transcription factor (59). It is possible that in *T. atroviride* SOD-1 may have an important role to contend against ROS by regulating ROS-related genes. Furthermore, HDA-2 could be altering heterochromatin on the promoters of negative regulators, which may influence the transcription of *sod-1*, *gpx,* and *gst-1* in the presence of menadione, which produces superoxide ($O_2^{\cdot-}$) by redox-cycling (60). Under this condition, SOD, GPx and GST-1 may detoxify cells of $O_2^{\cdot-}$ (Fig. 9). Together, our results indicate that HDA-2 participates in the regulation of genes that encode proteins necessary to contend against certain types of ROS in *T. atroviride*. It is important to highlight that, in the presence of H_2O_2 and menadione, *gpx* and *gst-1* were marginally upregulated in the Δ*blr-1* and *-2*, respectively, which could explain the resistance of these strains to such compounds, similar to behavior shown in other fungi (61–63)

Our results support a previous hypothesis that, based on the fact that Δ*blr-1* and *-2* mutants do not conidiate in response to carbon deprivation, assigns a role to the PAS and LOV domains of BLR-1 and BLR-2 in sensing ROS and redox states in *T. atroviride* (9). BLR-1 contains a putative flavin-binding motive that shares a consensus sequence with signal-transducing proteins that regulate gene expression in response to redox changes, oxygen, and blue light (14, 64–66). In *Trichoderma reesei*, recent results suggest that light perception and oxidative stress are integrated by the ENV1 photoreceptor, composed of a single LOV domain (67). Supporting this proposal, four of the nine predicted catalases and four of the six SODs encoding genes were downregulated in a Δ*env1* mutant upon growth in light and cellulose (68). A key element in that model is that oxidative stress promotes a sequestration of ENV1 in a homodimer, blocking formation of ENV1:BLR-1 and allowing activation of the BLR-1:BLR-2 complex (67). Overall, our results are inconsistent with this picture, because we observed that the absence of BLR-1 or BLR-2, with the subsequent absence of ENV1, does not preclude that the transcription of almost all ROS-related genes responds to the presence of both pro-oxidants. It is possible that significant differences in growing media and light conditions used in (67) compared to those used in this work could explain the discrepancies with that proposed model.

478 Histone acetylation and deacetylation parallel transcriptional gene activation or repression,
479 respectively, by altering the chromatin conformation (69, 70). In the wt, we observed up-regulation of
480 *gst-1* and *cat-3*, suggesting an increase in histone H3 acetylation at their promoters. In the Δ*hda-2*
481 mutant, transcription levels of *cat-3* were downregulated and low acetylation levels at the *cat-3*
482 promoter were found, indicating that *hda-2* plays a major role in the regulation of *cat-3* but it is not
483 acting directly in gene repression. These contrasting results could be explained by considering that
484 histone deacetylation may also activate transcription, by means of targeting repressors (71). In
485 particular, the absence of HDA-2 may have led to changes on heterochromatin structure by an
486 acetylation unbalance on the promoters of negative regulators of *cat-3*, including other HDACs.
487 Among negative regulators of the expression of ROS-related genes, histone methylases play a pivotal
488 role (72, 73). In *N. crassa* the histone methyltransferase DIM-5 and the non-histone protein
489 heterochromatin protein 1 (HP1) are essential for heterochromatin formation (74). In agreement with
490 our results, in this fungus H_2O_2 stimulates *cat-3* expression that correlates with histone H3 acetylation.
491 On the contrary, tri-methylation of histone H3 at proximal 5-kb heterochromatin region and *cat-3* locus
492 (H3K9me3) mediates repression of *cat-3* (73). Intriguingly, hpo^{KO} and dim^{KO} mutants, who lack of HP1
493 and DIM-5, show high resistance to H_2O_2, and high levels of *cat-3*. These results indicate that histone
494 methylation and proper formation of chromatin may regulate *cat-3* expression (73). Based on these
495 facts, we propose that histone H3 methylation and acetylation as well as the proper chromatin
496 formation could be important for *cat-3* regulation in *T. atroviride*. An alternative hypothesis is that
497 negative regulators are repressing the transcription of HATs that acetylate H3 on the *cat-3* promoter.
498 Further studies of chromatin conformation are needed in this fungus.

499 Regarding *gst-1*, Δ*hda-2* showed similar transcription levels as in wt, however, the H3 acetylation level
500 at the *gst-1* promoter was also low. This fact was also supported by the upregulation of *gpx* and *gst-1* in
501 the Δ*blr-1* and *-2* mutants, respectively, with the absence of acetylation at the promoters of *gst-1* and

502 *cat-3* in *blr* mutants. We speculate that additional histone modifications (such as methylation,
503 phosphorylation, etc.) could be involved in the regulation of *gst-1* in *T. atroviride*. An alternative
504 explanation is that BLR-1 and BLR-2 function as repressors on the promoters of some ROS-related
505 genes under certain stressful conditions. In this regard, it is known that BLR-2 locates on the promoter
506 of *phr-1* in the dark and a blue light pulse promotes unbinding. Unbinding of BLR-2 from the *phr-1*
507 promoter parallels induction of *phr-1* transcription, suggesting a role as repressor (40).

508 In conclusion, we have shown that HDA-2 and BLRC play a pivotal role in the regulation of mycelial
509 growth, conidiation, and resistance to H_2O_2, light perception, and gene regulation of blue-light and
510 ROS-related genes.

511

512 **ACKNOWLEDGEMENTS**

513 This work was supported by grant SEP-CONACYT CB-2013-01-220791 to Sergio Casas-Flores.
514 MOC, and GRCM are indebted to CONACYT for Ph.D. and Master of Science scholarships number
515 226637 and 333183, respectively. BGM is supported by CONACYT, grant SEP-CB-2013-01-223377.

516

517 **CONFLICT OF INTEREST**: The authors declare that they have no competing interests.

518

519 **REFERENCES**

520 1. **Rodriguez-Romero J**, **Hedtke M**, **Kastner C**, **Müller S**, **Fischer R**. 2010. Fungi, hidden in soil
521 or up in the air: light makes a difference. Annu Rev Microbiol **64**:585–610.

522 2. **Casas-Flores S**, **Herrera-Estrella A**. 2013. The influence of light on the biology of
523 Trichoderma, p. 43–66. *In* Mukherjee PK, Horwitz BA, Singh US, Mukherjee M, Schmoll M
524 (ed), *Trichoderma*: biology and applications. CABI, Wallingford.

525 3. **Casas-Flores S**, **Herrera-Estrella A**. 2016. The Bright and Dark Sides of Fungal Life, p. 41–
526 77. *In* Kubicek CP, Druzhinina IS (Ed), Environmental and Microbial Relationships. Springer
527 International Publishing, Cham.

4. **Howell CR**. 2003. Mechanisms Employed by *Trichoderma* Species in the Biological Control of Plant Diseases: The History and Evolution of Current Concepts. Plant Dis **87**:4–10.

5. **Benítez T**, **Rincón AM**, **Limón MC**, **Codón AC**. 2004. Biocontrol mechanisms of *Trichoderma* strains. Int Microbiol **7**:249–260.

6. **Monfil VO**, **Casas-Flores S**. 2014. Molecular Mechanisms of Biocontrol in *Trichoderma* spp. and Their Applications in Agriculture, p. 429–453. *In* Gupta V, Schmoll M, Herrera-Estrella A, Upadhyay R, Druzhinina I, Tuohy M (Ed), Biotechnology and Biology of Trichoderma. Elsevier, Amsterdam, The Neterlands.

7. **Gutter Y**. 1957. Effect of light in sporulation of *Trichoderma viride*. Bull Res Counc Isr Sect **D**:273–286.

8. **Gressel J**, **Galun E**. 1967. Morphogenesis in *Trichoderma*: Photoinduction and RNA. Dev Biol **15**:575–598.

9. **Casas-Flores S**, **Rios-Momberg M**, **Bibbins M**, **Ponce-Noyola P**, **Herrera-Estrella A**. 2004. BLR-1 and BLR-2, key regulatory elements of photoconidiation and mycelial growth in *Trichoderma atroviride*. Microbiology **150**:3561–3569.

10. **Gremel G**, **Dorrer M**, **Schmoll M**. 2008. Sulphur metabolism and cellulase gene expression are connected processes in the filamentous fungus *Hypocrea jecorina* (anamorph *Trichoderma* reesei). BMC Microbiol **8**:174.

11. **Castellanos F**, **Schmoll M**, **Martínez P**, **Tisch D**, **Kubicek CP**, **Herrera-Estrella A**, **Esquivel-Naranjo EU**. 2010. Crucial factors of the light perception machinery and their impact on growth and cellulase gene transcription in *Trichoderma reesei*. Fungal Genet Biol **47**:468–476.

12. **Osorio-Concepción M**, **Casas-Flores S**, **Cortés-Penagos C**. 2013. Efecto de la limitación de fosfato sobre la conidiación de *Trichoderma atroviride* y mutantes ciegas a la luz. Rev Mex Micol **37**:41–50.

13. **Rosales-Saavedra T**, **Esquivel-Naranjo EU**, **Casas-Flores S**, **Martínez-Hernández P**, **Ibarra-Laclette E**, **Cortes-Penagos C**, **Herrera-Estrella A.** 2006. Novel light-regulated genes in *Trichoderma atroviride*: A dissection by cDNA microarrays. Microbiology **152**:3305–3317.

14. **Taylor BL**, **Zhulin IB**. 1999. PAS Domains: Internal Sensors of Oxygen, Redox Potential, and Light. Microbiol Mol Biol Rev **63**:479–506.

15. **Aguirre J**, **Ríos-Momberg M**, **Hewitt D**, **Hansberg W**. 2005. Reactive oxygen species and development in microbial eukaryotes. Trends Microbiol **13**:111–118.

16. **Hansberg W**, **Aguirre J**. 1990. Hyperoxidant states cause microbial cell differentiation by cell isolation from dioxygen. J Theor Biol **142**:201–221.

17. **Takemoto D**, **Tanaka A**, **Scott B**. 2006. A p67Phox-like regulator is recruited to control hyphal branching in a fungal-grass mutualistic symbiosis. Plant Cell **18**:2807–21.

18. **Cano-Domínguez N**, **Álvarez-Delfín K**, **Hansberg W**, **Aguirre J**. 2008. NADPH oxidases NOX-1 and NOX-2 require the regulatory subunit NOR-1 to control cell differentiation and growth in *Neurospora crassa*. Eukaryot Cell **7**:1352–1361.

19. **Hernández-Oñate MA**, **Esquivel-Naranjo EU**, **Mendoza-Mendoza A**, **Stewart A**, **Herrera-Estrella AH**. 2012. An injury-response mechanism conserved across kingdoms determines entry of the fungus *Trichoderma atroviride* into development. Proc Natl Acad Sci U S A **109**:14918–23.

20. **Luger K**, **Richmond TJ**. 1998. The histone tails of the nucleosome. Curr Opin Genet Dev **8**:140–146.

21. **Shahbazian MD**, **Grunstein M**. 2007. Functions of Site-Specific Histone Acetylation and Deacetylation. Annu Rev Biochem **76**:75–100.

22. **Gregoretti IV.**, **Lee YM**, **Goodson HV.** 2004. Molecular evolution of the histone deacetylase family: Functional implications of phylogenetic analysis. J Mol Biol **338**:17–31.

23. **Blander G**, **Guarente L**. 2004. The Sir2 family of protein deacetylases. Annu Rev Biochem **73**:417–35.

24. **Baidyaroy D**, **Brosch G**, **Ahn JH**, **Graessle S**, **Wegener S**, **Tonukari NJ**, **Caballero O**, **Loidl P**, **Walton JD**. 2001. A gene related to yeast HOS2 histone deacetylase affects extracellular depolymerase expression and virulence in a plant pathogenic fungus. Plant Cell **13**:1609–1624.

25. **Tribus M**, **Bauer I**, **Galehr J**, **Rieser G**, **Trojer P**, **Brosch G**, **Loidl P**, **Haas H**, **Graessle S**. 2010. A novel motif in fungal class 1 histone deacetylases is essential for growth and development of *Aspergillus*. Mol Biol Cell **21**:345–53.

26. **Lee I**, **Oh J-H**, **Keats Shwab E**, **Dagenais TRT**, **Andes D**, **Keller NP**. 2009. HdaA, a class 2 histone deacetylase of *Aspergillus fumigatus*, affects germination and secondary metabolite production. Fungal Genet Biol **46**:782–790.

27. **Ding S-L**, **Liu W**, **Iliuk A**, **Ribot C**, **Vallet J**, **Tao A**, **Wang Y**, **Lebrun M-H**, **Xu J-R**. 2010. The Tig1 Histone Deacetylase Complex Regulates Infectious Growth in the Rice Blast Fungus *Magnaporthe oryzae*. PLANT CELL ONLINE **22**:2495–2508.

28. **Schmoll M**, **Dattenböck C**, **Carreras-Villaseñor N**, **Mendoza-Mendoza A**, **Tisch D**, **Alemán MI**, **Baker SE**, **Brown C**, **Cervantes-Badillo MG**, **Cetz-Chel J**, **Cristobal-Mondragon GR**, **Delaye L**, **Esquivel-Naranjo EU**, **Frischmann A**, **Gallardo-Negrete J de J**, **García-Esquivel M**, **Gomez-Rodriguez EY**, **Greenwood DR**, **Hernández-Oñate M**, **Kruszewska JS**, **Lawry R**, **Mora-Montes HM**, **Muñoz-Centeno T**, **Nieto-Jacobo MF**, **Nogueira Lopez G**, **Olmedo-Monfil V**, **Osorio-Concepcion M**, **Piłsyk S**, **Pomraning KR**, **Rodriguez-Iglesias A**, **Rosales-Saavedra MT**, **Sánchez-Arreguín JA**, **Seidl-Seiboth V**, **Stewart A**, **Uresti-Rivera EE**, **Wang C-L**, **Wang T-F**, **Zeilinger S**, **Casas-Flores S**, **Herrera-Estrella A**. 2016. The Genomes of

598 Three Uneven Siblings: Footprints of the Lifestyles of Three *Trichoderma* Species. Microbiol
599 Mol Biol Rev **80**:205–327.

600 29. **Grimaldi B**, **Coiro P**, **Filetici P**, **Berge E**, **Dobosy JR**, **Freitag M**, **Selker EU**, **Ballario P**.
601 2006. The *Neurospora crassa* White Collar-1 dependent blue light response requires acetylation
602 of histone H3 lysine 14 by NGF-1. Mol Biol Cell **17**:4576–83.

603 30. **Brenna A.**, **Grimaldi B**, **Filetici P**, **Ballario P**. 2012. Physical association of the WC-1
604 photoreceptor and the histone acetyltransferase NGF-1 is required for blue light signal
605 transduction in *Neurospora crassa*. Mol Biol Cell **23**:3863–3872.

606 31. **Sancar C**, **Ha N**, **Yilmaz R**, **Tesorero R**, **Fisher T**, **Brunner M**, **Sancar G**. 2015.
607 Combinatorial control of light induced chromatin remodeling and gene activation in *Neurospora*.
608 PLoS Genet **11**:e1005105.

609 32. **Vogel HJ**. 1956. A convenient growth medium for *Neurospora* (Medium N). Microb Genet Bull
610 **13**:42–43.

611 33. **Sambrook JF, Russell DW.** 2001. Molecular Cloning: A Laboratory Manual, 3rd edition. Cold
612 Spring Harb Lab Press **NY**:2344.

613 34. **Carroll AM**, **Sweigard JA**, **Valent B**. 1994. Improved Vectors for Selecting Resistance to
614 Hygromycin. Fungal Genet Newsl **41**:22.

615 35. **Casas-Flores S**, **Rios-Momberg M**, **Rosales-Saavedra T**, **Martínez-Hernández P**, **Olmedo-**
616 **Monfil V**, **Herrera-Estrella A**. 2006. Cross talk between a fungal blue-light perception system
617 and the cyclic AMP signaling pathway. Eukaryot Cell **5**:499–506.

618 36. **Raeder U**, **Broda P**. 1985. Rapid preparation of DNA from filamentous fungi. Lett Appl
619 Microbiol **1**:17–20.

620 37. **Baek JM, Kenerley CM**. 1998. The arg2 gene of *Trichoderma virens*: cloning and development
621 of a homologous transformation system. Fungal Genet Biol **23(1)**:34–44.

622 38. **Bubner B**, **Baldwin IT**. 2004. Use of real-time PCR for determining copy number and zygosity
623 in transgenic plants. Plant Cell Rep **23**:263–271.

624 39. **Berrocal-Tito G**, **Sametz-Baron L**, **Eichenberg K**, **Horwitz BA**, **Herrera-Estrella A**. 1999.
625 Rapid blue light regulation of a *Trichoderma harzianum* photolyase gene. J Biol Chem
626 **274**:14288–14294.

627 40. **Cervantes-Badillo MG**, **Muñoz-Centeno T**, **Uresti-Rivera EE**, **Argüello-Astorga GR**,
628 **Casas-Flores S**. 2013. The *Trichoderma atroviride* photolyase-encoding gene is
629 transcriptionally regulated by non-canonical light response elements. FEBS J **280**:3697–3708.

630 41. **Livak KJ**, **Schmittgen TD**. 2001. Analysis of relative gene expression data using real-time
631 quantitative PCR and. Methods **25**:402–408.

42. **Berrocal-Tito GM**. 1999. Caracterización del gen phr1, que codifica a la CPD-fotoliasa del hongo del suelo *Trichoderma harzianum*, y su papel en las respuestas a la luz azul. Centro de investigación y de estudios avanzados (CINVESTAV).

43. **Lledias F**, **Hansberg W**. 2000. Catalase modification as a marker for singlet oxygen. Methods Enzymol **319**:110–9.

44. **Yu PL**, **Chen LH**, **Chung KR**. 2016. How the pathogenic fungus *Alternaria alternata* copes with stress via the response regulators SSK1 and SHO1. PLoS One **11**:1–20.

45. **Esquivel-Naranjo EU**, **García-Esquivel M**, **Medina-Castellanos E**, **Correa-Pérez VA**, **Parra-Arriaga JL**, **Landeros-Jaime F**, **Cervantes-Chávez JA**, **Herrera-Estrella A**. 2016. A *Trichoderma atroviride* stress-activated MAPK pathway integrates stress and light signals. Mol Microbiol **100**:860–76.

46. **Proft M**, **Struhl K**. 2002. Hog1 kinase converts the Sko1-Cyc8-Tup1 repressor complex into an activator that recruits SAGA and SWI/SNF in response to osmotic stress. Mol Cell **9**:1307–17.

47. **de Nadal E**, **Zapater M**, **Alepuz PM**, **Sumoy L**, **Mas G**, **Posas F**. 2004. The MAPK Hog1 recruits Rpd3 histone deacetylase to activate osmoresponsive genes. Nature **427**:370–374.

48. **Laloraya MM**, **Chandra-kuntal K**, **Kumar GP**, **Laloraya M**. 1999. Active oxygen species in blue light mediated signal transduction in coleoptile tips. Biochem Biophys Res Commun **256**:293–8.

49. **Wen F**, **Xing D**, **Zhang L**. 2008. Hydrogen peroxide is involved in high blue light-induced chloroplast avoidance movements in *Arabidopsis*. J Exp Bot **59**:2891–901.

50. **Hansberg W**. 1996. A hyperoxidant state at the start of each developmental stage during *Neurospora crassa* conidiation. Cienc Cult **48**:68–74.

51. **Michán S**, **Lledías F**, **Hansberg W**. 2003. Asexual Development Is Increased in *Neurospora crassa cat - 3* -Null Mutant Strains **2**.

52. **Tribus M**, **Galehr J**, **Trojer P**, **Loidl P**, **Marx F**, **Haas H**, **Brosch G**, **Graessle S**. 2005. HdaA , a Major Class 2 Histone Deacetylase of *Aspergillus nidulans*, Affects Growth under Conditions of Oxidative Stress. Eukaryot Cell **4**:1736–1745.

53. **Green EM**, **Morrison AJ**, **Gozani O**. 2012. New marks on the block 335–339.

54. **Nutter LM**, **Ann-Lii C**, **Hsiao-Ling H**, **Ruey-Kun H**, **Ngo EO**, **Tsang-Wu L**. 1991. Menadione: Spectrum of anticancer activity and effects on nucleotide metabolism in human neoplastic cell lines. Biochem Pharmacol **41**:1283–1292.

55. **Thorpe GW**, **Fong CS**, **Alic N**, **Higgins VJ**, **Dawes IW**. 2004. Cells have distinct mechanisms to maintain protection against different reactive oxygen species: oxidative-stress-response genes. Proc Natl Acad Sci U S A **101**:6564–9.

666 56. **Michán S**, **Lledías F**, **Baldwin JD**, **Natvig DO**, **Hansberg W**. 2002. Regulation and oxidation
667 of two large monofunctional catalases. Free Radic Biol Med **33**:521–32.

668 57. **Chary P**, **Dillon D**, **Schroeder AL**, **Natvig DO**. 1994. Superoxide dismutase (sod-1) null
669 mutants of *Neurospora crassa*: oxidative stress sensitivity, spontaneous mutation rate and
670 response to mutagens. Genetics **137**:723–30.

671 58. **Jamieson DJ**, **Rivers SL**, **Stephen DWS**. 1994. Analysis of *Saccharomyces cerevisiae* proteins
672 induced by peroxide and superoxide stress. Microbiology **140**:3277–3283.

673 59. **Tsang CK**, **Liu Y**, **Thomas J**, **Zhang Y**, **Zheng XFS**. 2014. Superoxide dismutase 1 acts as a
674 nuclear transcription factor to regulate oxidative stress resistance. Nat Commun **5**:3446.

675 60. **Kappus H**, **Sies H**. 1981. Toxic drug effects associated with oxygen metabolism: redox cycling
676 and lipid peroxidation. Experientia **37**:1233–41.

677 61. **Huang K**, **Czymmek KJ**, **Caplan JL**, **Sweigard JA**, **Donofrio NM**. 2011. HYR1-mediated
678 detoxification of reactive oxygen species is required for full virulence in the rice blast fungus.
679 PLoS Pathog **7**:e1001335.

680 62. **Xiong C**, **Xia Y**, **Zheng P**, **Wang C**. 2013. Increasing oxidative stress tolerance and
681 subculturing stability of *Cordyceps militaris* by overexpression of a *glutathione peroxidase* gene.
682 Appl Microbiol Biotechnol **97**:2009–2015.

683 63. **Li C**, **Shi L**, **Chen D**, **Ren A**, **Gao T**, **Zhao M**. 2015. Functional analysis of the role of
684 glutathione peroxidase (GPx) in the ROS signaling pathway, hyphal branching and the
685 regulation of ganoderic acid biosynthesis in *Ganoderma lucidum*. Fungal Genet Biol **82**:168–
686 180.

687 64. **Hill S**, **Austin S**, **Eydmann T**, **Jones T**, **Dixon R**. 1996. *Azotobacter vinelandii* NIFL is a
688 flavoprotein that modulates transcriptional activation of nitrogen-fixation genes via a redox-
689 sensitive switch. Proc Natl Acad Sci U S A **93**:2143–8.

690 65. **Rebbapragada A**, **Johnson MS**, **Harding GP**, **Zuccarelli AJ**, **Fletcher HM**, **Zhulin IB**,
691 **Taylor BL**. 1997. The Aer protein and the serine chemoreceptor Tsr independently sense
692 intracellular energy levels and transduce oxygen, redox, and energy signals for *Escherichia coli*
693 behavior. Proc Natl Acad Sci U S A **94**:10541–6.

694 66. **He Q**, **Cheng P**, **Yang Y**, **Wang L**, **Gardner KH**, **Liu Y**. 2002. White collar-1, a DNA binding
695 transcription factor and a light sensor. Science (80-) **297**:840–843.

696 67. **Lokhandwala J**, **Hopkins HC**, **Rodriguez-Iglesias A**, **Dattenböck C**, **Schmoll M**, **Zoltowski**
697 **BD**. 2015. Structural Biochemistry of a Fungal LOV Domain Photoreceptor Reveals an
698 Evolutionarily Conserved Pathway Integrating Light and Oxidative Stress. Structure **23**:116–
699 125.

700 68. **Tisch D**, **Schmoll M**. 2013. Targets of light signalling in *Trichoderma reesei.* BMC Genomics
701 **14**:657.

69. **Grunstein M**. 1997. Histone acetylation in chromatin structure and transcription. Nature **389**:349–352.

70. **Lee KK**, **Workman JL**. 2007. Histone acetyltransferase complexes: one size doesn't fit all. Nat Rev Mol Cell Biol **8**:284–95.

71. **Bernstein BE**, **Tong JK**, **Schreiber SL**. 2000. Genomewide studies of histone deacetylase function in yeast. Proc Natl Acad Sci U S A **97**:13708–13.

72. **Chen A**, **Feldman M**, **Vershinin Z**, **Levy D**. 2016. SETD6 is a negative regulator of oxidative stress response. Biochim Biophys Acta - Gene Regul Mech **1859**:420–427.

73. **Wang Y**, **Dong Q**, **Ding Z**, **Gai K**, **Han X**, **Kaleri FN**, **He Q**, **Wang Y**. 2016. Regulation of *Neurospora* Catalase-3 by global heterochromatin formation and its proximal heterochromatin region. Free Radic Biol Med **99**:139–152.

74. **Rountree MR**, **Selker EU**. 2010. DNA methylation and the formation of heterochromatin in *Neurospora crassa*. Heredity (Edinb) **105**:38–44.

Tables

Table 1.

(a) Copy number of the *hda-2* gene by the 2^-ΔΔCt method

Strain	**Ct of endogenous control (Ct_e)**	**Ct of gene (Ct_t)**	**ΔCt (Ct_t-Ct_e)**	**ΔΔCt (ΔCt_s-ΔCt_c)**	**Copy number (2^-ΔΔCt)**
	tef-1	***hda-2***	***hda-2***	***hda-2***	***hda-2***
* wt	20.7	22.74	2.04	-0.17	1.1 ± 0.1
Δ*blr-1*	20.25	22.24	1.9	-0.04	1.03 ± 0.08
Δ*hda-2-1*	20.2	36.88	16.6	14.6	0.0 ± 0.0
Δ*hda-2-2*	20.29	36.57	16.2	14.6	0.0 ± 0.0
Δ*hda-2-3*	20.3	38.11	17.7	16.2	0.0 ± 0.0

* Calibrator for *hda-2* gene

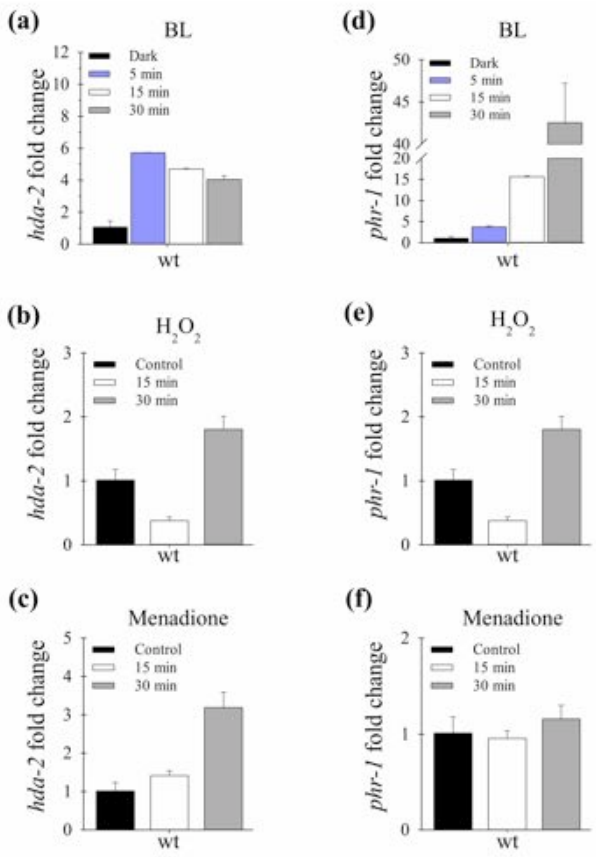
722 **(b)** Copy number of the *hph* gene by the 2^-ΔΔCt method

Strain	Ct of endogenous control (Ct_e)	Ct of transgene (Ct_t)	ΔCt (Ct_t-Ct_e)	ΔΔCt (ΔCt_s-ΔCt_c)	Copy number (2^-ΔΔCt)
	tef-1	***hph***	***hph***	***hph***	***hph***
wt	20.7	32.47	11.77	8.72	0.0 ± 0.0
** Δ*blr-1*	20.25	23.3	3.05	0	1 ± 0.2
Δ*hda-2-1*	20.2	23.34	3.14	0.09	0.9 ± 0.03
Δ*hda-2-2*	20.29	23.22	2.93	-0.11	1.08 ± 0.04
Δ*hda-2-3*	20.3	23.34	3.04	-0.006	1.0 ± 0.04

** Calibrator for *hph* gene

723

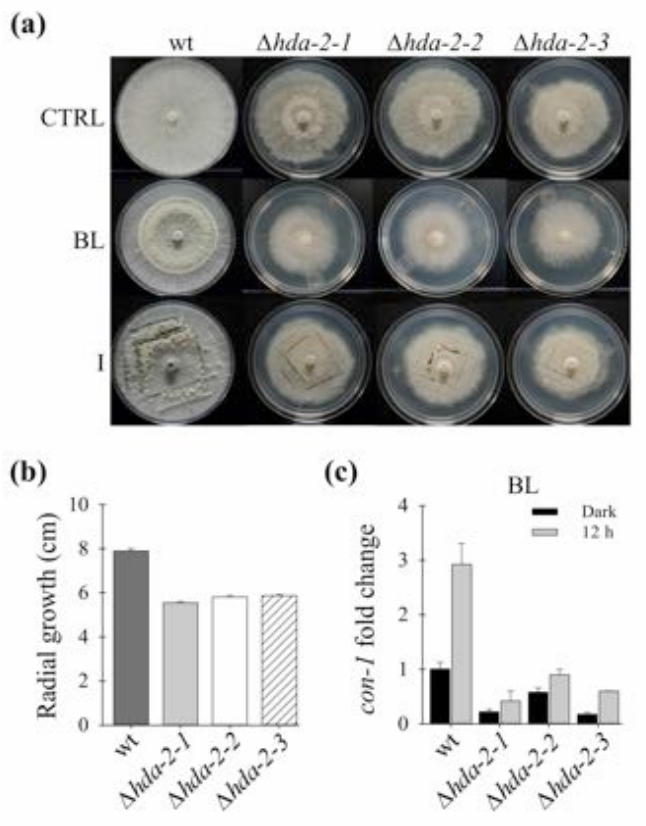
724 **Figures.**



725

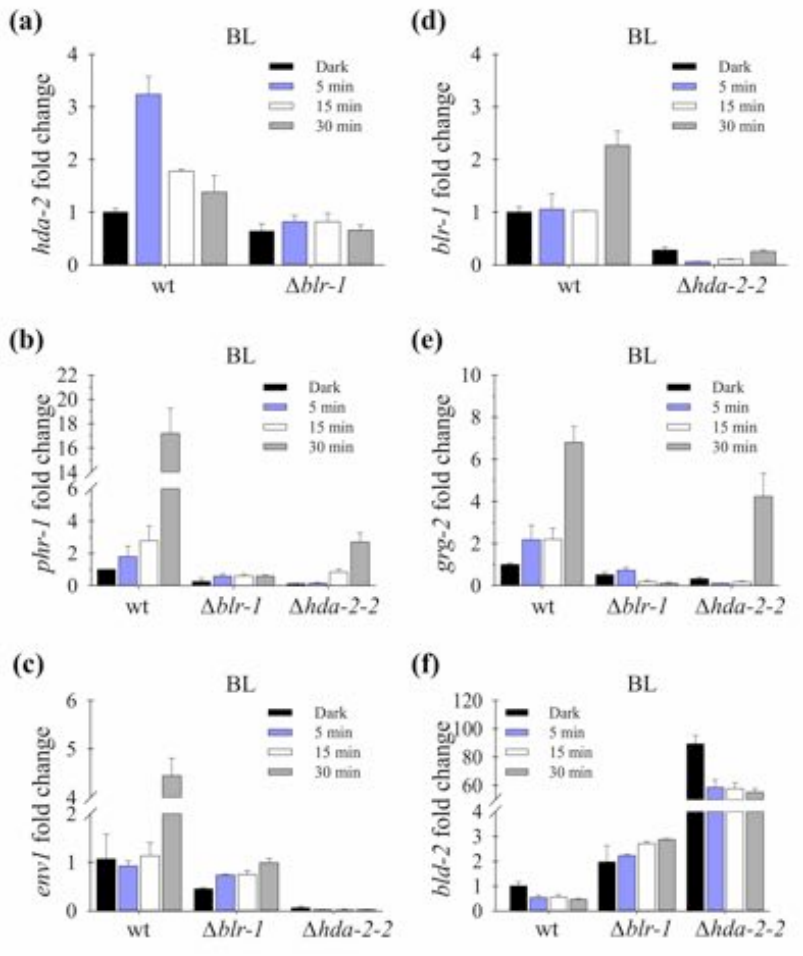
726 **Fig. 1**. **The *hda-2* and *phr-1* genes are induced by light and oxidative stress in *T. atroviride***.
727 Mycelium of *T. atroviride* wt strain was exposed to a blue light pulse (BL), and samples were collected
728 at the indicated times to determine *hda-2* (a) and *phr-1* (d) mRNA levels by RT-qPCR. Mycelium kept
729 in the dark was used as control. Mycelium of *T. atroviride* wt strain grown in the dark was treated with
730 30 mM H_2O_2 or 0.2 mM menadione, and 15 or 30 min after *hda-2* (b and c) and *phr-1* (e and f)
731 transcripts were measured by RT-qPCR. Untreated mycelium was used as control. Results are reported
732 as fold-change compared to the untreated sample. The translation elongation factor-encoding gene *tef-1*
733 was used as control gene to normalize the expression of *hda-2* and *phr-1* using the $2^{-\Delta\Delta Ct}$ method. The
734 graphs show the mean expression levels ± SD. The results are based on three repeats in two
735 independent experiments.

736



737

738 **Fig. 2. HDA-2 is required for proper growth and asexual reproduction in *T. atroviride*.** Pictures
739 show 48-h dark-grown colonies of wt, Δ*hda-2-1*, Δ*hda-2-2* and Δ*hda-2-3* kept in the dark as control
740 (CTRL), after a blue-light pulse (BL) or cut with a scalpel (injury, I), and photographed 48 h later (a).
741 Radial colony growth of wt (dark grey bars), Δ*hda-2-1*, Δ*hda-2-2* and Δ*hda-2-3* (light grey, white and
742 stripped bars, respectively) strains grown in the dark for 96 h was determined (b). *T. atroviride* wt and
743 Δ*hda-2* strains were kept in the dark or exposed to a blue-light pulse and 12 h later the *con-1* gene was
744 assessed by RT-qPCR (c). RT-qPCR results are reported as fold-change compared to the untreated
745 sample in the dark. *tef-1* was used as control gene to normalize the expression of *phr-1*, *hda-2*, and *con-*
746 *1* using the $2^{-\Delta\Delta Ct}$ method. The graphs show the mean expression levels ± SD. The results are based on
747 three repeats in two independent experiments.



748

749 **Fig. 3. Expression of blue light responsive genes is dependent on HDA-1.** Mycelia of wt, Δ*blr-1* and
750 Δ*hda-2* strains grown in the dark were exposed to a blue light pulse, collected at the indicated times
751 and used for expression analysis of *hda-2* (a), *blr-1* (b), *phr-1* (c), *grg-2* (d), *env1* (e) and *bld-2* (f).
752 Mycelia kept in the dark were used as control. *tef-1* was used as a control gene to normalize the
753 expression of blue light responsive genes, using the $2^{-\Delta\Delta Ct}$ method. The graphs show the mean
754 expression levels ± SD. The results are based on three repeats in two independent experiments.

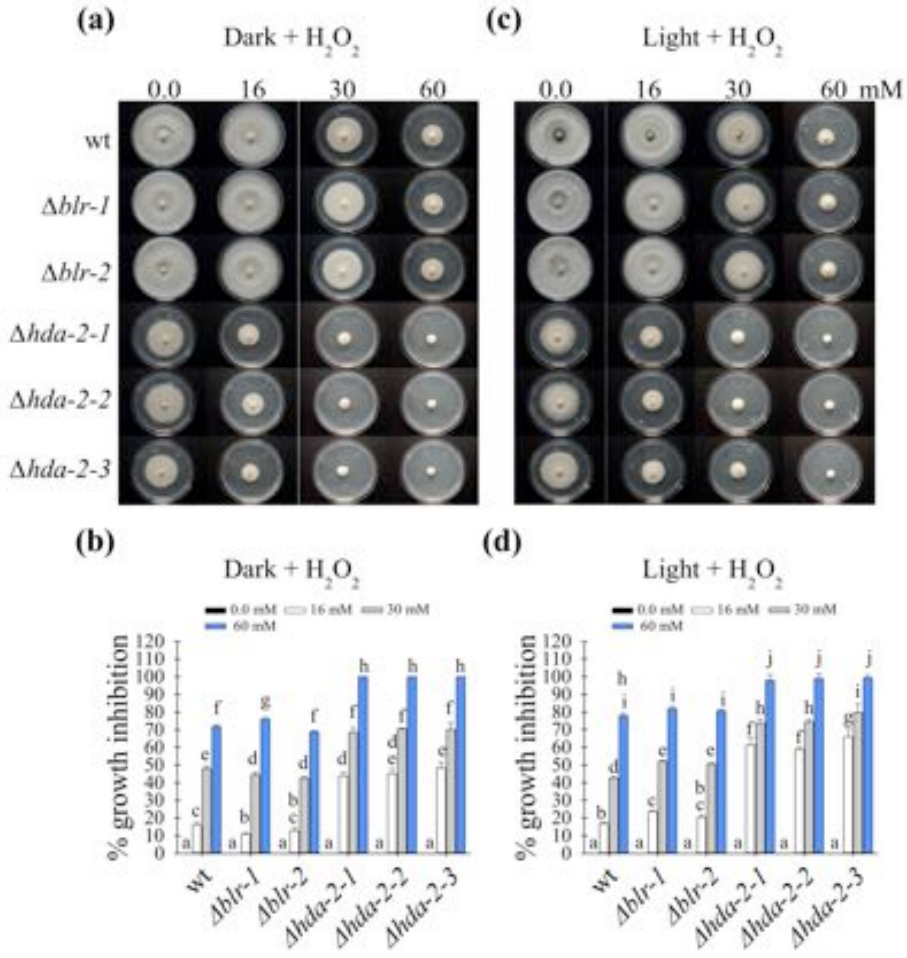
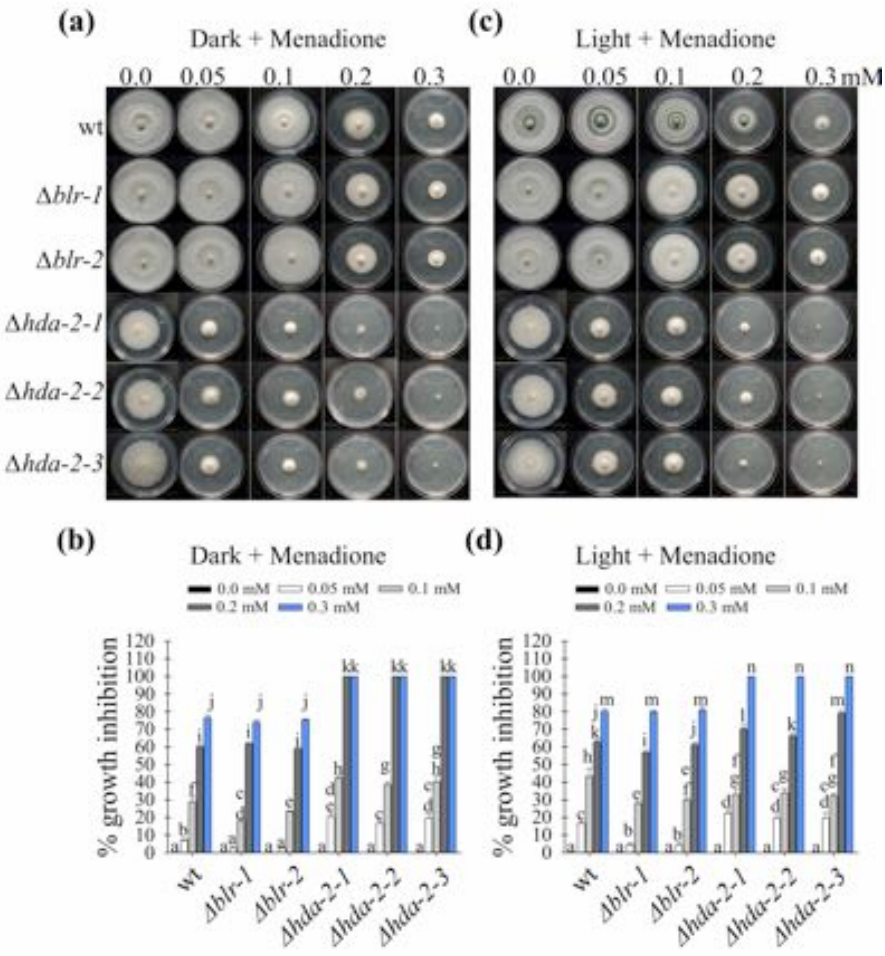


Fig. 4. The *hda-2, blr-1* and *blr-2* gene-lacking mutants differentially respond to H_2O_2. Growth inhibition phenotypes of wt, Δ*blr-1*, Δ*blr-2* and Δ*hda-2* strains after 96 h of growth in the dark in medium amended with different concentrations of H_2O_2 (a) or exposed to 12 h light/dark cycles (c). Graphs represent percentage of growth inhibition after 72 h of growth under different concentrations of H_2O_2 in the dark (b) or exposed to 12 h light/dark cycles (d) relative to the control condition. Control strains grown on medium without H_2O_2 in the dark or exposed to 12 h light/dark cycles were included. The graphs show the mean growth ± SD. The results are based on three repeats in three independent experiments.



765

766 **Fig. 5**. **The *hda-2, blr-1* and *blr-2* gene-lacking mutants differentially respond to menadione.**
767 Growth inhibition phenotypes of wt, Δ*blr-1*, Δ*blr-2* and Δ*hda-2* strains after 96 h of growth in the dark
768 amended with different concentrations of menadione (a) or exposed to 12 h light/dark cycles (c).
769 Graphs represent percentage of growth inhibition after 72 h of growth under different concentrations of
770 menadione in the dark (b) or exposed to 12 h light/dark cycles (d). Control strains grown on medium
771 without menadione in the dark or exposed to 12 h light/dark cycles were included. The graphs show the
772 mean growth ± SD. Different letters indicate statistically significant differences among mean values ($p < 0.05$). The results are based on three repeats in three independent experiments.
773

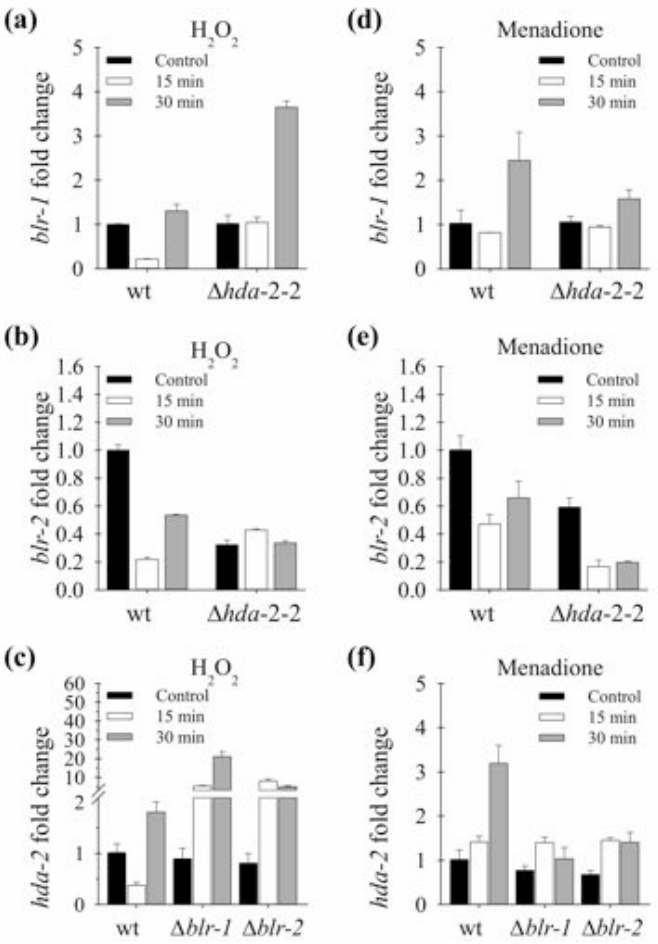
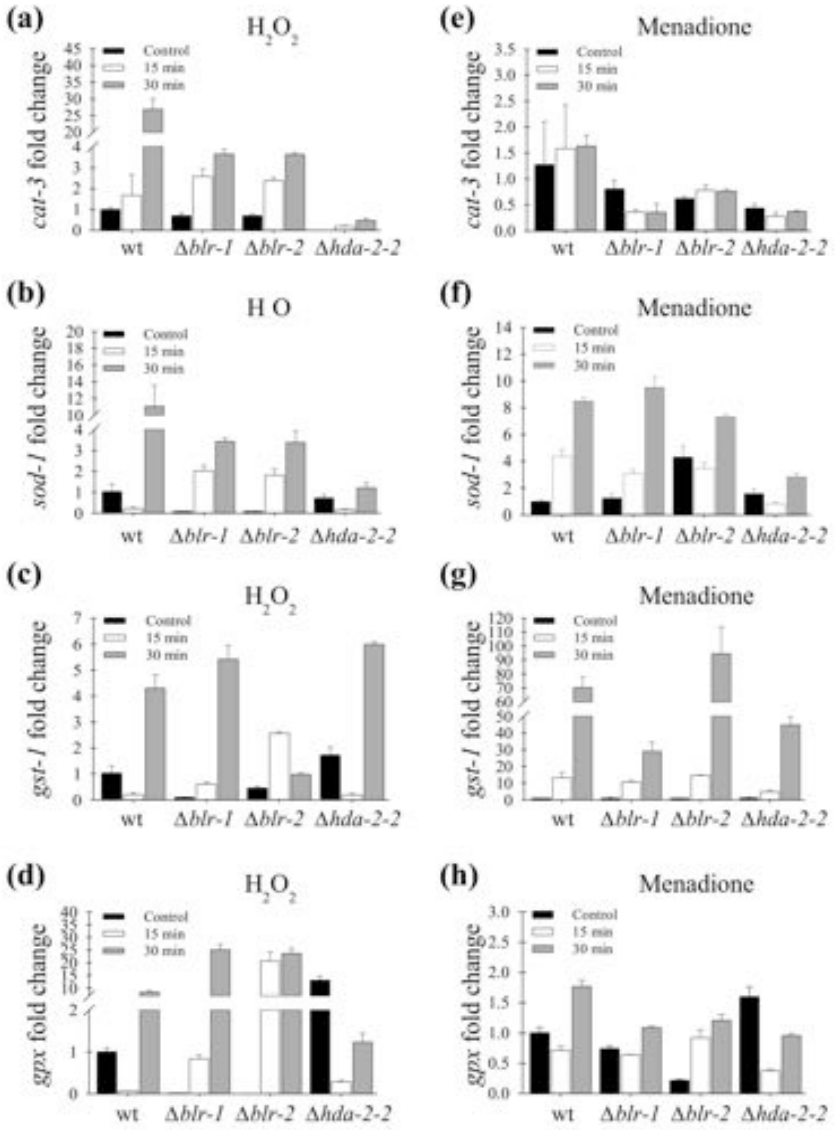
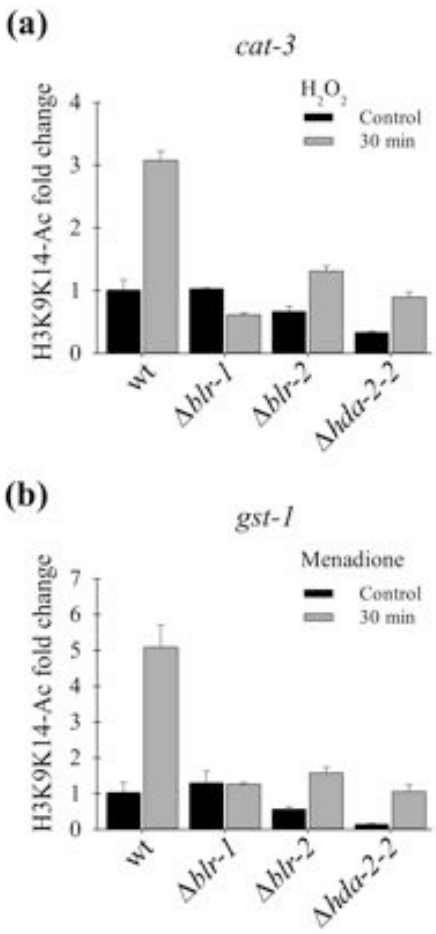


Fig. 6. Relative expression of *hda-2, blr-1* and *blr-2* in response to ROS-generating substrates. Mycelia of wt, Δ*hda-2*, Δ*blr-1* and Δ*blr-2* strains grown in the dark were treated with 30 mM H_2O_2 or 0.2 mM menadione and at the indicated times *blr-1* (a and d) and *blr-2* (b and e) transcripts were measured in wt and Δ*hda-2* strains by RT-qPCR, whereas *hda-2* (c and f) was measured in wt, Δ*blr-1* and Δ*blr-2*. Untreated mycelia of the different strains were used as control. *tef-1* was used as control gene to normalize the expression of *hda-2*, *blr-1* and *blr-2* using the $2^{-\Delta\Delta Ct}$ method. The graphs show the mean expression levels ± SD. The results are based on three repeats in two independent experiments.



783

784 **Fig. 7**. **HDA-2 and the BLR complex regulate the transcription of ROS-related genes in response**
785 **to H_2O_2 and menadione**. Relative expression of *cat-3*, *sod-1*, *gst-1*, and *gpx* genes in wt, Δ*blr-1*, Δ*blr-*
786 *2* and Δ*hda-2-2* strains under control conditions or exposed to 30 mM H_2O_2 (a-d) or 0.2 mM
787 menadione at indicated time were determined (e-h). The *tef-1* gene was used as control to normalize the
788 expression of ROS-related genes using the $2^{-\Delta\Delta Ct}$ method. The graphs show the mean expression levels
789 ± SD. The results are based on three repeats in two independent experiments.



790

791 **Fig. 8. HDA-2 and BLR complex are required under oxidative stress for histone H3 acetylation**
792 **on the promoter of ROS-related genes.** ChIP assays using specific histone H3-ac antiserum were
793 performed with crosslinked mycelia of the wt, Δ*hda-2-2*, Δ*blr-1* and Δ*blr-2* strains under control
794 conditions or 30 min after their exposure to 30 mM H_2O_2. Specific primers designed on *cat-3* (a) and
795 *gst-1* (b) promoters were used to amplify the crosslinked chromatin. The graphs show the mean
796 crosslinked chromatin levels ± SD. Antibody anti-H3 was used to normalize the acetylation enrichment
797 on *cat-3* and *gst-1* promoters, using the method $2^{-\Delta\Delta CT}$. The results are based on two independent
798 experiments.

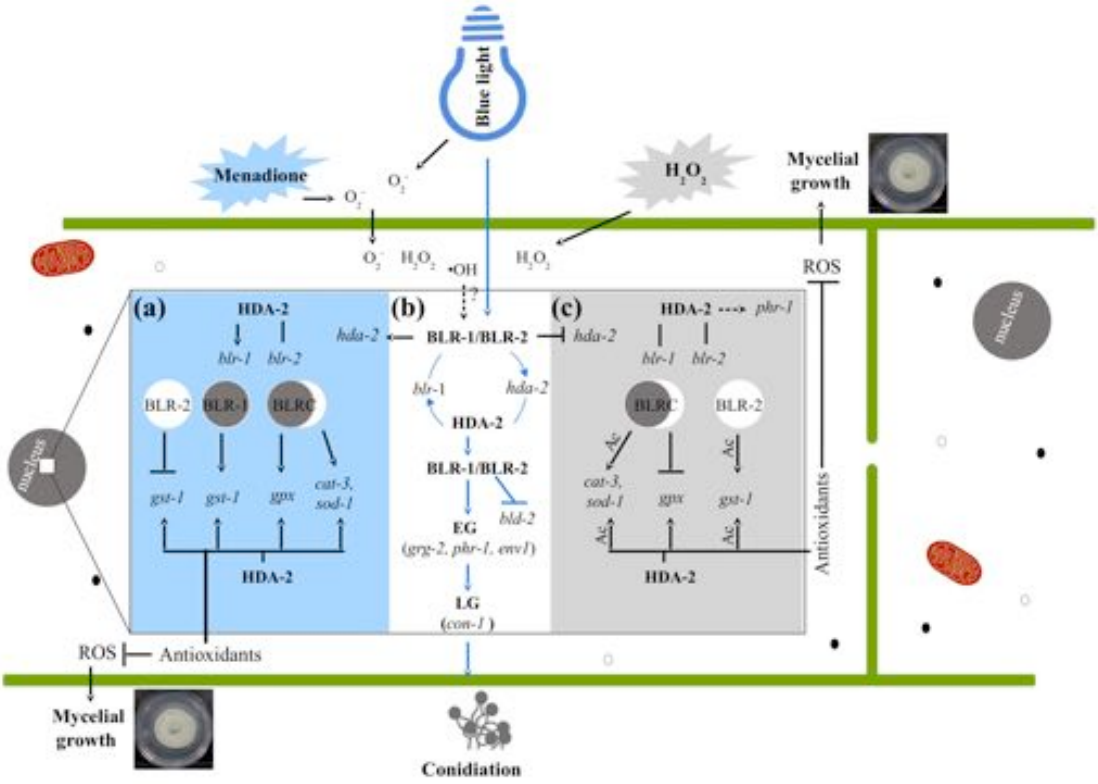


Fig. 9. Hypothetical model for HDA-2, BLR-1 and BLR-2 acting in blue light perception and oxidative stress in *T. atroviride*. Our results indicate an important role for HDA-2 in mycelial growth and conidia development, blue light gene induction and repression, as well as to contend against oxidative stress. Blue light activation of *hda-2* and *blr-1* are mutually dependent of their products HDA-2 and BLR-1, respectively, suggesting a regulatory feedback loop. Lack of the *hda-2* and *blr-1* genes has repercussions on the regulation of early (*phr-1*, *grg-2*, *env1*, *bld-2*) and late (*con-1*) blue-light responsive genes (white panel). ROS such as H_2O_2, light-induced and pro-oxidants can diffuse into the cell to activate *hda-2* expression, thus controlling the resistance to oxidative stress. When growing on medium amended with menadione, a superoxide-generating agent (blue panel), HDA-2 positively regulates the expression of *blr-1* and is necessary to maintain *blr-2* basal levels, and the products of

these genes positively regulate *hda-2* expression. BLR-1 and BLRC are positive regulators of *gpx*, and are necessary to maintain the basal levels of *cat-3*, whereas BLR-2 negatively controls *gst-1*. HDA-2 is necessary for the induction of *sod-1*, *gst-1* and *gpx*, and necessary to maintain *cat-3* basal levels. In the presence of menadione, the BLR proteins seem to play a negative role in colony growth, whereas HDA-2 plays the opposite role. Under H_2O_2 conditions (grey panel), HDA-2 is essential for *cat-3* and *sod-1* induction and it is necessary to maintain *gpx* basal levels, which seems to be important to contend against H_2O_2. Furthermore, HDA-2 is required for *blr-2* basal expression, whose product together with BLR-1 represses *hda-2* transcription. BLRC negatively controls the expression of *gpx*, whereas it seems to play a minor role in the regulation of *cat-3*, *sod-1*, and *gst-1*. HDA-1 and BLRC play a positive role in resistance to H_2O_2 for proper colony growth, with HDA-2 playing the major role. Finally, HDA-2 and BLRC appear to be necessary for proper acetylation (Ac) of H3 on the promoters of *cat-3* and *gst-1*.

Applied and Environmental Microbiology