Endohyphal Bacterium Enhances Production of Indole-3-Acetic Acid by a Foliar Fungal Endophyte

Michele T. Hoffman1, Malkanthis K. Gunatilaka1, Kithsiri Wijeratne2, Leslie Gunatilaka2, A. Elizabeth Arnold2

1 School of Plant Sciences, The University of Arizona, Tucson, Arizona, United States of America; 2 Southwest Center for Natural Products Research and Commercialization, School of Natural Resources and the Environment, Tucson, Arizona, United States of America

Abstract

Numerous plant pathogens, rhizosphere symbionts, and endophytic bacteria and yeasts produce the important phytohormone indole-3-acetic acid (IAA), often with profound effects on host plants. However, to date IAA production has not been documented among foliar endophytes — the diverse guild of primarily filamentous Ascomycota that live within healthy, above-ground tissues of all plant species studied thus far. Recently bacteria that live within hyphae of endophytes (endohyphal bacteria) have been detected, but their effects have not been studied previously. Here we show not only that IAA is produced in vitro by a foliar endophyte (here identified as Pestalotiopsis aff. neglecta, Xylariales), but that IAA production is enhanced significantly when the endophyte hosts an endohyphal bacterium (here identified as Luteibacter sp., Xanthomonadales). Both the endophyte and the endophyte/bacterium complex appear to rely on an L-tryptophan dependent pathway for IAA synthesis. The bacterium can be isolated from the fungus when the symbiotic complex is cultivated at 36°C. In pure culture the bacterium does not produce IAA. Culture filtrate from the endophyte-bacterium complex significantly enhances growth of tomato in vitro relative to controls and to filtrate from the endophyte alone. Together these results speak to a facultative symbiosis between an endophyte and endohyphal bacterium that strongly influences IAA production, providing a new framework in which to explore endophyte-plant interactions.

Introduction

Diverse plant-associated microbes synthesize phytohormones such as gibberellins, cytokinins, jasmonic acid, abscisic acid, ethylene, and indole-3-acetic acid (IAA), often with profound effects on growth, tissue differentiation, and reproduction of their hosts [1-8]. Microbial production of IAA is especially phylogenetically widespread, encompassing both plant-affiliated bacteria (e.g., Erwinia herbicola [9] and Pantoea agglomerans [10]) and diverse fungi (Mucoromycotina, Basidiomycota, and Ascomycota [11-13]). Most examples of IAA production by plant-associated microbes come from plant pathogens, mycorrhizal fungi, rhizosphere endophytes, and bacteria and yeasts that are endophytic in above-ground tissues [14-19]. IAA produced by rhizosphere fungi can stimulate production of plant biomass, enhance growth rate of roots, and promote disease resistance [7,19,20]. In some cases, IAA produced by fungi affiliated with plants also can inhibit hypersensitive responses, reducing the production of defensive enzymes such as chitinase and glucanase (reviewed in 6).

In both natural and human-made environments, plants consistently harbor filamentous fungi (primarily Pezizomycotina, Ascomycota) in their apparently healthy above-ground tissues [21]. These endophytes (Class 3, sensu [21]; hereafter, endophytes) are known from every major lineage of land plants in biomes ranging from tundra to tropical forests [22-26]. They are transmitted horizontally and form numerous, localized infections in asymptomatic tissues such as leaves and stems [27-30]. At the community level they are highly diverse and individual plants frequently harbor multiple species, with significant turnover in endophyte assemblages over plant species’ ranges [23,25,26,31-33]. Endophytes often are closely related to pathogens, with transitions between...
endophytism and pathogenicity occurring frequently in the evolution of the Ascomycota [25].

Recent studies have shown that foliar endophytes frequently harbor highly diverse endophyhal bacteria of unknown importance (e.g., [34]). These bacteria occur in living hyphae of phylogenetically diverse endophytes isolated from various plant lineages and in multiple biogeographic provinces [34]. Those found in foliar endophytes are phylogenetically distinct from the apparently obligate symbionts of other plant-affiliated fungi (e.g., Glomeromycota) [34]. Phylogenetic analyses of endophyhal bacteria associated with filamentous foliar endophytes reveal no clear signal of fungal phylogeny, host plant phylogeny, or geography, suggesting a facultative association [34]. However, endophyhal bacteria of many fungi have not previously been cultivated independently of their hosts, and the effects of endophyhal bacteria on foliar endophytic fungi have not been evaluated to date. More broadly, the benefits or costs that endophytes and endophyty-bacterial complexes extend to their hosts, and the mechanisms by which endophytes with or without bacterial symbionts can escape, tolerate, or prevent induction of plant defenses remain major questions [24,28,34,35]. Because IAA is produced by diverse plant-associated fungi, can decrease host hypersensitive responses, and can enhance plant growth, we anticipated that IAA production could be an important but unexplored aspect of foliar endophyte-plant symbioses.

Here we provide the first documentation of IAA production by a foliar endophyte representing the Pezizomycotina (identified as Pestalotiopsis sp., with affinity for Pe. neglecta) isolated from foliage of a coniferous host (Platycladus orientalis, Cupressaceae). Further, we demonstrate that IAA production by the endophyte in vitro is enhanced significantly when the endophyte hosts an endohyphal bacterium (here identified as Luteibacter sp., Xanthomonadaceae). We show that IAA production by the endophyte and the endophyte-bacterial complex requires L-tryptophan. The bacterium, which can be cultured axenically, does not produce IAA on a standard growth medium. Culture filtrate from the endophyte-bacterium complex significantly enhances growth of a model plant (tomato) relative to controls and to filtrate from the endophyte alone, suggesting a potentially important but previously overlooked aspect of plant-endophyte symbioses.

Materials and Methods

As part of a previous study [33], endophytic fungus 9143 was isolated on 2% malt extract agar (MEA) from surface-sterilized, asymptomatic foliage of a mature, healthy individual of Cupressaceae. Further, we demonstrate that IAA production could be an important but previously overlooked aspect of foliar endophyte-bacterium symbiosis.

Identification of endohyphal bacterium

Previous analyses revealed that endophyte 9143 harbored an endohyphal bacterium, which we identified previously on the basis of 16s rRNA sequencing and light microscopy as a member of the Gammaproteobacteria ( [34]; GenBank accession HM117737). The 16S rRNA sequence obtained by bidirectional Sanger sequencing using primers 27F and 1492R [47] was aligned manually in Mesquite v. 1.06 [43]. Bayesian analysis was implemented in MrBayes v. 3.1.2 [45] with 2 sets of 5 million generations each, initiated with random trees, four chains, and sampling every 1000th tree. After elimination of the burn-in, defined by assessment of ln li values, the remaining trees were used to infer a majority rule consensus. A complementary maximum likelihood (ML) inference was conducted with GARLI v1.0 [46] using default settings and GTR +I+G, followed by bootstrap analysis (100 replicates).

Production of bacterium-free clone

Three replicate subcultures of endophyte 9143 were cultivated on 2% MEA, and on 2% MEA amended with 40 µg ml⁻¹ of the antibiotic ciprofloxacin, for 10 d. Bacterial infection status in fresh mycelium was evaluated using light microscopy (400X), which ruled out external contaminants; Live-Dead stain, which established that bacteria and hyphae were viable; and DNA extraction, 16S rRNA PCR, and sequencing per above, which confirmed the presence or absence of Luteibacter following [34]. Cultures with (hereafter, 9143+) and without (9143-) the endohyphal bacterium were stored separately at -80 °C in 80% glycerol. Growth rates of 9143+ and 9143- were compared in triplicate on 2% MEA and water cultivation on MEA for 7 d (Figure S1) [36]. For confirmation, we compared sequence data from the nuclear ribosomal internal transcribed spacers and 5.8S gene of 9143, obtained by bidirectional Sanger sequencing in our previous work ( [33]; GenBank accession EF419899.1), with 35 sequences representing close relatives of Pe. neglecta [37-41], which were obtained from GenBank. The dataset, including Seiridium as the outgroup, was aligned automatically with default parameters in ClustalW 1.0 [42] and adjusted manually in Mesquite v. 1.06 [43]. The best-fitting model of evolution (GTR +I+G) was inferred using Modeltest 3.7 [44]. Bayesian analysis was implemented in MrBayes v. 3.1.2 [45] with 2 sets of 5 million generations each, initiated with random trees, four chains, and sampling every 1000th tree. After elimination of the burn-in, defined by assessment of ln li values, the remaining trees were used to infer a majority rule consensus. A complementary maximum likelihood (ML) inference was conducted with GARLI v1.0 [46] using default settings and GTR +I+G, followed by bootstrap analysis (100 replicates).
agiar at 22°C and 36°C, and at three pH levels on MEA (pH = 4.5, 6.8, and 8.0) as described in [50].

**Measurement of indole compound production in vitro**

Small (2mm²) fragments of mycelium from isolates of 9143+ and 9143- were plated on 2% MEA and allowed to grow for 7 d. Pieces removed from resulting cultures with a sterile cork borer were used to inoculate three flasks containing 80ml of Czapek Dox broth (CDB: Hymedia; pH 7.2) augmented with L-tryptophan (Sigma; 5 mM). Each treatment set, one flask containing only sterile CDB was used as a negative control. Flasks were checked for contamination after agitating at 120 rpm at 26 °C for 24 h.

After 72 h, three 1 ml aliquots were removed from each flask and centrifuged to remove cells from suspension. The Salkowski colorimetric technique then was used to estimate the concentration of indole compounds by treating the supematant with 2 ml of Salkowski reagent (1 ml, 0.5 mM FeCl₃, and 50 ml, 35% HClO₄) [51-53]. Samples were incubated at room temperature for 30 min, and then evaluated at 530 nm on a spectrophotometer. CDB supplemented with L-tryptophan (as above) was used as a blank. IAA concentrations were determined with an IAA standard curve using commercial IAA (Sigma) and sterile medium as a blank.

**Identification of the indole compound as IAA**

We used thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) to identify the indole compound revealed by the diagnostic colorimetric reaction with Salkowski reagent. Two cultures each of 9143+ and 9143- were grown for 14 d in 500 ml of sterile CDB supplemented with 5 mM L-tryptophan. One additional culture of each was grown in sterile CDB without L-tryptophan (1 L) to evaluate whether the pathway for IAA production is tryptophan-dependent. Each culture was filtered using Whatman No. 1 filter paper. Each filtrate (1 L) was extracted three times with 500 ml of ethyl acetate (EtOAc). The combined EtOAc layer was washed three times with 500 ml of H₂O, dried over anhydrous Na₂SO₄, and evaporated under reduced pressure to yield an EtOAc extract.

Normal phase TLC analysis of the EtOAc extract was performed on aluminum-backed plates coated with a 0.20 mm layer of silica gel 60 F₂₅₄ (E. Merck, Darmstadt). Spots were visualized by inspection of plates under UV (254 nm) and after spraying with Van Urk-Salkowski reagent [52] followed by heating. Analytical reversed phase TLC investigations were performed on aluminum-backed plates coated with a 0.20 mm layer of silica gel 60 RP-18 F₂₅₄S (E. Merck, Darmstadt; eluant: 20% MeCN in H₂O, R₂ 0.5). Analytical HPLC analysis of the EtOAc extracts was performed using a Kromasil 5 μm C-18 column (4.6 x 250 mm) on a Shimadzu HPLC system equipped with DGU-14A degasser, LC-10ADvp pump, SPD-M10Avp diode array detector and SCL-10Avp system controller utilizing Shimadzu LC-MS solution software. Samples were redissolved in MeOH (2.0 mg ml⁻¹) and injections (10 μl) were made with Shimadzu SIL-10ADvp auto injector. The mobile phase consisted of H₂O/MeCN/HCOOH (69:7.5:30.00:0.25) with a flow rate of 1.2 ml min⁻¹. Commercial IAA (Sigma) was used as an authentic sample (eluant: 8% MeOH in CH₂Cl₂, R₂ 0.3), with peak enhancement following injection confirming identity of putative IAA peaks in each analysis.

**Seedling assays**

Isolates of 9143+ and 9143- were grown for 14 d in 200 ml CDB with 5 mM L-tryptophan as described above and then vacuum-filtered through a 0.44 μm nylon filter. The pH of each filtrate (pH = 5.7 for 9143+; pH = 4.2 for 9143-; pH = 7.1 for CDB alone) was amended to 7.0-7.1 with 0.5 M NaOH as needed. Twenty-five ml of each filtrate was further filter-stereilized using 0.2 μm syringe filters for seedling assays.

Tomato seeds (ACE 55; The Home Depot) were surface-sterilized by agitating in 50% bleach for 12-15 minutes, rinsed in sterile water, and placed on sterile filter paper with 3ml of sterile water in 60 mm Petri dishes (20 seeds/dish). Plates were sealed with Parafilm and seeds were allowed to germinate at 25 °C for 5 d. Sets of 10 apparently healthy seedlings then were chosen randomly and transferred under sterile conditions to new 60 mm plates containing sterile filter paper. Each set of seedlings was treated with 3.5 ml of sterile water. Four plates per treatment (i.e., 40 seedlings/treatment) then received 50 μl per seedling of one of five treatments: (a) filter-sterilized CDB from 9143+; (b) filter-sterilized CDB from 9143-; (c) filter-sterilized CDB + 5 mM L-tryptophan; (d) filter-sterilized CDB + commercial IAA (0.1 mg ml⁻¹; pH 7.0); or (e) sterile water. Plates were sealed with Parafilm and incubated under fluorescent lights at room temperature for 5 d (12 h light/dark cycle). At harvest seedlings were stretched to full length by mounting to paper with transparent cellophane tape. Shoot and root lengths to longest points were measured using calipers. Data were analyzed in JMP® 8.0 using ANOVA after normalizing all measurements to the CDB + IAA treatment (treatment d).

**Isolation of the endophyhal bacterium**

Incubating 9143+ on 2% MEA at 36 °C for 7 d resulted in emergence of bacterial growth from the fungal mycelium. We sequenced 1100 bp of the 16S rRNA region of the emergent bacterium (BAC182) as above (see also 47). The sequence was identical to that of the bacterium sequenced directly from genomic DNA of endophyte 9143+. BAC182 was grown overnight in sterile LB broth and vouchedered in sterile glycerol at -80 °C. The bacterium was screened for IAA production as described above.

**Results**

After cultivation on 2% MEA, conidia of endophyte 9143 were observed as multisetulate, fusiform-shaped, and concolorous, without knobbed appendages (see 36). Phylogenetic analyses placed endophyte 9143 as sister to one of three taxa identified as *Pe. neglecta* by [40], congruent with morphological assessment (Figure 1, S1). Our results are congruent with previous studies (e.g., [40]) in placing two other putative *Pe. neglecta* sequences, which appear to represent misidentified sequences from GenBank, in a separate subclade. Based on morphology and phylogenetic placement
relative to a recognized and vouched isolate of \textit{Pe. neglecta},
we consider 9143 to be \textit{Pestalotiopsis aff. neglecta},
with further systematic revision to follow. In turn, phylogenetic analyses of
16S rRNA confirmed placement of the endohyphal bacterium
from 9143 in the Xanthomonadaceae, with strong support
within \textit{Luteibacter} (\textit{Luteibacter} sp.; Figure 2).

We found no difference between 9143+ and 9143- isolates in
growth rate on 2\% MEA or water agar at 22 °C, nor with regard
to pH of the growth medium (Figure S2). Neither 9143+ nor
9143- grew at 36 °C, but cultivation of 9143+ at this
temperature resulted in successful isolation of the endohyphal
bacterium (above).

Chromogenic testing indicated that 9143- produced an indole
compound when growing axenically \textit{in vitro} (Figure 3).
Production of that indole compound was enhanced significantly
when the mycelium of 9143 contained the endohyphal
bacterium (9143+; repeated measures ANOVA, $F_{1,4} = 358.7$; $p < 0.0001$; Figure 3). After 14 d, mean concentration of the
indole compound in CDB from 9143+ (104.8µg ml$^{-1}$) was 78 µg
ml$^{-1}$ greater than that produced by 9143- grown under the same
conditions (Figure 3).

TLC and HPLC identified the indole compound as indole-3-
acetic acid (IAA), and confirmed that 9143 produced
significantly more IAA when the endohyphal bacterium was
present vs. absent (Figure 4). No IAA was produced when
9143+ and 9143- isolates were grown in CDB without L-
tryptophan, nor by \textit{Luteibacter} when grown alone in CDB +
tryptophan (data not shown).

Tomato seedlings treated with filter-sterilized CDB from
9143- did not differ significantly in root length or total seedling
length relative to the CDB control (Figure 5, post-hoc Tukey-
Kramer test, alpha = 0.05). However, seedling length was
significantly increased by treatment with CDB from 9143+,
reflecting significantly longer roots relative to controls and
treatment with CDB from 9143- (Figure 5, Tukey-Kramer tests,
alpha = 0.05).

\textbf{Discussion}

Endohyphal bacteria have been found previously in living
hyphae of plant-associated Glomeromycota, \textit{Mucoromycotina},
and several ectomycorrhizal Dikarya (\textit{Tuber borchii};

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**Figure 1.** Majority rule consensus based on Bayesian analyses of ITSrDNA sequences representing \textit{Pestalotiopsis} spp.
with affiliation for \textit{Pe. neglecta}, with \textit{Seiridium} as the outgroup. Values indicate maximum-parsimony bootstrap ≥70\% (before slash) and Bayesian posterior probability ≥90\% (after slash).

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Ascomycota; Laccaria bicolor and Piriformospora indica; Basidiomycota) [54-59,61,62]. Recently, we reported their presence in foliar endophytic fungi representing four classes of Pezizomycotina, and demonstrated that they are both geographically widespread and phylogenetically diverse [34].

The present study is the first to identify the partners in a close ecological relationship between a foliar endophyte and an endohyphal bacterium, and the first to show that (a) a species of *Pestalotiopsis* (here identified as *Pestalotiopsis* sp. aff. *neglecta*) is capable of producing a phytohormone; (b) a filamentous endophyte from above-ground tissues can produce indole-3-acetic acid (IAA); (c) IAA produced by this endophyte appears to be dependent on the L-tryptophan pathway; (d) an endohyphal bacterium (*Luteibacter* sp.) significantly enhances this IAA production, but does not produce measurable IAA when grown axenically; and (e) exogenous culture filtrate of the endophyte with its endohyphal symbiont significantly enhances root growth of a model plant in *vitro* relative to controls and to filtrate from the endophyte alone. Moreover, our work shows that the endohyphal bacterium affiliated with 9143 can be isolated following heat-treatment of the host endophyte in culture.

The lack of IAA production by *Luteibacter* sp. in axenic culture contrasts with the ample production (ca. 40 µg ml⁻¹ of IAA) by axenic *Rhizobium radiobacter*, a culturable bacterial endosymbiont associated with the root endophyte *Piriformospora indica* (Sebacinales, Basidiomycota) [59]. It is possible that production could be observed by altering the growth medium on which *Luteibacter* was grown for the present work, perhaps through enhancement of tryptophan availability.

In the presence of endohyphal *Luteibacter* sp. and L-tryptophan, 9143+ cultivated in CDB produced ca. 100 µg ml⁻¹ of IAA. This amount is likely biologically significant, as suggested by seedling assays and by previous studies with plant-pathogenic and rhizosphere fungi: IAA production observed here was within the range produced by *Ustilago maydis* (75-262 µg ml⁻¹) as measured using the same colorimetric reagents [15]. Using GC-MS and HPLC-ESI_MS/MS, culture filtrate from *Piriformospora indica* yielded more IAA than was observed here [7]. In turn, isolates of *Colletotrichum* were found to generate 2-32 µg ml⁻¹ of IAA using TLC and GC-MS [5,17], consistent with the values observed in 9143-.

Recent work has shown that closely related or conspecific fungi can harbor different endohyphal bacteria [34]. These associations appear to be lost readily, as observed under heat treatment (above). The mechanism by which they are acquired...
has not yet been determined for foliar endophytes, although spore invasion in soilborne Glomeromycota has been observed [57]. Our results, coupled with phylogenetic analyses of endohyphal bacteria that reveal no signal of fungal phylogeny, host plant phylogeny, or geography [34], are consistent with a facultative association that contrasts with the obligate association between endohyphal bacteria and some arbuscular mycorrhizal fungi [54].

Although our study does not yet address bacterial-fungal-plant interactions during the endophyte symbiosis, it provides a first estimation of one way in which an apparently facultative bacterial endosymbiont can influence interactions between an endophytic fungus and its host. We attribute the observed differences in root growth to enhancement of IAA production by the endophyte/endohyphal bacterium relative to the endophyte alone, in part because of the large magnitude of change in IAA (Figures 3, 4) relative to other compounds (Figure 4). It is possible that other compounds detected by HPLC (Figure 4) might selectively inhibit growth; this is a focus for future study.

In the meantime we are interested to assess whether IAA production by the endophyte or endophyte/endohyphal bacteria complex can decrease host defensive responses to this and related endophytes [34], thus contributing substantively to the capacity of endophytic fungi to grow asymptptomatically host tissue.

Conclusions and perspectives

Our study provides the first evidence that bacterial associates of foliar endophytes can influence phytohormone production. We predict that the facultative nature of the endohyphal symbiosis may account for some of the diversity and ecological plasticity observed in endophyte-plant interactions [25]. More generally, increasing but still limited exploration of ectohyphal bacteria, endohyphal bacteria, and mycoviruses has begun to illustrate the powerful but often overlooked ways in which microbes associated with fungal hyphae can influence the outcome of plant-fungus associations [19,60-62]. Advancing our knowledge of endophyte interactions with plant hosts, other extrinsic microorganisms, and most recently, diverse endohyphal bacteria, will help define the functional biology of these diverse and ubiquitous symbionts.

Supporting Information

Figure S1. Pestalotiopsis neglecta asexual spore morphology coincides with the morphology of conidia from endophyte 9143. Conidia are fusiform, four-septate, a fuliginous brown in color, with end cells hyaline. The apical end is short with two or three spreading setulae, approximately 22 um long. The basal end contains a pedicel about 4-7 um long (Steyaert, 1953). Image depicts conidia from 9143- (400X) following cultivation on 2% MEA, showing fusiform cells with 4 septae.
We thank D.R. Maddison for sharing pre-release versions of data are not shown. Error bars indicate standard error of the three replicates performed for all experiments.

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Author Contributions

Conceived and designed the experiments: MTH AEA MKG EMKW. Performed the experiments: MTH MKG EMKW. Analyzed the data: MTH AEA MKG EMKW. Contributed reagents/materials/analysis tools: AEA EMKW MKG AALG. Wrote the manuscript: MTH AEA AALG.

References

1. Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol 21: 1-18. doi: 10.3109/10408419509113531. PubMed: 7576148.

2. Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41: 109-117. doi:10.1139/m95-015.

3. Koga J (1996) Structure and function of indolepyruvate decarboxylase, a key enzyme in indole-3-acetic acid biosynthesis. Biochim Biophys Acta 1249: 1-13. doi:10.1016/0167-4838(95)00111-I. PubMed: 7766676.

4. Lindow SE, Desumont C, Elkins R, McCourt G, Clark E et al. (1998) Occurrence of indole-3-acetic acid-producing bacteria on pear trees and their association with fruit russet. Phytopathology 88: 1149-1157. doi:10.1094/PHYTO.1998.88.11.1149. PubMed: 1894487.

5. Robinson M, Rivo J, Sharon A (1998) Indole-3-acetic acid biosynthesis in Colletotrichum gloeosporioides f sp aescynomone. Appl Environ Microbiol 64: 5030-5033. PubMed: 9836603.

6. Maco R, Haskin S, Levi-Kedmi H, Sharon A (2004) In planta production of indole-3-acetic acid by Colletotrichum gloeosporioides f sp aescynomene. Appl Environ Microbiol 70: 1852-1864. doi:10.1128/AEM.70.3.1852-1854.2004. PubMed: 15008816.

7. Sirrenberg A, Göbel C, Grond S, Czempsinski N, Ratzinger A et al. (2007) Piriformospora indica affects plant growth by auxin. Physiol Plant 131: 581-589. doi:10.1111/j.1399-3054.2007.00983.x. PubMed: 18251849.

8. Spaepe S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31: 425-448. doi:10.1111/j.1574-6976.2007.00072.x. PubMed: 17509086.

9. Yang S, Zhang Q, Guo J, Charkowski AO, Glick BR et al. (2006) Global effect of indole-3-acetic acid biosynthesis on multiple virulence factors of Erwinia chrysanthemi 9373. Appl Environ Microbiol 73: 1079-1088. PubMed: 17184441.

10. Barash I, Manulis-Sasson S (2009) Recent evolution of bacterial pathogens: The gall-forming Pantoea agglomerans case. Annu Rev Phytopathol 47: 133-152. doi:10.1146/annurev-phyto-080508-081903. PubMed: 18251849.

11. Franks D, Fester T, Hause B, Schliemann W, Walter MH (2003) The endophytic continuum. Mycol Res 109: 541-549. doi:10.1017/S0374180X0300062X. PubMed: 12507334.

12. Carroll G, Petrini O (1983) Patterns of substrate utilization by some fungal endophytes from coniferous foliage. Mycologia 75: 53-63. doi:10.2307/37929293.

13.Arnold AE, Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: Are tropical leaves biodiversity hotspots? Ecology 88: 541-549. doi:10.1890/05-1459.1. PubMed: 17503580.

14. Arnold AE (2007) Understanding the diversity of fungal endophytes: progress, challenges, and frontiers. Fungal Biol Rev 21: 51-66.

15.Arnold AE, Miadlikowska J, Higgins KL, Sarvate SD, Gugger P et al. (2009) A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? Syst Biol 58: 283-297. doi:10.1093/sysbio/syp001. PubMed: 19236579.

16. Arnold AE, Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: Are tropical leaves biodiversity hotspots? Ecology 88: 541-549. doi:10.1890/05-1459.1. PubMed: 17503580.

17. Arnold AE, Lutzoni F (2007) Diversity and host range of foliar fungal endophytes: Are tropical leaves biodiversity hotspots? Ecology 88: 541-549. doi:10.1890/05-1459.1. PubMed: 17503580.

18. Arnold AE (2007) Understanding the diversity of fungal endophytes: progress, challenges, and frontiers. Fungal Biol Rev 21: 51-66.

19. Arnold AE, Miadlikowska J, Higgins KL, Sarvate SD, Gugger P et al. (2009) A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? Syst Biol 58: 283-297. doi:10.1093/sysbio/syp001. PubMed: 19236579.

20. U'Ren JM, Lutzoni F, Miadlikowska J, Laetsch T, Arnold AE (2012) Host and geographic structure of endophytic and endolichefic fungi at a continental scale. Am J Bot 99: 898-914. doi:10.3732/ajb.1100459. PubMed: 22539807.

21. Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109: 661-686. doi:10.1017/S095375620500272X. PubMed: 16080390.

22. Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z et al. (2003) Fungal endophytes limit pathogen damage in a tropical tree. Proc Natl Acad Sci U S A 100: 15649-15654. doi:10.1073/pnas.2533483100. PubMed: 14671327.

23. Arnold AE, Herre EA (2003) Niche convergence and leaf age effect colonization by tropical fungal endophytes: ecological pattern and process in Theobroma cacao (Malvaceae). Mycologia 95: 388-398. doi:10.2307/3761880. PubMed: 21156627.
31. Lodge DJ, Fisher PJ, Sutton BC (1996) Endophytic fungi of Manilkara bidentata leaves in Puerto Rico. Mycologia 88: 733-738. doi: 10.2307/3760967.

32. Higgins KL, Arnold AE, Madiakowski J, Sarvate SD, Lutzoni F (2007) Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic-endophytes from three plant plant lineages. Appl Phylogenet Evol 42: 543-555. doi:10.1016/j.ympev.2006.07.012. PubMed: 17005421.

33. Hoffmann MT, Arnold AE (2008) Geographic locality and host identity shape fungal endophyte communities in cupressaceous trees. Mycol Res 112: 331-344. doi:10.1016/j.mycres.2007.10.014. PubMed: 18308531.

34. Hoffmann MT, Arnold AE (2010) Diverse bacteria inhabit living hyphae of phylogenetically diverse fungal endophytes. Appl Environ Microbiol 76: 4063-4075. doi:10.1128/AEM.02928-09. PubMed: 20435775.

35. Waller F, Achatz B, Baltruschat H, Fodor J, Becker K et al. (2005) The endophytic fungus Pinformospora indica reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc Natl Acad Sci U S A 102: 13386-13391. doi: 10.1073/pnas.0504423102. PubMed: 16174735.

36. Steyaert RL (1953) New and old species of Pestalotopsis. Trans Br Mycol Soc 36: 81-89. doi:10.1016/S0007-1356(53)80052-5.

37. Jeevon R, Liew ECY, Hyde KD (2004) Phylogenetic evaluation of species nomenclature of Pestalotopsis in relation to host association. Fungal Divers 17: 39-55.

38. Jeevon R, Liew ECY, Simpson JA, Hodgkiss JI, Hyde KD (2003) Phylogenetic significance of morphological characters in the taxonomy of Pestalotopsis species. Mol Phylogenet Evol 27: 372-383. doi: 10.1016/S1055-7903(03)00010-1. PubMed: 12742743.

39. Wei JG, Xu T, Guo LD, Liu AR, Zhang Y et al. (2007) Endophytic Pestalotiopsis species associated with plants of Podocarpaceae, Theaceae and Taxaceae in southern China. Fungal Divers 24: 55-74.

40. Watanabe K, Motohashi K, Ono Y (2010) Description of Pestalotopsis pallidotheae: a new species from Japan. Mycology 51: 182-188. doi:10.1056/S1055-7903(01)00202-5.

41. Suwannarach N, Sujarit K, Jumla J, Bussaban B, Lumyong S (2013) Stress tolerance, disease resistance, and higher yield. Proc Natl Acad Sci U S A 102: 13386-13391. doi:10.1073/pnas.1302923102. PubMed: 16174735.

42. Ethmann A (1977) The van Urk-Salkowski reagent - A sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. J Chromatogr 132: 267-276. doi:10.1016/S0021-9673(00)89300-0. PubMed: 188858.

43. Glickmann E, Dessaux Y (1995) A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61: 793-796. doi:10.1128/AEM.61.4.793-796.1995.

44. Bianciotto V, Bandi C, Minardi D, Sironi M, Tichy HV et al. (1996) An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. Appl Environ Microbiol 62: 3005-3010. PubMed: 8702293.

45. Barbieri E, Potenza L, Rossi I, Sisti D, Giomaro G et al. (2000) Phylogenetic characterization and in situ detection of a Cytophaga-Flexibacter-Bacteroides phylgroup bacterium in Tuber borchii Vittad ectomycorrhizal mycelium. Appl Environ Microbiol 66: 5035-5042. doi:10.1128/AEM.66.11.5035-5042.2000. PubMed: 11055961.

46. Bertaux J, Schmid M, Prevost-Boure NC, Churin JL et al. (2003) In situ identification of intracellular bacteria related to Paenibacillus sp in the mycelium of the ectomycorrhizal fungus Laccaria bicolor. S1328N. Fungal Divers 17: 39-55. doi:10.1023/A:1021797108784.

47. Lean DJ (1991) 16S/23S rRNA sequencing. In: E StackebrandtM & Glickmann E, Dessaux Y (1995) A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61: 793-796. doi:10.1128/AEM.61.4.793-796.1995.

48. Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of Gateway Computing Environments Workshop (GCE). New Orleans. pp. 1-8.

49. Swoford DL (2002) PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods). Sunderland, MA. Sinauer Associates.

50. Hoffman MT (2010) Bacterial endosymbionts of endophytic fungi: diversity, phylogenetic structure, and biotic interactions. Ph.D. Dissertation, the University of Arizona, Tucson, AZ.

51. Saikowsky SF (1979) Ultrastructure of the endosymbiotic fungus of Sebacinales. Cell Microbiol 10: 2235-2246. doi:10.1111/j.1087-4556.1977.tb01265.x. PubMed: 188858.

52. Ehmam A (1977) The van Urk-Salkowski reagent - A sensitive and specific chromogenic reagent for silica gel thin-layer chromatographic detection and identification of indole derivatives. J Chromatogr 132: 267-276. doi:10.1016/S0021-9673(00)89300-0. PubMed: 188858.

53. Glickmann E, Dessaux Y (1995) A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61: 793-796. doi:10.1128/AEM.61.4.793-796.1995.

54. Bianciotto V, Bandi C, Minardi D, Sironi M, Tichy HV et al. (1996) An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. Appl Environ Microbiol 62: 3005-3010. PubMed: 8702293.

55. Barbieri E, Potenza L, Rossi I, Sisti D, Giomaro G et al. (2000) Phylogenetic characterization and in situ detection of a Cytophaga-Flexibacter-Bacteroides phylgroup bacterium in Tuber borchii Vittad ectomycorrhizal mycelium. Appl Environ Microbiol 66: 5035-5042. doi:10.1128/AEM.66.11.5035-5042.2000. PubMed: 11055961.

56. Bertaux J, Schmid M, Prevost-Boure NC, Churin JL et al. (2003) In situ identification of intracellular bacteria related to Paenibacillus sp in the mycelium of the ectomycorrhizal fungus Laccaria bicolor. S1328N. Fungal Divers 17: 39-55. doi:10.1023/A:1021797108784.

57. Lean DJ (1991) 16S/23S rRNA sequencing. In: E StackebrandtM & Glickmann E, Dessaux Y (1995) A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. Appl Environ Microbiol 61: 793-796. doi:10.1128/AEM.61.4.793-796.1995.

58. Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of Gateway Computing Environments Workshop (GCE). New Orleans. pp. 1-8.

59. Swoford DL (2002) PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods). Sunderland, MA. Sinauer Associates.

60. Hoffman MT (2010) Bacterial endosymbionts of endophytic fungi: diversity, phylogenetic structure, and biotic interactions. Ph.D. Dissertation, the University of Arizona, Tucson, AZ.