The Putative Protein Methyltransferase LAE1 of *Trichoderma atroviride* Is a Key Regulator of Asexual Development and Mycoparasitism

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Abstract

In Ascomycota the protein methyltransferase LaeA is a global regulator that affects the expression of secondary metabolite gene clusters, and controls sexual and asexual development. The common mycoparasitic fungus *Trichoderma atroviride* is one of the most widely studied agents of biological control of plant-pathogenic fungi that also serves as a model for the research on regulation of asexual sporulation (conidiation) by environmental stimuli such as light and/or mechanical injury. In order to learn the possible involvement of LAE1 in these two traits, we assessed the effect of deletion and overexpression of *lae1* gene on conidiation and mycoparasitic interaction. In the presence of light, conidiation was 50% decreased in a Δlae1 and 30–50% increased in lae1-overexpressing (OElae1) strains. In darkness, Δlae1 strains did not sporulate, and the OElae1 strains produced much spores as the parent strain. Loss-of-function of *lae1* also abolished sporulation triggered by mechanical injury of the mycelia. Deletion of *lae1* also increased the sensitivity of *T. atroviride* to oxidative stress, abolished its ability to defend against other fungi and led to a loss of mycoparasitic behaviour, whereas the OElae1 strains displayed enhanced mycoparasitic vigor. The loss of mycoparasitic activity in the Δlae1 strain correlated with a significant underexpression of several genes normally upregulated during mycoparasitic interaction (proteases, GH16 β-glucanases, polyketide synthases and small cystein-rich secreted proteins), which in turn was reflected in the partial reduction of formation of fungicidal water soluble metabolites and volatile compounds. Our study shows *T. atroviride* LAE1 is essential for asexual reproduction in the dark and for defense and parasitism on other fungi.

Introduction

Comparison of the genomic inventory of *T. reesei*, *T. atroviride* and *T. virens* identified mycotrophy (i.e. successful feeding on either living or killed fungi) as the innate nature of the genus [1]. This lifestyle involves a combination of traits such as host recognition, attachment to and sometimes coiling around the host hyphae, and the secretion of antibiotic metabolites and cell-wall-degrading enzymes [2–4]. The molecular mechanisms involved have been studied mainly with regards to the possible involvement of hydrolytic enzymes (chitinases, glucanases and proteases) and secondary metabolites (gliotoxin, peptaibols, 6-pentyl-2H-pyran-2-one [6PP]) in antagonism, and of heterotrimeric G proteins and their receptors in sensing of host signals [5]. Mukherjee and Kenerley [6] reported the developmental regulator VEL1 (an orthologue of the *Aspergillus nidulans* veA) which encodes a conserved global regulator of development and secondary metabolism [7,8] regulates mycoparasitism that in *T. virens* [10,11], and later on shown to be also required for the biosynthesis of secondary metabolites in the industrially applied fungus *Penicillium chrysogenum* (e.g. penicillin) and the phytopathogenic fungi *Fusarium fujikuroi*, *F. verticillioides* and *Cochliobolus heterostrophus*, respectively [12–15]. Further evidence emerged that LaeA also controls numerous developmental events in fungi, such as conidiation and fruiting body formation [12–14]. In plant and human pathogenic fungi, LaeA has also been demonstrated to be a virulence factor [13,14,16,17]. We have recently studied the function of LAE1, the LaeA orthologue of *Trichoderma reesei* [18,19]. Interestingly, in this fungus that has specialized to saprotrophic growth on pre-decayed wood, LAE1 is a major regulator for the expression of cellulases and hemicellulases that are required for feeding on this substrate [18,19]. One may thus hypothesize that LAE1 controls different strategies to aid the fitness of the fungus in its environment.

As emphasized above, mycotrophy is the innate nature of *T. atroviride* [1]. In this work we have therefore tested the hypothesis that in *T. atroviride* LAE1 may be involved in mycoparasitic interaction of this species.
Materials and Methods

Fungal Strain and Culture Conditions

Trichoderma atroviride P1 (ATCC 74058) [20] was used throughout this work. For selected experiments, T. atroviride IMI 206040, and its bla1 and bbs2 deletion mutants [21] were also used. It was grown on PDA (Difco™ potato-dextrose-agar) plates at 25°C. Rhizoctonia solani C.P.K. 3753, B. cinerea C.P.K. 4679 and Alternaria alternata C.P.K. 3737 were grown on 2% (w/v) potato dextrose agar (PDA) under 12 h cycles of light and darkness at 25 C.

Escherichia coli JM109 (Promega, Madison, Wisconsin) was used for plasmid construction and amplification.

Manipulation of lae1 Gene Expression in T. atroviride

To obtain mutants not expressing the lae1 coding region was replaced by the hygromycin B phosphotransferase (hph) gene from E. coli under Trichoderma 5' and 3' regulatory signals [22]. To this end, 1.3 and 1.2 kb of the up- and downstream non-coding region of lae1 were amplified using the primer pairs Patro_FW_ConMeth_ApaI (5'-TGGGCCCCATCATCATCTGCTATTTTG TTCGAGCAGGTTCTGACCGCTTCAT-3'), and Tatro_FW_ConMeth_XhoI (5'-TCCGGGATCCGATGGTTCTTACTT-3'). The two resulting PCR fragments were digested with ApaI/XhoI and ligated into pBluescript SK(+) (upstream region) and SmaI/XhoI (downstream region), dephosphorylated and ligated into pHBluescript SK(+) (Stratagene, La Jolla, California), previously cut with ApaI/SmaI, followed by the insertion of the 2.4 kb XhoI/Sall fragment of hph cassette into the XhoI site resulting in pRKA_D 42103hph.

Vector pRKA OE41617hph, which bears the T. reesei lae1 gene under the constitutive expression signals of tef1 [23] was used to generate lae1 overexpressing strains of T. atroviride.

Transformation of Trichoderma

Transformation has been carried out as described by Guangtao et al [24]. The strains were purified twice to obtain mitotic stability, and integration of the expression cassettes was verified by PCR analysis (Fig. S1).

Assay for Growth and Conidiation

Cultures were grown on PDA at 25°C in a Sanyo incubator containing a Philips-master light source (TLD-15 W/840), either with illumination (12 hour cycles of light and dark; 1100 [+30] lux, 30 cm distance) or in full darkness (dark conditions), as specified. To this end, each plate was inoculated with a mycelial plug (5 mm diameter) taken from the edge of the plates. The respective colony radius was measured 3 times per day. After the fungi reached the edge of the plates, changes in radial growth were calculated using a calibration curve with T. reesei conidia.

Phenotype Microarrays

To test for sensitivity of the Trichoderma strains against hydrogen peroxide, they were grown on PDA plates supplemented with 0, 0.5, 1, 5 and 20 mM of H2O2, and incubated until the time that the fungi reached the edge of the plates. Changes in radial growth were measured 3 times per day.
colony, and expressed as ratio of the diameter of the halo to that of the fungal colony. A ratio of 1 indicates absence of cellulase formation.

**Quantitative PCR**

Following RNA isolation (using the RNeasy plant kit, Promega) 5 μg of the total RNA was treated with DNase (DNase I, RNase free; Fermentas) and reverse transcribed (RevertAid™ First Strand cDNA Kit, Fermentas) using a 1:1 mixture of oligo-dT and random hexamer primers. All quantitative RT-PCR experiments were performed on a Bio-Rad (Hercules, CA) iCycler IQ. For the reaction the IQ SYBR Green Supermix (Bio-Rad, Hercules, CA) was prepared for 25 ml assays with standard MgCl₂ concentration (3 mM) and a final primer concentration of 100 nM each. All assay were carried out in 96-well plates. Determination of the PCR efficiency was performed using triplicate reactions from a dilution series of cDNA (1; 0.1; 0.01; 0.001). Amplification efficiency was then calculated from the given slopes in the IQ Optical system Software v2.0. Primers, amplification efficiency and Tₘ values are given in Table S1. Expression of the reference gene tef1 was measured with both protocols for reference calculation. Expression ratios were calculated using REST® Software [28]. All samples were analyzed in at least two independent experiments with three replicates in each run.

**Results**

**Identification of the LAE1 Orthologue of *T. atroviride***

We have recently identified LAE1 from *T. reesei*, and shown that it forms a supported clade with putative orthologues of *T. viride* and *T. atroviride* [18]. The annotation of the *T. atroviride* orthologue (Triat2:302782; old number Triat1:42103) was manually corrected (correct sequence deposited under NCBI GeneBank accession number KC174792). LAE1 is encoded by a 1328 nt ORF that is interrupted by 5 introns and encodes a putative 363 aa protein. (correct sequence deposited under NCBI GeneBank accession number KC174792). LAE1 is encoded by a 1328 nt ORF that is interrupted by 5 introns and encodes a putative 363 aa protein.

**Phenotype of *T. atroviride* LAE1 Mutants**

To investigate the function of *T. atroviride* lae1, we prepared knock out- and overexpressing strains (see Materials and Methods). Several mitotically stable Δlae1 and OElae1 strains were obtained and verified by PCR (Fig. S1). Several Δlae1 strains and OElae1 strains bearing one additional copy were then investigated with respect to growth and conidiation. Strains bearing the same mutation (Δlae1 or OElae1 respectively; see below) displayed identical phenotypes. Despite several attempts we failed to introduce the wild-type lae1 copy into the Δlae1 strains. Two Δlae1 or OElae1 strains were thus selected for all further experiments, and gave essentially similar results (selected cases are shown in the supplementary information; see below). The Δlae1 strains showed a 25–30% reduced growth rate on plates with D-glucose or D-galactose as a carbon source, whereas growth of the OElae1 strain was the same as that of the parent strain (data not shown). Otherwise the mutants did not show any morphological differences from the parent strain.

**LAE1 is Essential for Cellulase Formation in *T. atroviride***

In *T. reesei* lae1 is essential for growth on cellulose and expression of cellulase and hemicellulase genes in *T. reesei* [18]. In order to learn whether this is also the case in *T. atroviride*, we grew the strains on plates with carboxymethyl cellulose as the only carbon source and analyzed cellulase secretion by Congo red staining. Indeed, *T. atroviride* P1 exhibited a ratio of halo diameter vs colony diameter of 1.29 (±0.011), which was slightly enhanced in the OElae1 strain (1.47±0.036). The Δlae1 strain, however, yielded a value close to 1 (1.07±0.016) indicating no or only very little cellulase secretion (which was also reflected in the very small colonies). Thus the observed LAE1-dependent regulation of cellulase formation in *T. reesei* also extends to *T. atroviride*.

**LAE1 Effects Conidiation in *T. atroviride* in a Carbon Source and Light/darkness Dependent Manner**

The loss-of-function of lae1 of *T. atroviride* significantly affected the intensity of conidiation, albeit the effect differed in light and darkness (Figure 1): when cultivated on PDA in light, conidiation intensity was reduced by approximately 50% in the Δlae1 strain. Conidiation in the dark, however, was reduced to almost zero. The OElae1 strains, on the other hand, exhibited an increased conidiation density under illumination, whereas it displayed the same level of conidiation as the parent strain P1 in darkness (Figure 1).

**Impairment of Conidiation in the *T. atroviride* Δlae1 Strain cannot be Rescued by Volatile Components from the Parent Strain**

Conidiation in *Trichoderma* has been shown to be triggered by volatile compounds (VOC) from neighboring *Trichoderma* colonies [29]. We therefore surmised that the loss of conidiation in darkness could be due to a loss of the ability to form VOC. Consequently we tested whether VOC released by the parent strain of *T. atroviride* would rescue conidiation in the darkness in the Δlae1 mutant. However, this hypothesis had to be rejected: using an...
upside-down sandwich of two plates, in which the Δlae1 mutant was growing in the plate on the top and the parent strain P1 on the bottom, the Δlae1 strain maintained being unable to form conidia (shown for two mutants, Δlae1-1 and Δlae1-2 in Fig. S2).

There is no Cross Talk between LAE1 and the Two Blue Light Receptors BLR-1 and BLR-2

The significant effect of the Δlae1 mutation on conidiation in response to light prompted us to investigate a possible cross-talk between LAE1 and the two blue light receptors BLR-1 and BLR-2, which form the top of the cascade that signals the presence of light to T. atroviride [21]. However, their transcripts were equally abundant in the parent, OElae1 and Δlae1 strains (Figure 2 A) indicating that their expression is unaffected by lae1 modulation. Also lae1 was expressed at the same level in Δblr1 and Δblr2 mutants (Figure 2 B). Hence, lae1 and bbl1/bbl2 do not influence the expression of each other.

LAE1 is also Required Triggering of Conidiation by Mechanical Injury

Conidiation in Trichoderma can also be induced by mechanical injury via generation of radical oxygen species (ROS) [21,30–32]. We have therefore investigated if LAE1 is also required for conidiation triggered by mechanical injury in darkness. As shown in Fig. S3, mechanical injury resulted in conidiation only in the parent and OElae1 strain but not in the Δlae1 strain, and LAE1 therefore influences sporulation also when triggered by mechanical injury.

LAE1 is Required for Oxidative Stress Tolerance in T. atroviride

Wu et al. [14] recently showed LAE1 is necessary for the oxidative stress response in the plant pathogen Cochliobolus heterostrophus. To find out whether LAE1 is required for the response to oxidative stress in T. atroviride, we tested the effect of hydrogen peroxide on the T. atroviride parent, the Δlae1 and the OElae1 strain (Figure 3). The parent strain P1 proved to be resistant to hydrogen peroxide up to a concentration of at least 5 mM, and displayed about 60% of its original growth rate at 20 mM. Similar data were obtained for the OElae1 mutant. The Δlae1 mutant, however, only showed 64% of its growth rate at 5 mM hydrogen peroxide, and exhibited only 39% of its original growth rate at 20 mM. Thus we conclude that LAE1 is partially involved in the defense against oxidative stress in T. atroviride.

LAE1 is Essential for T. atroviride Antagonism and Defense against Other Fungi

To analyze whether LAE1 would be relevant for the mycoparasitic activities of T. atroviride, we confronted the parent strain P1, and the Δlae1 and OElae1 mutants on plates with three standard model fungi used for antagonism experiments (i.e. Alternaria alternata, Rhizoctonia solani, and Botrytis cinerea). Their growth was monitored over the time in the presence and absence of T. atroviride and its lae1 mutants (Fig. S4 A). As can be seen, all three test fungi were initially able to grow in the presence of T. atroviride and its lae1 mutants at the same rate as in their absence, but stopped their growth when getting close (1–2 mm) to T. atroviride (plates for the second mutant strains shown in Fig S4 B). This was about 50 h for all three fungi when confronted by strains P1 and OElae1, whereas it occurred in the Δlae1 mutant only after 65 hrs with R. solani and B. cinerea and 85 hrs with A. alternata. Correspondingly, the final colony diameter of these three fungi was higher when confronted with the Δlae1 mutant than with P1 or OElae1, which also corresponded with a smaller colony diameter of the Δlae1 mutant strain. However, in addition to this slower growth of the Δlae1 strain, visual examination of the plates (Figure 4; Fig. S4 B) showed that it also failed to overgrow and feed on the tested plant pathogenic fungi, and in contrast its growth was suppressed by them. In confrontation with R. solani, T. atroviride Δlae1 almost completely also lost its ability to conidiate. In contrast, the mycoparasitic vigor of the OElae1 strain was even increased, and we particularly noted an increased formation of coils around mycelia of R. solani (data not shown).
LAE1 Regulates the Formation of Extracellular Antifungal Components

The dependence of mycoparasitism and antagonism on LAE1 prompted us to test whether this could be due to an involvement of LAE1 in the formation of water soluble extracellular compounds (WSC) that aid in the inhibition of growth of the plant pathogenic fungi. To this end, we grew T. atroviride P1, and its Δlae1 and OElae1 mutants on plates covered by cellophane. After T. atroviride had covered most of the plates, the fungal mycelium and the cellophane were removed, and Alternaria alternata (Aa), Rhizoctonia solani (Rs) and Sclerotinia sclerotiorum (Ss) were placed on these plates. The plates were photographed after 7 days.

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Discussion

The putative protein methyltransferase LaeA is still an enigmatic protein: it has originally been identified as a regulator of secondary metabolite (aflatoxin) biosynthesis [10], but subsequently was found to have – among other traits – also key functions in development: as an example, deletion of the laeA gene in A. fumigatus and A. flavus reduces conidiation [16,35]. In P. chrysogenum and in A. nidulans, the Olae1 mutant was relieved from repression of conidiation in the dark and produced numbers of conidia similar to wild-type in light [14]. In T. reesei, whose sporulation is not enhanced by light [18,36], formation of conidia is reduced to almost zero in both light and darkness. In the present study, we found that the T. atroviride Δlae1 mutant fail to conidiate in the dark on all carbon sources, while conidiation – which is stimulated by light [30,31,37] - was only reduced by 50% in light.

Loss of Function of lae1 Decreases the Expression of Mycoparasitism-associated Genes

In order to learn whether loss of function of LAE1 would be due to a decreased expression of genes known to be associated with mycoparasitism, we have investigated the expression of 13 genes that were recently shown to be strongly upregulated during interaction of T. atroviride with R. solani [26]. These were two GH16 β-1,3/1,4-glucanases, two aspartyl proteases, two subtilisin proteases, two polyketide synthases, two C-type lectins, one cyanovirin-type lectin and two small cysteine-rich secreted proteins. Figure 5 A shows that indeed 8 of these 13 genes were significantly underexpressed in the Δlae1 mutant. Interestingly, the expression of none of these genes was enhanced in the OElae1 strain, implying that the superior mycoparasitic activity of this mutant (vide supra) cannot be due to the increased expression of any of these genes. Nevertheless, the data demonstrate that in T. atroviride LAE1 is necessary for the expression of some of the genes encoding extracellular hydrolases, secondary metabolites and proteins that putatively interact with other organisms.

LAE1 Affects γ-pentyl-pyrone Formation by T. atroviride

One of the known antifungal metabolites produced by T. atroviride is 6PP [33,34], which exhibits an intensive coconut smell. During the antagonism experiments, we observed this aroma in plates of the parent strain and even more in plates of the OElae1 strain of T. atroviride, whereas it was absent from those of the two Δlae1 strains and only appeared faintly when this culture initiated its sporulation in the presence of light. To experimentally test whether LAE1 indeed regulates 6PP formation, we examined the expression of the lipoygenase gene (Triat2:33350) that is putatively involved in 6PP formation [3], and which is strongly upregulated during mycoparasitism [26]. In fact, the lipoxygenase transcript was strongly down regulated in Δlae1, both in ambient light and in dark. It was also upregulated in OElae1 in the dark but not in light (Figure 5 B). To test whether reduced 6PP formation would contribute to the reduced antagonistic activity in the T. atroviride Δlae1 strain, we tested the effect of VOC from the parent strain, the OElae1 and the Δlae1 strain on growth of the test fungi. The data show that VOC indeed reduce the growth of R. solani and also of A. alternata (data not shown), but only partially, and that this effect is much weaker in the Δlae1 strain (Figure 5 C).

Figure 4. Phenotype of confrontation of T. atroviride P1 and the lae1 mutants OElae1 and Δlae1 (all T) against B. cinerea (B), A. alternata (A) and R. solani (R) after termination of growth of the latter three fungi. Left plates are photographed from the backside, right plates are photographed from top. (B) Test for production of WSC: T. atroviride parent strain, and the Δlae1-1 and OElae1 mutants were grown on PDA agar covered by cellophane, and then removed and Alternaria alternata (Aa), Rhizoctonia solani (Rs) and Sclerotinia sclerotiorum (Ss) placed on these plates. The plates were photographed after 7 days.

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Thus, while LacA/LAE1 all affect asexual sporulation, the necessity for a functional LAE1 in *T. atroviride* predominates in darkness and appears to be partially counteracted by light, which is a unique case in the fungi studied so far.

One special trait of *Trichoderma* conidiation is that it can be induced by mechanical injury of the mycelium [21,30–32]. The present results showed that injury-triggered sporulation of *T. atroviride* in the dark was also dependent on LAE1 function, as no conidia were formed in its absence. Mechanical injury has been shown to be triggered by an oxidative stress response caused by NADPH oxidase-dependent production of radical oxygen species (ROS), for which the proteins NOX1 and NOXR are essential [32]. We have not tested whether the expression of *nox1* and *noxR* is affected by LAE1 loss-of-function in *T. atroviride* (the *T. reesei* orthologues are not) [19] but tested whether – as in *C. heterostrophus* [14] – the Δlae1 strain is affected in its sensitivity to oxidative stress provoked by hydrogen peroxide. While we found that this indeed the case, the reduction of resistance against hydrogen peroxide was not complete and required much higher concentrations than in *C. heterostrophus* [14]. We therefore conclude that – even in the absence of *lae1* function – *T. atroviride* can still respond to oxidative stress. The complete loss of conidiation upon mechanical injury in the Δlae1 strain must therefore be due to a requirement for LAE1 by other components needed for this process.

One of them could be VOC formation: sporulation by *T. atroviride* has been shown to be triggered by VOC from other *Trichoderma* colonies [29], and Roze et al. [38] showed that in *A. parasiticus*, the Velvet A protein VeA is required for production of VOC that mediates asexual conidiation and sclerotia formation. Because of the known interaction of VeA with LacA [8,9], which has also been shown in *T. reesei* [19], we tested whether LAE1 would be involved in the stimulation of *T. atroviride* sporulation by VOC. However, VOC from the parent strain were unable to overcome the reduced conidiation in Δlae1. Thus, either the effect of VeA on induction of sporulation is independent of LacA (e.g. in *P. chrysogenum*, the VeA and LacA orthologues PcVeA and PcLacA have different and independent roles in asexual development) [12], or this process is differentially regulated in *A. parasiticus* and *T. atroviride*.

The most striking and not yet reported phenotype of loss-of-function of LacA is that the *T. atroviride* Δlae1 strain had completely lost its mycoparasitic ability, and also the ability to defend itself against other fungi. This is similar to data that have been reported for Δvel1 strains of *T. virens*, suggesting that mycoparasitism is indeed controlled by the VEL1/LAE1/VEL2 (VeA/LaeA/VelB) complex [8,9]. We should like to stress that lae1 and vel1 are so far the only genes that have been identified as global regulators of *Trichoderma* antagonism, and whose loss-of-function is not compromised by severe growth defects: deletion or silencing of other genes, such as those encoding G-proteins or their receptors, while also leading to impaired mycoparasitic activity, also caused significant reductions in the growth rate of *Trichoderma* [5] and it is therefore difficult to assess whether their effect on mycoparasitism is direct or indirect. Although the *T. atroviride* Δlae1 strains showed some reduction of growth on some carbon sources, the effects did not exceed ±30% of that of the parent strain under conditions of antagonism with the test fungi, and particularly the hyphal morphology was not significantly altered (data not shown). We therefore do not believe that the loss of antagonistic abilities could be solely due to this fact.

Interestingly, qPCR analyses showed that this loss of mycoparasitic ability correlated with a loss of expression of genes encoding cell wall hydrolases (GH16 glucanases), secondary metabolites (PKSs), and proteins supposed to mediate hyphal contact to the host (lectins, SSCPRs). This would be in excellent agreement with the current view of mechanism of *Trichoderma* mycoparasitism [1]. However, none of these genes displayed enhanced expression in the Δlae1 strain, and the superior mycoparasitic activity in *OElae1* strains thus remains unexplained. We have recently observed that overexpression of *lae1* even converts other *Trichoderma* spp. that exhibit only weak antagonistic activities, into vigorous mycoparasites (R.A. Karimi, M. Marzouk and I.S. Druzhinina, unpublished data). LAE1 therefore must act at a target that is central to the mycoparasitic response that still awaits identification.

Work on LacA and its orthologues in several Aspergilli, but also in *P. chrysogenum, Fusarium fujikuroi, F. verticillioides* and *C. heterostrophus* has consistently proven that it regulates secondary metabolism [12–14,16,17,35]. However, only a few genes of secondary metabolism were affected by a loss of function of *lae1* in *T. reesei* [19], suggesting that the function of LAE1 may have
diverged in this genus. The present investigation with T. atroviride supports this view: while some of the secondary metabolism genes that have recently been shown to be upregulated during antagonism of T. atroviride against R. solani [26] were significantly underexpressed in the Δlae1 mutant, there was a less strong effect of LAE1 on the formation of inhibitory WSC and VOC, thus implying that the Δlae1 strain still can form WSC and VOC. Whether this is due to a decreased expression of several genes, or the complete blockage of expression of some of them remains to be determined. One must also bear in mind that the tests for WSC formation depends on the prior cultivation of T. atroviride in the absence of its prey, and it is possible that stronger effects may become apparent when the formation of these components is investigated under confrontation conditions. Yet it is clear from these studies that the lack of mycoparasitic activity in the Δlae1 strain cannot be explained by the observed changes in the expression of its secondary metabolism genes.

The present findings that LAE1 regulates the antagonistic and defensive reaction of the mycoparasite T. atroviride, is a further example of involvement of this protein in a specific response of a fungus to the environment. A similar conclusion has also been drawn by Sarikaya Bayram et al. [39], i.e. that LaeA is involved in the protective as well as the nutritional function for preparing the next generation for future life. Such a role would be in excellent agreement with an epigenetic function of LaeA/LAE1 [40], which however so far is only a speculation.

Supporting Information

Figure S1 Construction and proof for T. atroviride OElae1 and Δlae1 strains: (A) constructs used to disrupt lae1 (top) and to express it under the tef1 promoter (bottom). Numbers over the scheme indicate the size (in bp’s) of the promoter, ORF and terminator used; the number below the scheme of the nucleotide fragment amplified by the respective primers used. Bold numbers over the small bold arrows specify the scheme of the nucleotide fragment amplified by the respective primers used: 1, Patro_FW_ConMeth_ApaI; 2, Tatro_Rev_ConMeth_Smal; 3, tef1SC; 4, TrLae1TermHind. For primer sequences see Materials and Methods of the main manuscript. (B) Identification of two Δlae1 strains among 6 transformants; the two arrows point to the 5 and 4 kb marker (M) band (from top); (C) Identification of OElae1 strains among 6 transformants and the P1 parent strain (track 7). The two arrows point to the 3 and 2.5 kb marker (M) band.

Figure S2 Lack of induction of conidiation in T. atroviride Δlae1 by volatiles from strains P1, OElae1 and Δlae1-1 and Δlae1-2 (=control) in the presence of light (L) or in darkness (D).

Figure S3 Triggering of conidiation in the T. atroviride parent and lae1 mutant strains by mechanical injury. The mycelium of the strains shown was cut with a scalpel and incubated under periodic illumination condition for 24 hrs. Single plates from several (N>4) experiments are shown.

Figure S4 Effect of modulation of lae1 expression on the ability of T. atroviride to inhibit growth of R. solani, B. cinerea and A. alternata. A: (full △) indicates growth in the absence of T. atroviride; full A indicates growth in the presence of T. atroviride P1; full □ shows growth in the presence of T. atroviride OElae1; and × specifies growth in the presence of T. atroviride Δlae1. Full arrows define the time point where T. atroviride P1 and OElae1 stopped growth of the other fungi, whereas the dotted arrow specifies the time where T. atroviride Δlae1 strain stopped fungal growth. The solid and dotted horizontal line show the respective biomass formed by the three test fungi at the time of inhibition. B: confrontation of T. atroviride strain Δlae1-2 with R. solani, B. cinerea and A. alternata.

Table S1 Primers used for qPCR analysis.

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Author Contributions

Conceived and designed the experiments: RKA ISD CPK. Performed the experiments: RKA. Analyzed the data: RKA ISD CPK. Contributed reagents/materials/analysis tools: CPK ISD. Wrote the paper: CPK ISD.
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