Biosynthesis and Secretion of Indole-3-Acetic Acid and Its Morphological Effects on *Tricholoma vaccinum*-Spruce Ectomycorrhiza

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Fungus-derived indole-3-acetic acid (IAA), which is involved in development of ectomycorrhiza, affects both partners, i.e., the tree and the fungus. The biosynthesis pathway, excretion from fungal hyphae, the induction of branching in fungal cultures, and enhanced Hartig net formation in mycorrhiza were shown. Gene expression studies, incorporation of labeled compounds into IAA, heterologous expression of a transporter, and bioinformatics were applied to study the effect of IAA on fungal morphogenesis and on ectomycorrhiza. *Tricholoma vaccinum* produces IAA from tryptophan via indole-3-pyruvate, with the last step of this biosynthetic pathway being catalyzed by an aldehyde dehydrogenase. The gene *ald1* was found to be highly expressed in ectomycorrhiza and induced by indole-3-acetaldehyde. The export of IAA from fungal cells is supported by the multidrug and toxic extrusion (MATE) transporter Mte1 found in mycorrhiza and induced by indole-3-acetaldehyde. The export of IAA from fungal cells is supported by the multidrug and toxic extrusion (MATE) transporter Mte1 found in *T. vaccinum*. The addition of IAA and its precursors induced elongated cells and hyphal ramification of mycorrhizal fungi; in contrast, in saprobic fungi such as *Schizophyllum commune*, IAA did not induce morphogenetic changes. Mycorrhiza responded by increasing its Hartig net formation. The IAA of fungal origin acts as a diffusible signal, influencing root colonization and increasing Hartig net formation in ectomycorrhiza.

Fungi, mainly basidiomycetes, form a mutually beneficial symbiotic association, commonly known as ectomycorrhiza, with the roots of woody plants (1). After establishing contact with the host, the fungus initially grows around the roots to form a mass of mycelium called the fungal mantle. From there, some hyphae penetrate the roots to grow between cortical cells to form the Hartig net, which acts as a surface for the exchange of nutrients and signals between the two symbiotic partners (1). Variation in host specificity occurs within ectomycorrhizal fungi; some have a broad host range, whereas others form host-specific ectomycorrhiza. In contrast to ectomycorrhizal fungi such as *Pisolithus tinctorius* and *Paxillus involutus*, *Tricholoma* species often show host specificity; their fruiting bodies are found only below compatible host trees. For example, *Tricholoma vaccinum* fruiting bodies occur only near spruce, which is the compatible host (2). Under laboratory conditions, *Tricholoma* species form ectomycorrhiza with nonhost trees, but the mycorrhization process in such low-compatibility interactions requires more time and features spotty Hartig net formation (3). Thus, *T. vaccinum*, a slow-growing, late-stage, and highly host-specific mycorrhizal fungus was chosen for the investigation of fungus-derived phytohormone effects on both partners. These interactions were expected to show the importance of indole-3-acetic acid (IAA) in the establishment and functioning of the symbiosis. We hypothesized that a long period of incubation might be necessary to observe changes in the morphogenetic responses that are not easily visualized with fast-growing, early-stage mycorrhiza. In order to generalize our findings, we included experiments with other mycorrhizal fungi in our analysis of IAA formation by basidiomycetes.

Indole-3-acetic acid (IAA) was hypothesized, by proponents of the so-called “auxin theory” (4), to regulate ectomycorrhiza formation through the IAA produced by ectomycorrhizal fungi (5, 6). Mostly, biosynthesis takes place from tryptophan. In accordance with this hypothesis, we carried out a cytological examination of mycorrhiza between an IAA-overproducing transformant of *Hebeloma cylindrosporum* and *Pinus pinaster* seedlings and found a hypertrophic Hartig net (7). The authors suggested that fungal IAA affects the physiology of the host, thereby inducing the formation of the Hartig net.

Some studies have supported the role for IAA in mycorrhiza (6, 8, 9), whereas others have refuted it (10–12). This controversy remains unresolved, and explanations of the mechanisms involved remain elusive. It has been hypothesized that IAA acts as a signal to induce the expression of genes involved in mycorrhiza differentiation (13). Gea et al. (7) speculated that IAA facilitates the loosening of plant cell walls, allowing the fungus to enter the host root and to manipulate the host physiology to form a Hartig net. We propose that the phytohormone, in addition to influencing the morphogenesis of the host root, also affects the morphogenesis of the fungal partner.

Although many ectomycorrhizal fungi have been shown to produce IAA, the biosynthetic pathways and enzymes involved have not been investigated (14). Two different auxin biosynthetic pathways have been described for plants. One produces the phytohormone via indole-3-glycerophosphate; the other, which is
tryptophan dependent, has branches that differ in the intermediates indole-3-pyruvate (IPA) (15–19), tryptamine (TAM) (20), indole-3-acetaldoxime (IAOx) (21), and indole-3-acetamide (IAM) (22–24). A route converting indole-3-acetonitrile (IAN) into IAA has been proposed (21) but is controversial (25). In pathogenic bacteria, IAA production seems to be realized via IAM, whereas plant growth-promoting bacteria prefer the IPA pathway (26–28). In fungi, IAA synthesis was shown for the basidiomycetes *Ustilago maydis* (15, 29, 30), *Rhizoctonia* (31), and *Piriformospora indica* (32) and for the ascomycetes *Colletotrichum gloeosporioides* (33) and *Fusarium proliferatum* (34).

Here, we aimed to comprehensively investigate IAA involvement in ectomycorrhizal morphogenesis, following our hypothesis that IAA is a crucial component in ectomycorrhizal signaling between fungus and tree. The ability of *T. vaccinum* to produce IAA was verified, possible biosynthetic pathways were determined, and the involvement of a previously identified aldehyde dehydrogenase gene, *ald1*, was shown (3, 35). Indeed, the gene is strongly upregulated in *T. vaccinum* colonizing spruce seedling roots. IAA export from fungal cells was linked to transport via a member of the multidrug and toxic compound extrusion (MATE) family.

**MATERIALS AND METHODS**

**Culture conditions.** Fungal isolates used in this study are *T. vaccinum* Gk6514 (FSU 4731; Jena Microbial Resource Collection, Jena, Germany), *Armillaria mellea* MG091013_14, *Heterobasidion annosum* MG091028_02, *Leucoagaricus leucothites* MG090924_04, *Lyophyllum brevatum* MG091108_01, *Paecilomyces variotii* MG091013_13, *Schizophyllum commune* 1-116, *S. commune* 12-43 (ura*′*), and *T. vaccinum* 1.16 (35). Fungi were cultured as described previously (3).

To test for IAA production, Pachlewski medium (36) (0.25 g of di-NH4-tartrate, 0.5 g of KH2PO4, 0.25 g of MgSO4 ·7 H2O, 2.5 g of maltose, 5 g of glucose, 42 μL of thiamine hydrochloride [1.2 mg mL−1], 1 μg of FeCl3 · 3 H2O, pH 7, 1 ml of trace element solution) (37) was used to exclude potential IAA sources in the medium. To quantify IAA biosynthesis by *T. vaccinum* and investigate the effect of IAA on fungal growth and hyphal ramification, the MMNb medium (38) was supplemented with tryptophan (Trp), indole-3-acetaldehyde (IAD), indole-3-pyruvate (IPA), indole-3-acetamide (IAM), tryptamine (TAM), indole-3-acetonitrile (IAN), tryptophol (TOL), or IAA in concentrations of 0.05 to 2.5 μM. The IAA transport inhibitor 2,3,5-triiodobenzoic acid (TIBA; Sigma-Aldrich, Munich, Germany) was applied (0.01 mM) to test for IAA functions.

**Fungal growth, morphogenesis, and mycorrhiza formation.** The effect of IAA on fungal growth was investigated in fungal cultures established on MMNb plates containing tryptophan (0.5 mM), IAA (0.1 mM), and/or TIBA (10 μM). Mycelial radial growth was quantified by measuring the diameter of fungal colonies in biological triplicates starting after 1 week, when aerial mycelium had begun to form, and continuing up to 4 weeks. To test the effect of the phytohormone on fungal morphogenesis, fungal cultures were grown for 4 weeks (*T. vaccinum, A. mellea, and P. variotii*) or for 2 weeks (*S. commune, L. leucothites, L. loricatum, and *H. annosum*) with Trp, IPA, IAD, and IAA (0.1, 0.25, and 0.5 mM). Fungal mycelium was observed in three to five replicates under an Axioplan 2 microscope (Zeiss, Jena, Germany), and cell lengths and ramifications were quantified in 100 cells each. The length of each cell was measured (SPOT Advanced; Diagnostic Instruments, Sterling Heights, MI, USA), and the number of branches per hypha was microscopically counted.

Chitin was stained with 1 mM calcofluor (Sigma-Aldrich, Taufkirchen, Germany), nucleic acids were stained with 1 mM 4’,6-diamidino-2-phenylindole (DAPI; Roth, Karlsruhe, Germany) solution, and immunofluorescence was performed using anti-actin (rabbit) and Cy3-labeled goat anti-rabbit or anti-a-tubulin (mouse) and fluorescein (FITC)-labeled goat anti-mouse as described previously (41). Fluorescence microscopy using filter sets 02 for calcofluor and DAPI 15 for Cy3, and 10 for FITC was performed, and images were processed with SPOT Advanced software (Diagnostic Instruments, Sterling Heights, MI, USA).

Fungal mantle development around mycorrhized roots, as the first evidence of mycorrhiza formation, was monitored using a binocular microscope (KL 1500; Zeiss, Jena, Germany). Microtome sections (6 to 8 μm) were obtained from lateral roots of three trees for biological replicates. After 4 months in coculture, multiple root tips were analyzed for each replicate (rotation microtome HM 355; Microm, Walldorf, Germany), using embedding medium (Technovit 7101; Heraeus, Hanau, Germany) and toluidine blue O (0.1%, wt/vol, in H2O) for observing Hartig net development (41), which was visualized with a confocal laser scanning microscope (LSM 780; Zeiss, Jena, Germany), with tile scanning and three-dimensional stack optimization.

**Analysis of gene expression in mycorrhizal cultures.** The genomic sequence of *ald1* (GenBank accession number HM363121) was used for expression analyses. Competitive PCR was performed on mycorrhiza tissues from different hosts and after the mycelium mantle and the root parts were microdissected to account for *ald1* expression in the mantle and Hartig net. Total RNA was extracted from lyophilized material, and competitive PCR was performed with *tef1* (42) as a control in cDNA (Oligotex mRNA Batch Protocol [Qiagen, Hilden, Germany], Superscript II RNase H Reverse Transcriptase [Invitrogen, Darmstadt, Germany], or iScript cDNA synthesis kit [Bio-Rad, Munich, Germany]) with the respective controls and using the same kit in each experiment. Degenerate primers *tef0* (AAG AAG GTY GGN TAY AAY) and *tef3* (GTY CTR CAV ATG TTC TAR) were designed, and the resulting PCR products from fungus (240 bp) and plant (204 bp) were compared to be detected by using fungal cDNA in the mycorrhiza samples.

For competitive PCR, primers *rfl3a* (GCA AGA AAG GCA TAC AAA ACT) and *rfl3b* (GGG TCG CTG GTG AAT AA) were used to simultaneously amplify an 826-bp cDNA fragment and a 1,297-bp competitor fragment (a fragment comprised of genomic DNA with seven introns carried by a plasmid). The signal intensities of the resulting PCR products were measured after agarose gel electrophoresis to estimate the relative amount of the transcript. Real-time quantitative PCR (qPCR) was performed using SYBR green/6-carboxy-X-rhodamine (ROX) qPCR master mix (Thermo Scientific, Waltham, MA, USA) with primers *aldTRIPCRF* (GAA AGC TCT TGG AGG AGG TG) and *aldTRIPCRR* (TGG ACT GTA GCA CCC TCC TT) in a MiniOpticon cycler (Bio-Rad, Munich, Germany) from RNA isolated with an RNasey Plant Kit (Qiagen, Hilden, Germany). Subsequent cDNA synthesis from 0.5 μg of RNA was performed using iScript cDNA synthesis kit (Bio-Rad, Munich, Germany) or QuantiTect reverse transcription kit (Qiagen, Hilden, Germany). The accumulation of IAA mRNA after cells were fed IAA precursors was determined in absolute terms by comparing transcript levels to a standard curve generated by using *ald1* cDNA clones in plDrive. For the *ald1*-overexpressing transformant, expression was normalized to the reference genes *act1, cist1*, and *tefl* coding for actin, citrate synthetase, and the translation elongation factor EF1α, respectively. Three biological and two technical replicates were performed in each case.

**Identification of genes for IAA synthesis.** Candidate genes for IAA synthesis were identified in *silico* from *T. vaccinum* genomic DNA Illumina reads (GATC, Constance, Germany) after conceptual translation and alignment (using MAFFT, for alignments and citations, see Fig. S1 in the supplemental material). The genes *tam1* (GenBank accession number...
KP096350), idp1 (GenBank accession number KP096351), and iah1 (GenBank accession number KP096352) were identified. For homologous transformation and overexpression, aldl was introduced by Agrobacterium tumefaciens-mediated transformation using the constitutive glycerol-aldehyde-3-phosphate dehydrogenase promoter (Ppgd) of Agaricus bisporus on the pBGHg plasmid backbone (35).

Production of IAA by T. vaccinum. IAA concentrations were determined after T. vaccinum was cultivated in the dark at room temperature for about 4 weeks. Culture filtrates from three or four individual cultures, supplemented with labeled precursors, were sampled weekly and assessed for about 4 weeks. Culture filtrates from three or four individual cultures, mined after 210°C, and at 15°C min

with concentrations higher than 3 µM were found following supplementation of the medium with 13C6-labeled i-tryptophan after 1 month of incubation (Fig. 1).

These data were further verified by feeding cells 2H5-labeled precursors, showing a time-dependent conversion. One week after the addition of 2H5tryptophan (0.5 mM), traces of unlabeled IAA and 2H5IAA were released to the culture medium. Mass spectrometry revealed that the released IAA consisted of approximately 50% natural IAA and 50% labeled 2H5IAA. After 2 weeks, 2H5IAA prevailed (73%), while natural, unlabeled IAA dropped to 23% ± 4%. After 4 weeks, the ratio of 2H5IAA to natural IAA approached 4:1.

Labeled intermediates en route from tryptophan to IAA, easily recognized by an intense methyl-indole fragment at m/z 130, were not observed. The administration of other IAA precursors (0.25 mM), such as TAM, IAM, IAN, or TOL, did not enhance the production of IAA. Experiments with IPA remained inconclusive as the precursor proved to be unstable and generated IAA spontaneously (46, 47). Interestingly, the incubation with 0.2 mM IAD yielded the largest amount of IAA, 140.39 µM. Figure 2 shows the mass spectra (methyl ester) of natural and labeled IAA determined by GC-MS after 4 weeks. After extraction and esterification with diazomethane, both compounds displayed an intense molecular ion (m/z 189 for natural IAA and m/z 194 for 2H5IAA) and a base peak at m/z 130 or m/z 135, corresponding to the methylindole cation after the loss of O=C-OCH3. The fragments at m/z 130 and m/z 135 or m/z 136 result from an indole-typical loss of HCN or 2HCN, respectively. Aromatic core fragments at m/z 277 and m/z 81 indicate that the labeled fragment still contains four of the five deuterium atoms of the administered 2H5tryptophan.

In order to check whether IAA is produced only by ectomycorrhizal fungi, five additional fungi were investigated: one fast-growing, early-stage mycorrhizal species and four nonmycorrhizal species. Supernatants of three samples for each fungus were analyzed with a Salkowski assay after treatment with 0.1 mM tryptophan to evaluate the amount of IAA produced that might induce morphogenetic changes in fungi and/or trees. In addition to T. vaccinum, which produced approximately 7 µM IAA in this experiment, the mycorrhizal fungus P. involutus produced 17 µM IAA in Ectomycorrhiza

FIG 1 GC-MS quantification of IAA from T. vaccinum cultures supplemented with labeled tryptophan. IAA was purified weekly over a period of 4 weeks and analyzed by GC-MS. IAA peaks were obtained by tracing [13C6]IAA. The IAA amounts shown represent IAA only from labeled tryptophan. Bars denote standard errors, and letters indicate significant differences in IAA amounts (P < 0.05). dpi, days postinoculation.

RESULTS

IAA biosynthesis in T. vaccinum. The fungal biosynthesis of IAA was investigated using labeled precursors. Mass spectrometry verified the results of thin-layer chromatography and Salkowski assays, namely, that there was no detectable production of IAA in pure cultures of T. vaccinum. Considerable amounts of [13C6]IAA
IAA. Orthologs to the biosynthesis genes in the IPA pathway could be identified from *S. commune* (see Table S1 in the supplemental material), and IAA production was shown for single isolates of that fungus (48). However, under growth conditions similar to those used for *T. vaccinum*, none of the phytopathogenic or sap-robic basidiomycetes, *H. annosum, S. commune, L. leucothites*, and *L. loricatum*, produced IAA, with the exception of 4.1 /\text{mM measured for *A. mellea*.

**Regulation of ald1 in IAA synthesis and mycorrhization.** The pathways of IAA synthesis via IPA and conversion of IAD to IAA,
Changes in cell length and hyphal ramification were also found with the ectomycorrhizal fungus *P. involutus*; in contrast, the nonmycorrhizal species *A. mellea*, *H. annosum*, *S. commune*, *L. leucothites*, and *L. loricatum* did not respond to IAA. Thus, IAA was seen to have a signaling function in ectomycorrhizal fungi such as *T. vaccinum* and to affect hyphal morphology and growth, connections which have not previously been observed.

**Ectomycorrhiza development is supported by IAA secretion from the fungus.** To examine the effect of IAA in symbiotic interactions, homologous transformation of *ald1* was performed, and *ald1* overexpression was established in the transformant *T. vaccinum* 1.16 (Fig. 6 gives qPCR data). The strain was used to form ectomycorrhizas, which in turn formed an enhanced Hartig net and a thicker hyphal mantle (Fig. 7). Thus, an effect of Ald1, likely through IAA formation, was verified.

For IAA to influence the morphogenesis of both plants and fungi, it must be secreted to allow a transkingdom signaling function. Involvement of a multidrug and toxic compound extrusion (MATE) transporter, *mte1* of *T. vaccinum*, was tested since, for this transporter gene, mycorrhiza-specific expression had been established (3). We tested the hypothesis that Mte1-overexpression, heterologous yeast transformants should show better growth in the presence of an inhibitory high concentration of IAA. Indeed, *mte1* rescued growth of yeast transformants (Fig. 8). Our results showed that IAA was indeed a substrate for the tested transporter. Taking these observations together, the production, potential secretion, and effect of IAA on the fungus as well as on the tree partners were shown, and genes crucial for this mycorrhiza-specific IAA signaling between both partners were identified.

**DISCUSSION**

In this study, we identified the biosynthesis pathway for IAA production in the ectomycorrhizal fungus *T. vaccinum* and presented candidate genes for enzymes involved in this IAA biosynthesis pathway. Although ectomycorrhizal fungi have been reported to produce IAA, mainly with tryptophan as an IAA precursor (5, 14, 49), no single study has investigated the potential biosynthetic pathways and genes involved. Based on precursor feeding and isotope studies, we propose that *T. vaccinum* produces IAA through a tryptophan-indole pyruvate-indole acetaldehyde biosynthetic pathway. Free intermediates were not detected. Nevertheless, feeding cells intermediates resulted in labeled IAA being

| TABLE 1 Relative amounts of *ald1* transcript in mycorrhiza, determined by competitive PCR |
|-----------------------------------------------|-------------------------------------------|
| Type of *T. vaccinum* interaction (time posttreatment) | Fold increase in *ald1* expression* |
| Control | 1 |
| Mycorrhizal stage of compatible interaction | |
| Spruce (3 weeks) | 30 |
| Spruce (3 mo) | 45 |
| Mycorrhizal tissues of compatible interaction | |
| Hartig net | 40 |
| Fungal mantle | 50 |
| Low-compatible interaction | |
| Pine (3 mo) | 30 |

* Relative amount of the transcript using 1 ng of cDNA.
released from the fungal mycelium. The genes identified as involved in this biosynthetic pathway further support the findings for the IPA and IAM pathway available for *T. vaccinum* (see Fig. S1 and Table S1 in the supplemental material). Orthologs from *Lacccaria bicolor* and *T. matsutake* were identified, thus supporting the notion that other ectomycorrhizal fungi also use IAA signaling in mycorrhiza formation.

One of the candidate genes for biosynthesis, the aldehyde dehydrogenase gene *ald1*, was identified previously in an earlier attempt to understand ectomycorrhiza-specific gene expression (3). Increased expression after cultures were fed precursors of the suggested pathway suggests that the enzyme might be involved in the oxidation of IAD to IAA, the last step in the proposed Trp-dependent biosynthetic pathway (19, 25). The variation in *ald1* expression after feeding cells IAD compared to that after feeding cells IPA or tryptophan might result from the toxicity of the aldehyde that necessitates rapid detoxification by oxidation. Also, a spontaneous nonenzymatic breakdown cannot be ruled out. This pathway for IAA biosynthesis has been shown to be unlikely for plants (50) and might be specific to the fungal kingdom. Another basidiomycete, the smut fungus *Ustilago maydis*, has been shown to produce IAA via IPA (30). Interestingly, the product of *ald1* has a significant amino acid identity of 58% to *U. maydis* indole-3-acetaldehyde dehydrogenase, *iad1*, especially in the conserved motifs (15, 35), supporting the role of *ald1* in ectomycorrhizal IAA-dependent morphogenesis (51). Alternative pathways have been suggested for other fungi in different environmental settings (15, 33); however, these await detailed investigation.

In our study, the mycorrhizal fungi produced appreciable IAA amounts even when grown in axenic culture. *T. vaccinum* responded to IAA by increasing average cell length and ramification. In *T. vaccinum*, the addition of IAD did not result in cell elongation, perhaps because of the toxicity of the aldehyde and/or the involvement of other metabolic pathways. A reorganization of the cytoskeleton was not detected in our study after treatment with IAA precursors. However, a slight modification, as has been described for ectomycorrhiza (52), cannot be ruled out. For saprobic fungi, IAA production seems to be dispensable, or the concentrations necessary to exert a change in morphology

![FIG 5](http://aem.asm.org/images/5.jpg)

**FIG 5** *T. vaccinum* cell length and ramification in response to feeding cells external IAA or precursors at different concentrations. A total of 100 cells were counted (n = 3 replicates). Bars denote standard errors.

![FIG 6](http://aem.asm.org/images/6.jpg)

**FIG 6** Expression of *ald1* in *T. vaccinum* wild-type (*T.v.-WT*) and the *ald1*-overexpressing strain *T. vaccinum* 1.16 (*T.v.-116*). The regulation was evaluated in three biological replicates by qPCR and normalized to three reference genes (*act1*, *cis1*, and *tef1*). Bars denote standard errors, and letters indicate significantly different expression levels (P < 0.05).
might differ from those of mycorrhizal fungi. With saprobic fungi such as *S. commune*, IAA production is strain dependent (48) and depends on induction through interaction with other organisms or media.

The upregulation of *ald1* in *T. vaccinum* ectomycorrhiza indicated that fungal IAA biosynthesis is relevant to the symbiosis. Comparing different mycorrhizal tissues, stages, and fungal-host compatibility, we did not observe significant differences in the expression of *ald1*, which confirms the hypothesis that IAA is involved in different steps of the mycobiont-plant interaction. At early stages of mantle formation, but also within the Hartig net, hyperbranching is necessary to form the pseudoparenchymatic tissues (53). By feeding *T. vaccinum* with IAA precursors and IAA, we showed that IAA increases fungal cell length and hyphal ramification. The lack of increased colony diameter either indicates sufficient IAA production by the fungus under *in vitro* conditions, where the fungus is not additionally stimulated by plant compounds, or is the result of a denser mycelium as a response to ramification.

The induction of fungal morphogenetic changes by IAA during the mycobiont-plant interaction supports the hypothesis that phytohormones play a role in enhancing ectomycorrhiza formation and efficiency (54). Our results, which show increased mantle and Hartig net formation using an *ald1* overexpression strain, confirm that hypothesis and support the findings of Gay et al. (54), which show that IAA affects mycorrhiza morphology in pine when *Hebeloma* overproduces IAA. Additionally, the *ald1*-overexpressing strain, combined with feeding experiments, supported the hypothesis that *ald1* catalyzes the last step in the IPA pathway in IAA biosynthesis in *T. vaccinum*. Knockout mutants, which would be desirable for future investigation, are not feasible since gene deletion is not available in the dikaryotic fungus *T. vaccinum*. Nor have gene knockout or knock-down techniques yet been established for this slow-growing fungus. With tryptophan a typical component of root exudates present at relevant concentrations (55), conversion into IAA can increase the speed and efficiency of mycorrhization. Indeed, root exudates were reported to stimulate the hyphal growth of ectomycorrhizal fungi (56). Additionally, auxin was thought to be involved in host recognition during the premycorrhizal phase (57). The functional complexity of IAA explains why the controversy over IAA involvement in ectomycorrhiza formation has continued for so long.

For IAA to act as a signaling compound between host and fungus, it must be exported by the fungus and imported into either organism. The phytohormone might act as a diffusible signal in the communication between the mycobiont and the plant (51). IAA transport mechanisms are known in plants, where TIBA acts as a polar auxin transport inhibitor and reverses the effects of IAA on spruce-*Laccaria bicolor* ectomycorrhiza (58). We observed similar TIBA effects although an ortholog to the plant transporters is not encoded within the *T. vaccinum* genome. Nevertheless, we cannot rule out the possibility that TIBA targets other mechanisms. Instead, we suggest that IAA is transported by Mte1, a transporter belonging to the multidrug and toxic compound extrusion (MATE) family and known to be upregulated in the compatible mycorrhiza of *T. vaccinum* (45). In this study, IAA was shown to play a role in ectomycorrhizal morphogenesis, linking Hartig net formation to the response of the fungus to IAA by increased hyphal ramification. Thus, IAA is a signal in the mycobiont-plant interaction, and fungal IAA can contribute to ectomycorrhizal functions. Genes involved in biosynthesis (such as *ald1*) and in transport (such as *mte1*) were indeed identified as mycorrhiza specific. This identification further
contributes to the finding that IAA plays a versatile role in mycorrhiza, with the phytohormone regulating ectomycorrhiza formation and morphology.

ACKNOWLEDGMENTS

This research was supported by the Max Planck Society, Germany and by the Excellence Graduate School GSC 214 and the Research Training Group GRK 1257 funded by the German Science Foundation. We thank Paulina Dabrowska and Anja David for assistance and Maritta Kunert, Ines Schlunk, and Elke-Martina Jung for support. Petra Mitscherlich is acknowledged for technical help. We thank Emily Wheeler for editorial assistance.

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