Histone acetyltransferase TGF-1 regulates *Trichoderma atroviride* secondary metabolism and mycoparasitism

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Abstract

Some filamentous fungi of the *Trichoderma* genus are used as biocontrol agents against airborne and soilborne phytopathogens. The proposed mechanism by which *Trichoderma* spp. antagonizes phytopathogens is through the release of lytic enzymes, antimicrobial compounds, mycoparasitism, and the induction of systemic disease-resistance in plants. Here we analyzed the role of TGF-1 (*Trichoderma Gcn Five-1*), a histone acetyltransferase of *Trichoderma atroviride*, in mycoparasitism and antibiosis against the phytopathogen *Rhizoctonia solani*. Trichostatin A (TSA), a histone deacetylase inhibitor that promotes histone acetylation, slightly affected *T. atroviride* and *R. solani* growth, but not the growth of the mycoparasite over *R. solani*. Application of TSA to the liquid medium induced synthesis of antimicrobial compounds. Expression analysis of the mycoparasitism-related genes *ech-42* and *prb-1*, which encode an endochitinase and a proteinase, as well as the secondary metabolism-related genes *pbs-1* and *tps-1*, which encode a peptaibol synthetase and a terpene synthase, respectively, showed that they were regulated by TSA. A *T. atroviride* strain harboring a deletion of *tgf-1* gene showed slow growth, thinner and less branched hyphae than the wild-type strain, whereas its ability to coil around the *R. solani* hyphae was not affected. Δ*tgf-1* presented a diminished capacity to grow over *R. solani*, but the ability of its mycelium -free culture filtrates (MFCF) to inhibit the phytopathogen growth was enhanced. Intriguingly, addition of TSA to the culture medium reverted the enhanced inhibition growth of Δ*tgf-1* MFCF on *R. solani* at levels compared to the wild-type MFCF grown in medium amended with TSA. The presence of *R. solani* mycelium in the culture medium induced similar proteinase activity in a Δ*tgf-1* compared to the wild-type, whereas the chitinolytic activity was higher in a Δ*tgf-1* mutant in the absence of *R. solani*, compared to the parental strain. Expression of mycoparasitism- and secondary metabolism-related genes in Δ*tgf-1* was differentially regulated in the presence or absence of *R. solani*. These results indicate that histone acetylation may play important roles in the biocontrol mechanisms of *T. atroviride*. 

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Introduction

Nucleosome, the basic unit of chromatin, consists of ~146 base pairs (bp) of DNA wrapped around a histone octamer composed of two copies of each of the core histones H2A, H2B, H3, and H4 [1]. In eukaryotes, gene transcription is strongly dependent on chromatin compactness. Relaxed chromatin (euchromatin) comprises most of the active genes, whereas tightly packed chromatin (heterochromatin) comprehends most of the repressed genes [2,3]. Chemical modifications of histone N-terminal tails, including phosphorylation, methylation, ADP-ribosylation, and acetylation, among others, strongly correlate with chromatin structure and gene regulation [3]. Among all known histone tails’ chemical modifications, probably histone acetylation is the best understood to date. Histone acetylation levels result from the balance of the activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). Acetylation of lysine residues in histone N-terminal tails is related to a relaxed chromatin leading to gene transcription, whereas deacetylation is tightly associated with heterochromatin resulting in gene repression [4]. Several studies have revealed important roles of histone acetylation in gene transcription of various cellular processes [4]. However, some reports have shown that HATs and HDACs are also required for gene repression and activation, respectively [5–7]. Histone acetylation is achieved by larger multisubunit complexes that are recruited by specific gene promoters to modify local chromatin structure and, thus, regulate transcription [8]. The HAT Gcn5 (general control nondepressible-5) is part of the SAGA complex (Spt/Ada/Gcn5 L-acetyltransferase) and is the most studied member of the GNAT family (N-acetyltransferase family related to Gcn5), which preferentially acetylates lysines 9, 14, 18, and 27 of histone H3 [9]. In the budding yeast *Saccharomyces cerevisiae*, histone acetylation has been widely studied; however, in filamentous fungi this regulation mechanism has been poorly approached. Orthologous proteins to Gcn5 have been identified in filamentous fungi [5, 10–14]. In *Neurospora crassa*, the orthologous to Gcn5, NGF-1 acetylates lysine 14 of histone H3 through its association with the photoreceptor White Collar-1 for light activation of the *al-3* promoter [5, 15]. Recently, the participation of histone acetylation in development, as well as in primary and secondary metabolism through the HDAC HdaA, and the orthologous to Gcn5, GcnE has been described in *Aspergilli* [11, 16–18]. In *Ustilago maydis*, a maize pathogen, Gcn5 plays important roles in gene regulation, affecting virulence and dimorphic transition of yeast to mycelium [13, 14]. Furthermore, in *Trichoderma reesei*, TrGcn5 regulates mycelial growth, conidiation, and cellulase gene expression [10].

The *Trichoderma* genus comprises a group of cosmopolitan microorganisms with a variety of lifestyles and high plasticity to adapt to different ecosystems. For instance, *Trichoderma* spp. are commonly found growing in soil or as saprophytes in bark or decaying wood, as well as in many other substrates [19]. Furthermore, it has been suggested that these fungi have, as an ancestral lifestyle, the ability to antagonize other fungi by means of mycoparasitism [20]. This is one of the many reasons why they are used in biological control of soil- and air-borne phytopathogenic fungi [21]. In addition to the mycophagy behavior of *Trichoderma* to antagonize phytopathogenic microorganisms, other mechanisms such as competition for space and nutrients, antibiosis, and the activation of plant systemic disease-resistance have been proposed [22–25]. Mycoparasitism initiates by the recognition and adhesion of *Trichoderma* to the host cell wall, followed by the hydrolysis of host hyphae by means of lytic enzymes, concluding with the uptake of the host cellular content [26]. The main known activities implicated in host cell wall degradation include chitinase, glucanase, N-acetylglucosaminidase, and protease activities [19, 27]. Integration of multiple copies of cell wall degrading enzymes or proteases encoding genes in the *Trichoderma* genome, has provided increased biocontrol activity. For instance, *Trichoderma* strains overexpressing either ech-42, encoding an endochitinase of the family 18
of the glycosyl hydrolases, or prb-1, which codes for a basic protease of the subtilisin type, provide more protection to plants against root and foliar pathogens [28–31].

Intriguingly, the cooperative effect between lytic enzymes and antibiotics to disrupt cell walls has been proposed [32]. In this sense, Trichoderma spp. use non-ribosomal peptide synthetases (NRPS) to produce linear peptides called peptaibols, which exert antibacterial, anti-fungal, and occasionally antiviral activities [33–35]. The peptaibol synthetase PBS-1 of T. atroviride contains 19 typical peptide synthetase modules with the required additional modifying domains at its N- and C-termini [36]. In fungi, the 4-phosphopantetheinyl transferase (PPTase) activates enzymes involved in primary and secondary metabolism [37]. In T. virens, a Δppt1 mutant was unable to synthesize peptaibols, and was also incapable of inhibiting the growth of phytopathogenic fungi and oomycetes [38]. Moreover, in T. virens, a secondary metabolite deficient mutant showed a reduced expression of genes whose products are related to secondary metabolism. In agreement with these results, the fungus was incapable of synthesizing the antibiotics viridiol and viridin [39].

The search to identify regulators of mycoparasitism and secondary metabolism-related genes in the presence of external stimuli, such as the presence of a host, needs to consider the way that leads to chromatin modifications in Trichoderma.

In this work, we analyzed the effect of Trichostatine A (TSA), an inhibitor of HDACs from class I and II [40], in mycoparasitism, in the production of antibiotics, and in the expression of mycoparasitism- and secondary metabolism-related genes in T. atroviride during its interaction with the phytopathogenic fungus Rhizoctonia solani. The role of TGF-1, the orthologous to Gcn5 of S. cerevisiae in T. atroviride, on mycoparasitism and on the transcriptional regulation of mycoparasitism and secondary metabolism-related genes was also assessed. Our results provide new insights into the biological control mechanisms of phytopathogens by Trichoderma.

Materials and methods

Microorganisms and growth conditions

T. virens Gv29-8 [41], T. atroviride IMI206040 and Δtgf-1 (Uresti-Rivera et al., in preparation) were used throughout this study. Rhizoctonia solani, Trichoderma citrinoviride and Trichoderma harzianum strains were isolated from a tomato field in San Luis Potosi, Mexico (22° 38’ 39.84” N, 100° 50’ 56.4” W), and identified by PCR amplification of the internal transcribed spacer (ITS) of ribosomal deoxyribonucleic acid (rDNA) sequences, using the oligonucleotides ITS1 and ITS4 [42]. Fungal strains were routinely grown in potato dextrose agar (PDA) or potato dextrose broth (PDB) (both from Difco™, BD Becton, Dickinson and Company, New Jersey, USA), as indicated for each experiment. When indicated, 300 nM of TSA (Sigma-Aldrich, Taufkirchen, Germany) was added to the media. All the strains were incubated at 28 °C at the indicated times for each experiment.

Dual cultures of T. atroviride wild-type and Δtgf-1 strains versus R. solani and other Trichoderma species

A plug of actively growing mycelium of T. atroviride wild-type (wt) strain was placed at one edge of the Petri dish, whereas at the opposite edge a plug of actively growing mycelium of R. solani, T. virens, T. citrinoviride or T. harzianum was placed. The dual cultures were incubated at 28 °C for 36, 48, 60, 72, 96, 120, 168, and 196 h and photographed. Radial growth inhibition of R. solani by T. atroviride strains was determined after 36, 48, 60, 72 and 96 h post-inoculation. The Δtgf-1 inocula were obtained by growing the fungus at 28 °C for 96 h in PDB at 250
rpm. The mycelium was centrifuged 5 min at 2000 rpm and used to inoculate PDA plates at 3 cm from the middle, and allowed to grow for 72 h before inoculating with the other fungal strains, as described above. Two independent experiments were performed in triplicate. Photographs were taken at the indicated times. In addition, dual cultures of *T. atroviride* wt or Δtgf-1 against *R. solani* were carried out on PDA plates with or without TSA (300 nM). The different fungal strains were grown alone on PDA plates with or without TSA as controls of the interactions.

**Antimicrobial activity of *T. atroviride* wt and Δtgf-1 mycelium-free culture filtrates versus *R. solani***

*T. atroviride* wt and Δtgf-1 strains were grown in PDB or Vogel minimal medium supplemented with 1.5% sucrose [43] in the presence or absence of 300 nM TSA for 7 days. Then, cultures were filtered using Corning® 500 ml bottle top vacuum filter, 0.2 μm-pore 33.2 cm² nylon membrane (Corning Life Sciences, Massachusetts, USA). Mycelium-free culture filtrates (MFCF) were used to prepare 1 × PDA plates containing 60% of MFCF and 40% of sterile distilled water. A mycelial plug of an actively growing colony of *R. solani* was inoculated on the center of a PDA-MFCF plate, incubated at 28 °C, and radial growth was measured after 12, 24, 36, 48, and 60 h post-inoculation. PDA plates without MFCF inoculated with *R. solani* were included as controls. Two independent experiments were performed in triplicate.

**Induction of Trichoderma cultures with *R. solani* mycelium***

*R. solani* mycelium was grown in PDB medium for 7 days and filtered as described for MFCF. After filtration, *R. solani* mycelium was lyophilized, frozen in liquid nitrogen, and ground. For the induction of *T. atroviride* wt or Δtgf-1, 0.7 g of *R. solani* sterilized powder was added to 500 ml of PDB previously autoclaved. The induction medium was inoculated with 10 plugs (0.5 cm²) of *T. atroviride* wt or Δtgf-1 strains and incubated at 28 °C with agitation at 250 rpm for 7 days in the darkness. The wt and Δtgf-1 induced MFCF were obtained by filtration as described above for *R. solani*. The mycelium was discarded and the MFCF were concentrated by lyophilization. The wt and Δtgf-1 lyophilized samples were resuspended in 500 μl of phosphate buffer, and protein concentration was determined as described by [44].

**Relative expression of mycoparasitism (ech-42 and prb-1) and secondary metabolism-related genes (tps-1 and pbs-1) in dual culture experiments***

Dual cultures of *T. atroviride* wt and Δtgf-1 against *R. solani* were performed as described above, but placing a sterile cellophane sheet on the PDA plates before inoculation of the fungi. Dual cultures were incubated for 36, 48, and 60 h at 28 °C. Mycelia of the different *Trichoderma* strains were collected at the indicated times, frozen in liquid nitrogen, and ground for total RNA extraction by the Trizol® method (Invitrogen, New York, USA). Total RNA (5 μg) was treated with Turbo DNase as described by the manufacturer (Ambion, Life Technologies, New York, USA). cDNA was synthesized using the Superscript® kit (Invitrogen), following the manufacturer’s protocol. Subsequently, cDNA was quantified using a Nanodrop spectrophotometer (ND-1000, Thermo Scientific, Delaware, USA). Relative gene expression was assessed by quantitative reverse transcription PCR (RT-qPCR), using specific oligonucleotides (Table 1) in an Applied Biosystems 7500/7500 Fast Real-Time PCR System, using the Fast SYBR® Green Master Mix (Applied Biosystems, Foster City, California), in a final volume of 20 μl per reaction. Reactions were set up by using the standard settings of the system, except for the oligonucleotides annealing temperature (Table 1), which was 62 °C. Two independent
experiments were performed in triplicate. Relative genes expression was calculated using the $2^{\Delta\Delta Ct}$ method [45].

**Scanning Electron Microscopy (SEM) micrographs of T. atroviride wt and Δtgf-1 versus R. solani**

For SEM analysis, dual cultures of T. atroviride wt and Δtgf-1 strains against themselves or against R. solani were performed as described in the previous section. For SEM analysis, 0.5-cm² agar pieces of the different confrontations at the indicated times were fixed in 3% glutaraldehyde in PBS (phosphate buffered saline) for 2 h. Samples were rinsed three times with cold PBS for 15 min each. Afterwards, samples were post-fixed with 1% OsO₄ in PBS solution for 1 h, followed by three rinses in PBS, 15 min each, and then dehydrated in graded ethanol. Critical point drying was done in a Tousimis Samdri-PVT-3D, mounted, and gold-coated sputtering in the Cressington Model 108 auto, and examined in a FEI model Quanta 200 SEM. The SEM was adjusted at 18 kV, spot 5, WD 10 mm, and the photomicrographs were taken with Everhart Thornley Detector (ETD).

**Proteinase activity**

Proteinase activity was determined in a 500-μl reaction mix containing 100 μl of Trichoderma atroviride wt and Δtgf-1 strains induced MFCF, 0.5 mM Suc-Ala-Ala-Pro-Phe-pNA (Sigma, Cat number S7388) in 50 mM MOPS, pH 7.0. The reaction mix was incubated at 37 °C for 20 min, and 500 μl of ice-cold water was added to stop the reaction, and measured spectrophotometrically at 405 nm. The activity was expressed as millimoles of p-nitroanilide released per minute [46]. Specific activity was referred to 1 mg of protein.

**Chitinolytic activity**

Chitinolytic activity was assayed by estimating the reducing ends of sugars as described elsewhere [47]. The reactions were performed in a 600-μl mix containing 300 μl of 1% colloidal chitin, pH 6.6, and 300 μl of a 1/100 dilution of the wt or Δtgf-1 concentrated induced MFCF. The reaction mix was incubated 30 min at 37 °C, and the hydrolysis reactions were terminated and analyzed by adding 600 μl of dinitrosalicylic acid reagent (DNS). Then, the reaction mix was boiled for 15 min, and the insoluble chitin was removed by centrifugation, absorbance was measured spectrophotometrically at 540 nm. One unit of chitinase activity is defined as the amount of enzyme required to release 1 μmol of detectable reducing sugars in 1 min at 37 °C.

Table 1. Oligonucleotide used in this study.

| Gene name | JGI Protein ID | Primer sequence (5’ to 3’) | Primer name | Annealing temperature | Product size (bp) |
|-----------|----------------|----------------------------|-------------|-----------------------|------------------|
| ech-42    | 176466         | atgtagtctccccttgcggaaatcc  | Ech-42 fw   | 60 °C                 | 237              |
|           |                | ccagtttctgaggctggagaattt   | Ech-42 Rv   |                       |                  |
| prb-1     | 268415         | atgaccagcattcgtctgctcgtc  | Prb-1 fw    | 60 °C                 | 206              |
|           |                | gcagtttcgctcgttcggttaagaa | Prb-1 Rv    |                       |                  |
| pbs-1     | 317938         | cccagagaagctgcgaagga       | Pbs-1 fw    | 60 °C                 | 152              |
|           |                | gctctctgctcgtgtt           | Pbs-1 Rv    |                       |                  |
| tps-1     | 31441          | ccattttctggctctgtctcctaa   | Tps-1 fw    | 60 °C                 | 150              |
|           |                | gcacgtctgtctgttaagcc        | Tps-1 Rv    |                       |                  |
| act-1     | 297070         | tcaccgaggcccc caccaacc     | Act fw      | 60 °C                 | 127              |
|           |                | gcacgggaggctcag ggacaga    | Act Rv      |                       |                  |

*JGI, Joint Genome Institute

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Data analysis
Experiments were statistically analyzed in the SPSS 10 program (SPSS, Chicago). Multivariate analyzes with a Tukey's post hoc test were used for testing differences in antimicrobial activity of MFCF, RT-qPCR and biochemical analysis (proteinase and chitinolytic activity). Linear regression analysis was done using the SPSS 10 program (SPSS, Chicago). Different letters are used to indicate means that differ significantly ($P \leq 0.05$).

Results

Trichostatin A (TSA) did not affect the growth of *T. atroviride* over *R. solani*

To elucidate the involvement of chromatin acetylation on the biocontrol capacities of *T. atroviride*, we first assessed the effect of the histone deacetylase (HDAC) inhibitor Trichostatin A (TSA) on the growth of *T. atroviride* over *R. solani* in dual cultures. *T. atroviride* was grown alone or in confrontation with *R. solani* on PDA plates amended or not with TSA (300 nM). Dual cultures were visually inspected and photographed after 36, 48, 60, 72, and 96 h of co-culture (Fig 1A and 1B). Addition of TSA to the growing medium slightly affected the growth of *T. atroviride* and *R. solani* strains (Figs 1B and 2) compared to the control without TSA (Figs 1A and 2). However, the capacity of *T. atroviride* to growth over *R. solani* was not affected by the presence (Fig 1B) or absence of TSA (Fig 1A).

Addition of TSA to *T. atroviride*-growing medium enhanced its inhibitory effect on *R. solani* growth

As byproducts of their metabolism, most *Trichoderma* species are capable of synthetizing a wide range of antimicrobial compounds, used to combat phytopathogenic fungi. To determine whether TSA has an effect on the synthesis of antimicrobial compounds in *T. atroviride*, the fungus was grown for 7 days in PDB amended or not with 300 nM TSA. Thereafter, *Trichoderma* cultures were filtered to remove the mycelium. Plugs of *R. solani* were inoculated at the center of PDA plates amended with mycelium-free culture filtrates (MFCF) (final concentration of 60%) and the radial growth of *R. solani* was measured at 12, 24, 36, 48, and 60 h. As shown in Fig 2, addition of MFCF to PDA plates (crosshatched bars) inhibited *R. solani* radial growth compared to *R. solani* growth on PDA control plates with (arrow filled bars) or without TSA (black bars) (Fig 2). Furthermore, addition of MFCF plus 300 nM TSA to PDA plates (white dotted bars) enhanced the inhibition of *R. solani* radial growth compared to PDA-MFCF medium without TSA (crosshatched bars) (Fig 2).

TSA and *R. solani* presence negatively affected *T. atroviride* ech-42, prb-1, pbs-1, and tps-1 gene expression

To investigate whether the addition of TSA to the culture medium affected the expression of *T. atroviride* mycoparasitism-related genes ech-42 and prb-1, and those related to secondary metabolism, pbs-1 and tps-1, dual cultures of this fungus against *R. solani* were performed on PDA plates amended or not with 300 nM TSA. In the absence of TSA but in the presence of *R. solani*, expression of ech-42 remained unalterable after 36 and 48 h of co-culture, whereas prb-1 and tps-1 were induced after 36 h. However, all three genes were repressed after 48 h and upregulated after 60 h of co-culture (grey bars) (Fig 3A, 3B and 3D). In contrast, pbs-1 was downregulated at all tested times in the presence of the phytopathogen (grey bars) (Fig 3C). Addition of TSA to the culture medium in the absence of *R. solani* induced the expression of ech-42, and tps-1 after 36 h (black bars); but ech-42 was downregulated in the presence of *R.
Furthermore, expression of \textit{tps-1} but no \textit{prb-1}, \textit{ech-42}, and \textit{pbs-1} was considerably high at 48 h. However, \textit{prb-1}, \textit{tps-1}, and \textit{pbs-1} expression was significantly high 60 h after the addition of TSA in the absence of \textit{R. solani} (black bars), but down-regulated or almost abolished in presence of the phytopathogen (grey bars), except \textit{ech-42} (Fig 3F, 3G and 3H). The presence of TSA and the phytopathogen marginally downregulated the expression of \textit{ech-42} after 36 and 48 h, which was marginally upregulated after 60 h of co-culture compared to their respective times in the presence of TSA alone (grey bars) (Fig 3E).

**Deletion of \textit{tgf-1} did not affect \textit{T. atroviride} mycoparasitic effect against \textit{R. solani}**

The mutant growing phenotype of $\Delta\textit{tgf-1}$, whose wild-type gene encodes for a histone acetyltransferase, was analyzed by inoculating \textit{T. atroviride} \textit{wt} and $\Delta\textit{tgf-1}$ on PDA plates during 72 h under 12:12 light:dark regime (Fig 4A and 4B). The $\Delta\textit{tgf-1}$ strain showed a slow growth.

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**Fig 1. Trichostatin A did not affect the overgrowth of \textit{Trichoderma atroviride} on \textit{Rhizoctonia solani}**

Dual confrontation plates of \textit{T. atroviride} (left side) against \textit{R. solani} (right side) on PDA plates in the absence (A) or presence (B) of 300 nM Trichostatin A, incubated at 28 °C and photographed at 36, 48, 60, 72, and 96 h. Ta = \textit{T. atroviride}, Rs = \textit{R. solani}, Trichostatin A = TSA. Images are representative of similar results from three independent trials.

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phenotype and lack of the typical green colored conidia compared to the wt strain (Fig 4A and 4B). SEM photomicrographs of the Δtgf-1 strain showed a thin, flat and less branched mycelium compared to the wt strain (Fig 4C and 4D).

To determine whether the product of tgf-1 is involved in the antagonistic capacity of T. atroviride against phytopathogenic fungi, dual cultures of wt or Δtgf-1 strains against R. solani were conducted. The wt strain was able to growth over R. solani, whereas the Δtgf-1 strain was scarcely able to come in contact (Fig 5). Moreover, in confrontation assays of Δtgf-1 against the phytopathogen, the growth of the latter was slightly delayed as compared when R. solani was co-cultured with the wt strain (Fig 5). Linear regression analysis was used to find the relationship between the strains used in dual cultures and the growth inhibition of R. solani (S1 Table).

Positive relationships were found between growth inhibition of R. solani by the wt, and growth inhibition of R. solani by the Δtgf-1 mutant. A strong significant positive ($R^2 = 0.802$, Sig. = 0.000) relationship was observed in growth inhibition of R. solani by the wt, followed by a low, but significant positive relationship in growth inhibition of R. solani by Δtgf-1 mutant ($R^2 = 0.382$, Sig. = 0.014). Since the Δtgf-1 mutant was barely able to enter in contact with the phytopathogen at the different times tested, the 192 h interaction zone of both dual cultures was taken and analyzed by SEM. Fig 6 shows that both, the Δtgf-1 and the wt, strains were able to coil around the phytopathogen hyphae (Fig 6C and 6D).

Dual cultures of Δtgf-1 against itself (Fig 7B), its parental strain (Fig 7C), or other Trichoderma species (Fig 7D–7I) showed that Δtgf-1 could be overgrown by other Trichoderma species compared to its parental strain. Also, the Δtgf-1 mutant was able to stop the growth of other species, showing, in some cases, an enhanced brown color in the contact area, which is indicative of lysed hyphae (Fig 7G and 7I). Confrontation of tgf-1 against itself did not show
Fig 3. TSA and *R. solani* presence negatively affected *T. atroviride* ech-42, prb-1, pbs-1, and tps-1 gene expression. *T. atroviride* and *R. solani* were co-cultured on PDA (A, B, C, and D) or PDA amended with 300 nM TSA (E, F, G, and H) at 28 °C. Total RNA was extracted from *T. atroviride* mycelium collected at 36, 48, and 60 h. Relative expression was calibrated using *act-1* as housekeeping gene and normalized against the wt strain in the absence of the phytopathogen. *ech-42* = 42-kDa endochitinase gene, *prb-1* = basic proteinase gene, *pbs-1* = peptaibol synthase gene, and *tps-1* = terpene synthase gene. Black bars, *T. atroviride* wt strain growing alone (Ta); grey bars, *T. atroviride* vs *R. solani* (Ta+Rs) co-cultures. The bars show the mean ± SD of three independent biological replicates. Different letters are used to indicate means that differ significantly (*P* < 0.05). This assay was repeated twice per triplicate with similar results.

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Fig 4. Colony morphology and development of *T. atroviride* wt and Δtgf-1 strains. *T. atroviride* wt (A) and Δtgf-1 (B) were grown on PDA plates for 7 days at 28 °C and photographed. Both strains were co-cultured on PDA medium, fixed, dehydrated, and analyzed by SEM at 100 (C) and 400 (D) magnifications. The wt is shown in the right side whereas the Δtgf-1 is shown in the left side of the photomicrographs. Images are representative of similar results from two independent trials, including three replicate plates.

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Fig 5. Dual culture assays of *T. atroviride* wt and Δtgf-1 strains versus *R. solani*. wt and Δtgf-1 strains were inoculated at the left side of the Petri dish, whereas *R. solani* was inoculated at the right side. Dual cultures were incubated at 28 °C, and photographed at 72, 96, 120, 168, and 192 h. Ta = *T. atroviride* wt strain, Δtgf-1 = *T. atroviride* tgf-1 mutant strain, Rs = *Rhizoctonia solani*. This assay was repeated twice including three replicate plates.

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Fig 6. Deletion of tgf-1 did not affect T. atroviride mycoparasitic effect against R. solani. Dual culture assays of T. atroviride wt (A) and Δtgf-1 (B) strains versus R. solani were analyzed by SEM (C and D, respectively). Blue arrowheads show R. solani hyphae, whereas red arrowheads show wt and Δtgf-1 coiling hyphae on R. solani. SEM photomicrographs were magnified 1500 ×. The experiments were repeated twice and representative photographs are shown.

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Fig 7. Antagonistic ability of Δtgf-1 strain against other Trichoderma species. Dual cultures of T. atroviride wt and Δtgf-1 strains against other species of T. atroviride, indicated on the right side of the figure, were grown at 28 °C for 96 h. Ta = T. atroviride, Δtgf-1 = T. atroviride tgf-1 mutant strain, Tv = T. virens, Tc = T. citrinoviride, Th = T. harzianum. Images are representative of similar results from three independent trials.

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significant changes in any of the two colonies (Fig 7B); however, when the Δtgf-1 strain was confronted against its parental strain, a lysing zone was observed (Fig 7C).

**MFCF obtained from a Δtgf-1 strain shows enhanced inhibition of *R. solani* growth**

Since the Δtgf-1 strain inhibited the growth of *R. solani* slightly more than the wt strain before they entered in contact (Fig 5 and S1 Table), we decided to assess the capacity of MFCF from Δtgf-1 to inhibit *R. solani* growth. PDA or PDA plus MFCF plates were inoculated in the center with mycelial plugs of *R. solani* and the radial growth of the phytopathogen was measured at 12, 24, 36, 48, and 60 h post-inoculation. MFCF from Δtgf-1 inhibited the growth of *R. solani* more effectively than that obtained from the wt strain (dotted black and black bars, respectively) (Fig 8). Intriguingly, addition of TSA to the Δtgf-1 growing medium diminished the negative effect of MFCF on the phytopathogen radial growth (arrow filled bars), compared to its respective control without TSA (dotted black bars), but MFCF from Δtgf-1 reached the inhibition effect observed with the wt MFCF plus TSA (cross-hatched bars) (Fig 8). Furthermore, the MFCF from the wt and Δtgf-1 grown in liquid Vogel’s minimal medium showed similar results compared when they were grown in PDB medium (S1 Fig and Fig 8, respectively). Addition of TSA to the wt, grown in Vogel’s minimal medium showed also similar results to those observed in PDB (Fig 8). Contrastingly, addition of TSA to the Vogel’s minimal medium

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**Fig 8. Antibiosis assay using MFCF obtained from *T. atroviride* wt or Δtgf-1 strains grown in the presence or absence of TSA against *R. solani*.** The Δtgf-1 and wt strains were grown for 7 days in PDB medium amended or not with 300 nM TSA, at 28 °C. MFCFs obtained from each of these cultures were added to PDA 1× medium at a final concentration of 60%. *R. solani* was inoculated into the different media and its radial growth was determined at 12, 24, 36, 48, and 60 h. Radial growth of *R. solani* on PDA containing *T. atroviride* wt strain MFCF without TSA (black bars) or amended with TSA (crosshatched bars) was determined at the indicated times. Radial growth of *R. solani* on PDA containing *T. atroviride* Δtgf-1 strain MFCF without TSA (black dotted bars) or with TSA (arrow filled bars) was determined at the indicated times. The bars show the mean ± SD of three independent biological replicates. Different letters are used to indicate means that differ significantly (*P* < 0.05). Three replicate plates were established for each treatment, and the experiment was repeated twice.

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increased the growth inhibition effect of the Δtgf-1 MFCF on *R. solani* compared when the mutant was grown in PDB plus TSA (S1 Fig).

**The Δtgf-1 strain shows constitutive chitinolytic but inductive proteolytic activity**

Lysing enzymes such as chitinases and proteases are used by some *Trichoderma* strains as biocontrol mechanisms against phytopathogenic fungi and oomycetes [23]. Aimed at elucidating whether chitinolytic and proteolytic activities were affected in the Δtgf-1 strain, and if they could be contributing to the enhanced inhibition of growth observed against *R. solani*, these activities were determined in cultures of the wt and Δtgf-1 strains induced by the presence of *R. solani* mycelium (Fig 9). Wild-type and Δtgf-1 MFCFs showed similar proteolytic activity under control conditions, whereas in the presence of *R. solani*, this activity increased at similar levels in both strains (Fig 9A). In contrast, the wt strain presented an increased chitinolytic activity in the presence of *R. solani*, compared to the mocked control, whereas the Δtgf-1 MFCF showed enhanced chitinolytic activity in control conditions, which was significantly high than that observed for the wt MFCF under the induced condition (Fig 9B). Addition of *R. solani* to the growing medium did not increase the chitinolytic activity of Δtgf-1 as compared to its own control (Fig 9B).

**TGF-1 and TSA differentially regulate ech-42, prb-1, pbs-1, and tps-1 expression in the presence or absence of *R. solani***

To investigate whether TGF-1 plays a role in the regulation of mycoparasitism- (*ech-42* and *prb-1*) and secondary metabolism-related genes (*pbs-1* and *tps-1*), their expression levels were analyzed in a Δtgf-1 background and compared to its parental strain during their co-culture with *R. solani*, in media amended or not with TSA (300 nM). In the absence of TSA and *R. solani*, *ech-42* showed significantly changes in its expression after 48 and 60 h (black bars) (Fig 10A), whereas *pbs-1*, and *tps-1* were considerably upregulated after 36 and 48 h, but downregulated at 60 h (black bars). *prb-1* was upregulated after 48 h but downregulated at 60 h (Fig 10B, 10C and 10D). The presence of *R. solani*, increased ~2-fold the expression of *ech-42* after 36 h of co-culture, but it was downregulated after 48 and 60 h (gray bars) (Fig 10A). *prb-1* expression suffered no significant changes in the presence of *R. solani* (Fig 10B). However, *pbs-1*, and *tps-1* were downregulated in the presence of the phytopathogen after 36 and 48 h compared to their controls in absence of *R. solani* (gray bars) (Fig 10B, 10C and 10D). In the presence of TSA, *ech-42* did not increase its expression after 36 h; however, it was marginally upregulated after 48 and 60 h (black bars) (Fig 10E). *pbs-1* was downregulated in the presence of TSA after 60 h, whereas *prb-1* and *tps-1* were repressed 48 and 60 h after the addition of TSA, respectively (black bars) (Fig 10E, 10F and 10G). The presence of both, the phytopathogen and TSA, upregulated the expression of *ech-42* and *pbs-1* after 36 and 48 h (gray bars) (Fig 10E and 10G). *prb-1* and *tps-1* were marginally upregulated in the presence of TSA and *R. solani* after 48 h, and downregulated after 60 h (gray bars) (Fig 10G and 10H).

**Discussion**

Some species of the *Trichoderma* genus are used as biocontrol agents of phytopathogenic fungi and oomycetes that affect negatively important crops [22]. The molecular mechanisms governing mycoparasitism and secondary metabolism in *Trichoderma* have been intensely investigated. However, the role of histone acetylation in the biocontrol repertoire shown by these fungi is poorly understood.
Fig 9. TGF-1 plays a negative role in chitinolytic activity but a minor role in proteolytic activity in T. atroviride.

Chitinolytic activity was determined using colloidal chitin by estimating the reducing sugars with dinitro-salicylic acid (DNS) method, measuring absorbance spectrophotometrically at 540 nm. One unit of chitinase activity was defined as the amount of enzyme required to increase absorbance at 540 nm by 1 OD unit ml⁻¹ h⁻¹. Proteinase activity was determined using mM Suc-Ala-Ala-Pro-Phe-pNA (0.5 mM) as substrate, the liberation of pNA was measured spectrophotometrically at 405 nm. The activity was expressed as p-nitroanilide released per min. Specific activity was referred to 1 mg of protein. The effect of induced MFCF of the wt and Δtgf-1 strains was tested over both activities. The bars show the mean ± SD of three independent biological replicates. Different letters are used to indicate means that
differ significantly ($P < 0.05$). Chitinolytic and proteolytic activities were tested in three replicates and repeated two times.

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**Fig 10.** TGF-1 and TSA differentially regulate *T. atrovirese ech-42, prb-1, pbs-1* and *tps-1* in the presence or absence of *R. solani*. Dual culture assays of *T. atrovirese* wt and Δtgf-1 against *R. solani*, or grown alone as control were performed on PDA in absence (A, B, C, D) or in presence of 300 nM TSA (E, F, G, H) at 28 °C. Total RNA was extracted from *T. atrovirese* mycelium collected at 36, 48, and 60 h. Relative expression was calibrated using *act-1* as housekeeping gene and normalized against the wt strain in absence of the phytopathogen. *ech-42* = 42-kDa endochitinase gen, *prb-1* = basic proteinase gene, *pbs-1* = peptaibol synthase gene, *tps-1* = terpene synthase gene. Black bars represent Δtgf-1 strain, whereas gray bars represent Δtgf-1 vs *R. solani* interaction. Addition of TSA is indicated at the top of the panels. The bars show the mean ± SD of three independent biological replicates. Different letters are used to indicate means that differ significantly ($P < 0.05$). This assay was repeated twice per triplicate with similar results.

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Trichostatin A (TSA) did not affect the overgrowth of *T. atroviride* on *R. solani*

In this work, we reported that TSA, a potent inhibitor of histone deacetylases class I and II, slightly affected the growth of *T. atroviride* and *R. solani*; however, the capability of *T. atroviride* to grow over the phytopathogen was not affected. Since TSA is *per se* an antifungal compound, it may have a negative effect on the growth of both fungi [48]. We also speculate that the inhibition of growth in both fungi could be due to the enhanced production of antimicrobial compounds promoted by the addition of TSA. In this regard, addition of TSA to the growing medium increases antimicrobial activity in a number of fungi against facultative bacterial and yeast pathogens [49]. Furthermore, *T. virens* strains that overproduce the antimicrobial compound gliovirin grow slower than the wt strain [49, 50]. Contrastingly, mutants lacking genes whose products are involved in the synthesis of antimicrobial compounds, such as the potent antimicrobial and cytotoxic compound gliotoxin grow faster than the wt strain [38, 51, 52].

**Addition of TSA to *T. atroviride*-growing medium enhanced its inhibitory effect on *R. solani* growth**

Fungi are excellent producers of biological active compounds, as a consequence of their secondary metabolism, including antibiotics, plant growth-regulating molecules, cell toxic compounds, mutagenic, immunosuppressants, enzyme inhibitors, and molecules with other biological effects [25,53,54]. Here, we show that the addition of TSA enhanced the ability of *T. atroviride* MFCF to inhibit *R. solani* growth. These data suggest that inhibition of HDACs class I and II, such as Rpd3p and Hda1p, by TSA may be affecting the production of antimicrobial compounds in *T. atroviride*, probably by an increase in the acetylation of histone tails. This increased acetylation could de-repress silenced chromatin regions that contain genes involved in secondary metabolism and/or in the synthesis of antimicrobial compounds in this fungus. A genomic analysis of three *Trichoderma* species, including *T. atroviride*, showed that all of them contain the orthologous genes to *RPD3* and *HDA1* of *S. cerevisiae* [12]. It was recently demonstrated that the addition of HDAC inhibitors, including TSA, induces the antimicrobial activity of extracts on several fungi [49]. Moreover, *A. nidulans* mutants lacking components involved in chromatin compaction, such as HepA (heterochromatin protein 1) show de-repression of genes involved in the biosynthesis of secondary metabolites. In agreement with these results, the silent sterigmatocystin gene cluster shows low levels of repressive histone marks (trimethylated H3K9), whereas histone H3 acetylation is increased [55].

**TSA and *R. solani* presence negatively affected *T. atroviride* ech-42, prb-1, pbs-1, and tps-1 gene expression**

Expression analysis of mycoparasitism and secondary metabolism-related genes showed that *R. solani* induced the expression of ech-42 and prb-1, as previously reported [28,56]. In addition, the presence of *R. solani* also induced the transcription of *tps-1* and the repression of *pbs-1*. A plausible explanation for the repression of *pbs-1* in the presence of *R. solani* is that the phytopathogen is trying to block the production of the peptaibols produced by *T. atroviride* through the synthesis of effector molecules. However, this is not the case for *tps-1*, which is involved in the synthesis of volatile terpenes. This fact, points to different regulation pathways for *pbs-1* and *ech-42*, *prb-1* and *tps-1* in the presence of the host. In this regard, it is well-known the strong battle that takes place between parasites and their hosts to counteract each other by means of lysing enzymes as well as effector molecules [57]. For instance, fungal effectors that
promote pathogen virulence by suppressing the chitin-triggered immunity in plants, including LysM (effectors that carry no recognizable protein domains other than lysin motifs) have been identified also in saprophytic and beneficial fungi [58]. In this case, probably the phytopathogen is attempting to evade the mycophagous behavior of T. atroviride by producing effectors that, in the presence of TSA, enhance their production in the phytopathogen to silence genes such as tps-1.

Intriguingly, pbs-1 and ech-42, prb-1 and tps-1 were positively affected by the presence of TSA. These results indicate that TSA may be affecting the transcription of these genes, by inhibiting Rpd3- and Hdal-encoding genes through histone acetylation in silenced chromatin regions as reported for other organisms [59,60]. In this sense, it is well known that TSA induces histone acetylation, which is often associated with an increased expression of a number of genes [61–63]. Frequently, these changes impact the biology of the organism, such as virulence and pathogenicity [64–66]. Moreover, the presence of both TSA and the phytopathogen downregulated the expression of all four genes, suggesting a positive effect of TSA in the synthesis of effector molecules in R. solani to counteract the mycoparasitic response of T. atroviride. Another plausible explanation is that both, the presence of TSA and the phytopathogen, but none of them alone enhances the expression of a negative regulator of mycoparasitism and secondary metabolism-related genes in this fungus.

**Deletion of tgf-1 did not affect T. atroviride mycoparasitism effect against R. solani**

Dual cultures of Δtgf-1 and R. solani showed that the mutant coiled around the phytopathogen hyphae; however, Δtgf-1 was deficient to grow over the pathogen. This result suggests that TGF-1 is not involved in coiling, but the reduced growth rate observed in the Δtgf-1 strain could be affecting its capacity to grow over R. solani. Probably, the Trichoderma growth rate is an important trait for competence. In this regard, it has been determined that growth inhibition of fungi by Trichoderma is directly proportional to the growth rate of the antagonists under a given condition [67].

**MFCF obtained from a Δtgf-1 strain shows enhanced inhibition of R. solani growth**

Antimicrobial analysis of MFCF obtained from a Δtgf-1 strain, as well as dual cultures against R. solani, showed an enhanced growth inhibition of the phytopathogen compared to MFCF obtained from the wt strain. These results point out to a negative role of TGF-1 in secondary metabolism in T. atroviride. In this regard, TGF-1, orthologous in other organisms, also regulates negatively gene expression. For instance, in N. crassa and in T. atroviride, the orthologous to Gcn5 positively regulates gene photoinduction, but negatively the expression of the al-3 gene in darkness [5] (Uresti-Rivera et al., in preparation). In S. cerevisiae, stress response adaptation requires Gcn5 activity for the activation or repression of genes that are physically associated with this protein. This fact provides support for a role of Gcn5 and its orthologous proteins as co-activators as well as co-repressors [6].

Addition of TSA to the Δtgf-1 growing medium (PDB) reverted the effect of the lack of TGF-1 in the antibiosis assays, which suggests that histone acetylation induced by TSA is enough to totally revert the effect of tgf-1 deletion, and that probably its product regulates negative elements. In this regard, it has been demonstrated that addition of TSA rescues the phenotypes of HAT mutants in yeast and HeLa cells [68–70]. Intriguingly, addition of TSA to the Vogel’s minimal medium, did not revert the effect of Δtgf-1 MFCF on R. solani to wild-type phenotype, on the contrary, it was enhanced. This result indicates that the components of the
PDB medium could be affecting the acetylation pattern on the promoters of secondary metabolism-related genes in a Δtgf-1 background, but not in the wt.

The Δtgf-1 strain shows constitutive chitinolytic but inductive proteolytic activity

Our results indicate that TGF-1 is a negative regulator of chitin degrading proteins, but not of those implicated in protein degradation, since chitinase activity showed an increased basal activity in the Δtgf-1 as compared to the wt. These results together with those of growth inhibition of R. solani may explain in part the augmented growth inhibition exerted by Δtgf-1 MFCF on the phytopathogen compared to wt MFCF, since some Trichoderma chitinases are secreted to the medium [71]. Trichoderma strains overexpressing either ech-42 or prb-1 provide more protection to plants against root and foliar pathogens [29–31]. In addition, mutants in the mitogen-activated protein kinase encoding gene, tvk1, from T. virens, showed increased transcription levels of mycoparasitism-related genes, high production of lytic enzymes and were considerably more effective in disease control than the wt strain [72].

TGF-1 and TSA differentially regulate ech-42, prb-1, pbs1, and tps-1 expression in the presence or absence of R. solani

Expression analysis of mycoparasitism- and secondary metabolism-related genes in the Δtgf-1 strain showed that probably the product of tgf-1 negatively regulates prb-1, tps-1, ech-42 and pbs-1 in absence of the phytopathogen, since all tested genes were expressed in almost all tested times. Based on this, we can propose that TGF-1 could be a direct repressor through its putative histone acetyltransferase activity. Or it could be also an indirect repressor of these genes through acetylation of the promoter of a negative regulator-encoding gene, whose transcript is absent by the loss of TGF-1. In this regard, a negative role of Gcn5 has been observed in yeast and filamentous fungi in a number of processes [5, 6, 72] (Uresti-Rivera et al., in preparation).

Dual cultures of Δtgf-1 against R. solani showed that the expression of all tested genes in the mutant strain was downregulated at almost all tested times. A plausible explanation for these results is that TGF-1 is necessary to maintain the homeostasis with the effector molecules produced by R. solani, which in the mutant background favors the suppression of Trichoderma genes by the phytopathogen. Together, these results indicate that TGF-1 is necessary for the induction of all four genes in the presence of the phytopathogen or to counteract the negative effect of R. solani on T. atroviride. The exceptions in gene repression by R. solani at all tested times were ech-42 and pbs-1. Probably, the induction of such genes under this condition may be due to the activity of other HATs such as Sas3 (ID Ta_223094; [12]), which has been shown to present overlapped roles with Gcn5 in S. cerevisiae [73]. Addition of TSA to the culture medium induced the expression of all tested genes in the Δtgf-1 strain, but at different times compared with its control. Probably this is a result of the promotion of histone acetylation, but it could be also a compensatory effect of other HAT in absence of TGF-1. The addition of TSA in the presence of R. solani induced the transcription of ech-42 and pbs-1, which were repressed at 60 h of co-culture. Nevertheless, the expression of prb-1 and tps-1 genes was downregulated.

A possible explanation for these results is that the sole presence of R. solani is not enough to fully repress the four genes at early times as it happened during confrontation with the wt strain in the presence of TSA and that such suppression was delayed by the absence of TGF-1.

The fact that other Trichoderma strains can grow over the Δtgf-1 mutant, and that some of them, including its parental strain, showed a marked lysing zone, could be explained as due to the slow growth of the Δtgf-1 strain. It is feasible that Δtgf-1 cannot compete with the other...
Trichoderma strains, although it showed an enhanced production of antibiotics that led to the lysis of other fungi hyphae [74], including its parental strain.

A detailed study focused on the interaction of HATs/HDACs with chromatin and acetylation balance, by ChIP on chip and ChIP-Seq assays, will be required to fully understand the role of chromatin modifications that regulate the antagonistic activity of T. atroviride and to determine if TGF-1, in these processes, acts as a co-activator as well as a co-repressor.

Supporting information
S1 Fig. Antibiosis assay using MFCF obtained from T. atroviride wt or Δtgf-1 strains grown in Vogel’s minimal medium in the presence or absence of TSA against R. solani. The Δtgf-1 and wt strains were grown for 7 days in Vogel’s minimal medium amended or not with 300 nM TSA, at 28 °C. MFCFs obtained from each of these cultures were added to PDA 1× medium at a final concentration of 60%. R. solani was inoculated into the different media and its radial growth was determined at 12, 24, 36, and 48 h. Radial growth of R. solani on PDA containing T. atroviride wt strain MFCF without TSA (black bars) or amended with TSA (crosshatched bars) was determined at the indicated times. Radial growth of R. solani on PDA containing T. atroviride Δtgf-1 strain MFCF without TSA (black dotted bars) or with TSA (arrow filled bars) was determined at the indicated times. The bars show the mean ± SD of three independent biological replicates. Different letters are used to indicate means that differ significantly (P < 0.05). Eight replicate plates were established for each treatment, and the experiment was repeated twice.

(TIF)

S1 Table. Relation between growth inhibition of R. solani and the T. atroviride strain used in dual cultures assays.

(DOCX)

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References

1. Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. Nature. 1997; 389: 251–60. https://doi.org/10.1038/38444 PMID: 9305837

2. He H, Lehming N. Global effects of histone modifications. Brief Funct Genomic Proteomic. 2003; 2: 234–43. Available: http://www.ncbi.nlm.nih.gov/pubmed/15239926 PMID: 15239926

3. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997; 389: 349–352. https://doi.org/10.1038/38664 PMID: 9311776

4. Shahbazian MD, Grunstein M. Functions of Site-Specific Histone Acetylation and Deacetylation. Annu Rev Biochem. 2007; 76: 11: 200. https://doi.org/10.1146/annurev.biochem.76.052705.162114 PMID: 17362198

5. Macario Osorio-Concepción, Cristobal-Mondragon Gema Rosa G-M B and C-F S. Histone Deacetylase HDA-2 Regulates Trichoderma atroviride Growth, Conidiation, Blue Light Perception, and Oxidative Stress Responses. Appl Environ Microbiol. 2017; 83: 1–19.

6. Grimaldi B, Ciofi P, Filetici P, Berge E, Dobosy JR, Freitag M, Selker EU B P. The Neurospora crassa White Collar-1 dependent Blue Light Response Requires Acetylation of Histone H3 Lysine 14 by NGF-1. Mol Biol Cell. 2006; 17: 4576–4583. https://doi.org/10.1091/mbc.E06-03-0232 PMID: 16914525

7. Xue-Franzén Y, Johnsson A, Brodin D, Henriksson J, Bürglin TR, Wright APH. Genome-wide characterisation of the Gcn5 histone acetyltransferase in budding yeast during stress adaptation reveals evolutionarily conserved and diverged roles. BMC Genomics. 2010; 11: 200. https://doi.org/10.1186/1471-2164-11-200 PMID: 20338033

8. Martínez-Soto D, González-Prieto JM, Ruiz-Herrera J. The UmGcn5 gene encoding histone acetyltransferase from Ustilago maydis is involved in dimorphism and virulence. Fungal Genet Biol. 2014; 71: 86–95. https://doi.org/10.1016/j.fgb.2014.09.002 PMID: 25242418

9. Martínez-Soto D, González-Prieto JM, Ruiz-Herrera J. Transcriptomic analysis of the GCN5 gene reveals mechanisms of the epigenetic regulation of virulence and morphogenesis in Ustilago maydis. FEMS Yeast Res. 2015; 15: 1–14. https://doi.org/10.1093/femsyr/fov055 PMID: 26126523

10. Brenna A, Grimaldi B, Filetici P, Ballario P. Physical association of the WC-1 photoreceptor and the histone acetyltransferase NGF-1 is required for blue light signal transduction in Neurospora crassa. Mol Biol Cell. 2012; 23: 3863–3872. https://doi.org/10.1091/mbc.E12-02-0142 PMID: 22875992

11. Lee I, Oh JH, Shwab E Keats, Dagenais TRT, Andes D, Keller NP. Histone deacetylase activity regulates chemical diversity in Aspergillus. Eukaryot Cell. 2007; 6: 1655–1664. https://doi.org/10.1128/EC.00186-07 PMID: 17616629

12. Reyes-Dominguez Y, Narendja F, Berger H, Gallmetzer A, Fernandez-Martín R, García I, et al. Nucleosome positioning and histone H3 acetylation are independent processes in the Aspergillus nidulans
Wei X, Yang F, Straney DC. Multiple non-ribosomal peptide synthetase genes determine peptaibols.

Komon-Zelazowska M, Neuhof T, Dieckmann R, Von Doehren H, Herrera-Estrella A, Kubicek CP, et al. Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of Trichoderma. Genome Biol. 2011; 12. https://doi.org/10.1186/gb-2011-12-4-r15

Olmedo-Monfil V, Casas-Flores S. Molecular Mechanisms of Biocontrol in. In: Gupta Vijai G., Schnoll Monika, Herrera-Estrella Alfredo, Upadhay Ray R. S., Druzhinina Irina T. M, editor. Biotechnology and Biology of Trichoderma. 2014. pp. 429–453. https://www.elsevier.com/books/biotechnology-and-biology-of-trichoderma/gupta/978-0-444-59576-8

Howell CR. Mechanisms Employed by Trichoderma Species in the Biological Control of Plant Diseases: The History and Evolution of Current Concepts. Plant Dis. 2003; 87: 4–10. https://doi.org/10.1094/PDIS.2003.87.1.4

Harman GE. Overview of Mechanisms and Uses of Trichoderma spp. Phytopathology. 2006; 96: 190–194. https://doi.org/10.1094/PHYTO-96-0190 PMID: 18943924

Shoresh M, Harman GE, Mastouri F. Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents. Annu Rev Phytopathol. 2010; 48: 21–43. https://doi.org/10.1146/annurev-phytopath-073009-114450 PMID: 20197257

Salas-Marina MA, Silva-Flores MA, Uresti-Rivera EE, Castro-Longoria E, Herrera-Estrella A, Casas-Flores S. Colonization of Arabidopsis roots by Trichoderma atroviride promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. Eur J Plant Pathol. 2011; 131. https://doi.org/10.1007/s10658-011-9782-6

Inbar J, Chet I. The role of lectins in recognition and adhesion of the mycoparasitic fungus Trichoderma spp. to its host. 1st ed. Kahane I, Ofek I, editors. Toward Anti-Adhesion Therapy for Microbial Diseases. New York: Springer US; 1996. https://doi.org/10.1007/978-1-4613-0415-9_27

Sanz L, Montero M, Grondona I, Vizcaíno JA, Llobell A, Hermosa R, et al. Cell wall-degrading isoenzyme profiles of Trichoderma biocontrol strains show correlation with rDNA taxonomic species. Curr Genet. 2004; 46: 277–286. https://doi.org/10.1007/s00294-004-0532-6 PMID: 15480677

Carsolio C, Gutiérrez A, Jiménez B, Van Montagu M, Herrera-Estrella A. Characterization of ech42, a Trichoderma harzianum endochitinase gene expressed during mycoparasitism. Proc Natl Acad Sci U S A. 1994; 91: 10903–10907. https://doi.org/10.1073/pnas.91.23.10903 PMID: 7971981

Carsolio C, Benhamou N, Haran S, Cortés C, Gutiérrez A, Chet I, et al. Role of the Trichoderma harzianum endochitinase gene, ech42, in mycoparasitism. Appl Environ Microbiol. 1999; 65: 929–935. 0099–2240 PMID: 10049644

Flores A, Chet I, Herrera-Estrella A. Improved biocontrol activity of Trichoderma harzianum by overexpression of the proteinase encoding gene prb1. Curr Genet. 1997; 31: 30–37. https://doi.org/10.1007/s002940050173 PMID: 9003738

Szekeres A, Kredics L, Antal Z, Kevei F, Manczinger L. Isolation and characterization of protease overproducing mutants of Trichoderma harzianum. FEMS Microbiol Lett. 2004; 233: 215–222. https://doi.org/10.1016/j.femsle.2004.02.012 PMID: 15063489

Lorito M, Farkas V, Rebuffat S, Bodo B, Kubicek CP. Cell wall synthesis is a major target of mycoparasitism by Trichoderma. Cell Wall Synthesis Is a Major Target of Mycoparasitism. Antagonism by Trichoderma harzianum. J Bacteriol. 1996; 178: 6382–6385. 0021–9193 PMID: 8892847

Mathivanan N, Prabavathy VR V VR. The Effect of Fungal Secondary Metabolites on Bacterial and Fungal Pathogens. In: Karlovsky Petr, editor. Secondary Metabolites in Soil Ecology. 1st ed. New York: Springer; 2008. pp. 129–140.

Mukherjee PK, Wiest A, Ruiz N, Keightley A, Moran-Diez ME, McCluskey K, et al. Two classes of new peptaibols are synthesized by a single non-ribosomal peptide synthetase of Trichoderma virens. J Biol Chem. 2011; 286: 4544–4554. https://doi.org/10.1074/jbc.M110.159723 PMID: 21213172

Wei X, Yang F, Straney DC. Multiple non-ribosomal peptide synthetase genes determine peptaibol synthesis in Trichoderma virens. Can J Microbiol. 2005; 51: 423–429. https://doi.org/10.1139/w05-008 PMID: 16088338

Komon-Zelazowska M, Neuhoft R, Dieckmann R, Von Döhren H, Herrera-Estrella A, Kubicek CP, et al. Formation of atroviridin by Hypocrea atroviridis is condensation associated and positively regulated by blue light and the Q protein GNA3. Eukaryot Cell. 2007; 6: 2332–2342. https://doi.org/10.1128/EC.00143-07 PMID: 17933910
38. Velazquez-Robledo R, Contreras-Cornejo H a, Macias-Rodriguez L, Hernendez-Morales a, Aguirre J, Casas-Flores S, et al. Role of the 4-phosphopantetheiny l transferase of Trichoderma virens in secondary metabolism and induction of plant defense responses. Mol Plant Microbe Interact. 2011; 24: 1459–71. https://doi.org/10.1094/MPMI-02-11-0045 PMID: 21830953

39. Mukherjee M, Horwitz BA, Sherkhane PD, Hadar R, Mukherjee PK. A secondary metabolite biosynthesis cluster in Trichoderma virens: Evidence from analysis of genes underexpressed in a mutant defective in morphogenesis and antibiotic production. Curr Genet. 2006; 50: 193–202. https://doi.org/10.1007/s00294-006-0075-0 PMID: 16804721

40. Yoshida M, Horinouchi S, Beppu T. Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. Bioessays. 1995; 17: 423–430. https://doi.org/10.1002/bies.950107510 PMID: 7768288

41. Baek J-MM, Kenerley CM. The arg2 gene of Trichoderma virens: cloning and development of a homologous transformation system. Fungal Genet Biol. 1998; 23: 34–44. https://doi.org/10.1006/fgbi.1997.1025 PMID: 9501476

42. White T, Bruns T, Lee S T J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M. A., Gelfand D. H., Sninsky J. J. and W TJ. PCR Protocols: A Guide to Methods and Applications. New York: Academic Press, Inc.; 1990. pp. 315–322.

43. Vogel HJ. A convenient growth medium for Neurospora crassa. Microb Genet Bull. 1956; 13: 42–43.

44. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin-Phenol Reagent. J Biol Cemistr y. 1951; 193: 265–275. Available: https://doi.org/10.1002/febs.12113 PMID: 23289754

45. Staskawicz BJ, Mudgett MB, Dangl JL. Common and Contrasting Themes of Plant and Animal Disease. Science (80-). 2001; 292: 2285–2289. https://doi.org/10.1126/science.1062013 PMID: 11423652

46. Seidl-Seiboth V, Zach S, Frischmann A, Spadut O, Dietzsch C, Herwig G, et al. Spore germination of Trichoderma atroviride is inhibited by its LygM protein TAL6. FEBS J. 2013; 280: 1226–1236. https://doi.org/10.1111/febs.12113 PMID: 23289754
59. Sanchez del Pino MM, Lopez-Rodas G, Sendra R, Tordera V. Properties of the yeast nuclear histone deacetylase. Biochem J. 1994; 303 (Pt 3): 723–729. Available: http://www.ncbi.nlm.nih.gov/pubmed/7980438

60. Carmen AA, Rundlett SE, Grunstein M. HDA1 and HDA3 are components of a yeast histone deacetylase (HDA) complex. J Biol Chem. 1996; 271: 15837–15844. https://doi.org/10.1074/jbc.271.26.15837 PMID: 8663039

61. Ekwall K, Olsson T, Turner BM, Cranston G, Allshire RC. Transient inhibition of histone deacetylation alters the structural and functional imprint at fission yeast centromeres. Cell. 1997; 91: 1021–1032. https://doi.org/10.1016/S0092-8674(00)80492-4 PMID: 9428524

62. Bernstein BE, Tong JK, Schreiber SL. Genomewide studies of histone deacetylase function in yeast. Proc Natl Acad Sci. 2000; 97: 13708–13713. https://doi.org/10.1073/pnas.250477697 PMID: 11095743

63. Ali IK, Ehrenkaufer GM, Hackney JA S U. Growth of the protozoan parasite Entamoeba histolytica in 5-azacytidine has limited effects on parasite gene expression. BMC Genomics. 2007; 8: 7. https://doi.org/10.1186/1471-2164-8-7 PMID: 17207281

64. Isakov E, Siman-Tov R, Weber C, Guilien N, Anki S. Trichostatin A regulates peroxiredoxin expression and virulence of the parasite Entamoeba histolytica. Mol Biochem Parasitol. 2008; 158: 82–94. https://doi.org/10.1016/j.molbiopara.2007.11.014 PMID: 18191469

65. Simonetti G, Passariello C, Rotili D, Mai A, Garaci E, Palamara AT. Histone deacetylase inhibitors may reduce pathogenicity and virulence in Candida albicans. FEMS Yeast Res. 2007; 7: 1371–1380. https://doi.org/10.1111/j.1567-1364.2007.00276.x PMID: 17827775

66. Klar AJS, Srikantha T, Soll DR. A histone deacetylase inhibitor and mutant promote colony-type switching of the human pathogen Candida albicans. Genetics. 2001; 158: 919–924. PMID: 11404352

67. Summerbell RC. the Inhibitory Effect of Trichoderma Species and Other Soil Microfungi on Formation of Mycorrhiza By Laccaria-Bicolor Invitro. New Phytol. 1987; 105: 435–448

68. Choy JS, Kron SJ. NuA4 Subunit Yng2 Function in Intra S Phase DNA Damage Response NuA4 Subunit Yng2 Function in Intra S Phase DNA Damage Response. Mol Cell Biol. 2002; 22: 8215–8225. https://doi.org/10.1128/MCB.22.23.8215-8225.2002 PMID: 12417725

69. Choy JS, Tobe BTD, Huh JH, Kron SJ. Yng2p-dependent NuA4 Histone H4 Acetylation Activity Is Required for Mitotic and Meiotic Progression. J Biol Chem. 2001; 276: 43653–43662. https://doi.org/10.1074/jbc.M102531200 PMID: 11544250

70. Taipale M, Rea S, Richter K, Vilar A, Lichter P, Imhof A, et al. hMOF Histone Acetyltransferase Is Required for Histone H4 Lysine 16 Acetylation in Mammalian Cells hMOF Histone Acetyltransferase Is Required for Histone H4 Lysine 16 Acetylation in Mammalian Cells. Mol Cell Biol. 2005; 25: 6798–6810. https://doi.org/10.1128/MCB.25.15.6798-6810.2005 PMID: 16024812

71. Herrera-Estrealla A, Chet I. Chitinases in biological control. Exs-BASEL. 1999; 87: 171–184. https://doi.org/10.1007/978-3-0348-8757-1_12

72. Mendoza-Mendoza A, Pozo MJ, Grzegorski D, Martínez P, García JM, Olmedo-Monfli V, et al. Enhanced biocontrol activity of Trichoderma through inactivation of a mitogen-activated protein kinase. Proc Natl Acad Sci U S A. 2003; 100: 15965–70. https://doi.org/10.1073/pnas.2136716100 PMID: 14673101

73. Ricci AR, Genereaux J, Brandl CJ. Components of the SAGA histone acetyltransferase complex are required for repressed transcription of ARG1 in rich medium. Mol Cell Biol. 2002; 22: 4033–4042. https://doi.org/10.1128/MCB.22.12.4033-4042.2002 PMID: 12024017

74. Reaves, Jimmy L.; Crawford RH. In vitro colony interactions among species of Trichoderma with inference toward biological control. Res Pap PNW-RP-474 Portland, OR US Dep Agric For Serv Pacific Northwest Res Stn. 1994; 8p. https://www.treeresearch.fs.fed.us/pubs/20586