# *DE NOVO* ASSEMBLY AND TRANSCRIPTOME CHARACTERIZATION OF AN *ARMILLARIA SOLIDIPES* MYCELIAL FAN

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## INTRODUCTION

*Armillaria* (Fr.) Staude is a widely distributed fungal genus comprising approximately 40 species (Volk and Burdsall 1995) that display diverse ecological behaviors ranging from beneficial saprobe to virulent pathogen. *Armillaria solidipes* (formerly *A. ostoyae*; Burdsall and Volk 2008; pending vote to conserve *A. ostoyae*; Redhead et al. 2011), one of the causal agents of Armillaria root disease, is a virulent primary pathogen with a broad host range in northern temperate latitudes (Kile et al. 1991). This fungal pathogen attacks sapwood as mycelial fans under the bark, and grows between trees as rhizomorphs. The pathogen causes a white rot of infected wood and is responsible for reduced forest yields as a result of direct tree mortality and non-lethal cryptic infections (Cruickshank et al. 2011).

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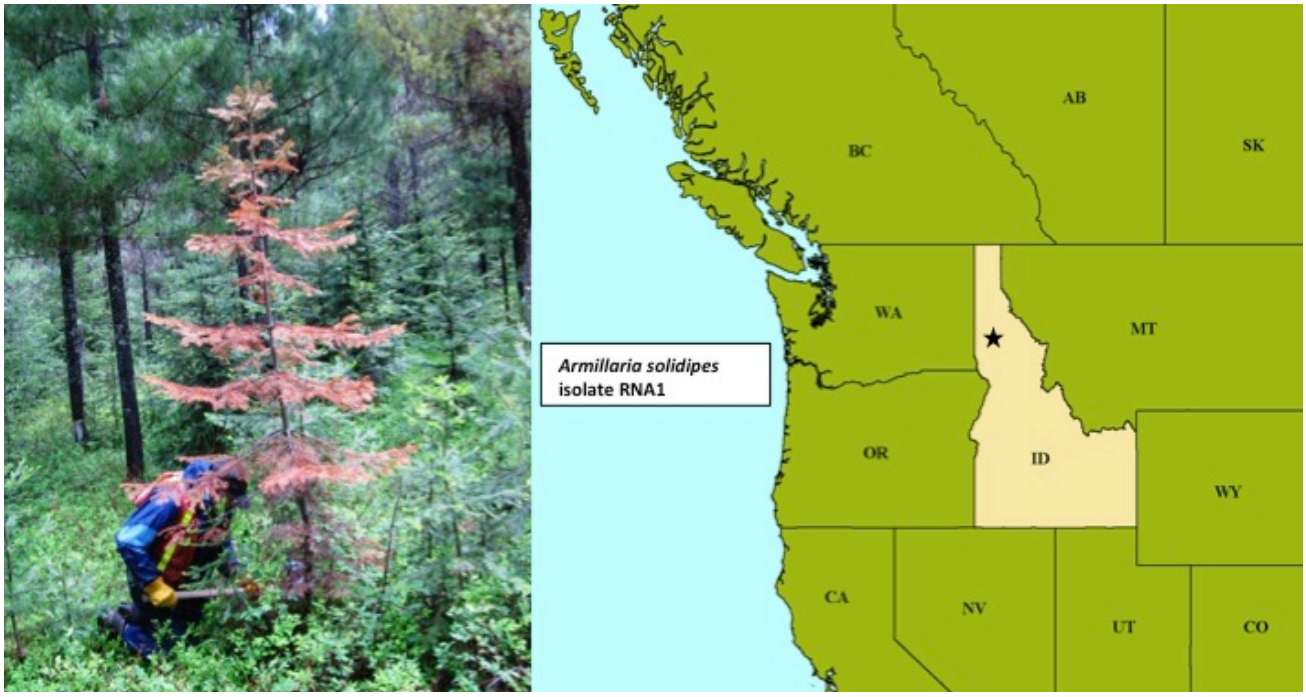


**Figure 1:** Diseased grand fir (*Abies grandis*) infected with *Armillaria solidipes* (left) collected near Elk River, Idaho (right).

The purpose of this research is to present the first assembly and characterization of a transcriptome from a root disease pathogen during pathogenesis. Specifically, our objective is to identify a large number of genes expressed by an active mycelial fan of *A. solidipes*, focusing on genes that may be associated with pathogenicity (i.e., those that result in a loss or reduction in disease symptoms when disrupted). Detection of putative genes that show homology to annotated genes involved in infection, cuticle and cell wall degradation, response to host environment, production of fungal toxins, and signaling will ultimately help inform forest management decisions.

## METHODS

We assembled and characterized a transcriptome of an active mycelial fan of *Armillaria solidipes* infecting *Abies grandis* near Elk River, Idaho (Figure 1; Table 1). The stand from which this isolate was collected has been well-characterized in that genets have been mapped and collected from several different hosts over several years.

**Table 1:** Assembly statistics.

| | Count | Average length | Total bases |
|---|---|---|---|
| Reads | 24,166,534 | 76.77 | 1,855,146,290 |
| Matched | 20,281,443 | 76.77 | 1,556,994,617 |
| Not matched | 3,885,091 | 76.74 | 298,151,673 |
| Contigs | 39,943 | 551 | 22,027,774 |

cDNA was generated from polyA$^+$ purified total RNA and then sequenced using a paired-end read approach on the Illumina GAII platform. A total of 24,166,534 reads was generated and assembled *de novo* into 39,943 contigs using the CLC Genomics Workbench 4.7.2. Significant alignments were identified using the NCBI NR database using a BLASTx search with a threshold expectation or e-value of 1e$^{-5}$. Hits were coded by taxon (Figure 2) and functional annotations relating to pathogenicity were assigned if known (Table 2). Signal peptides were identified using SignalP 4.0.

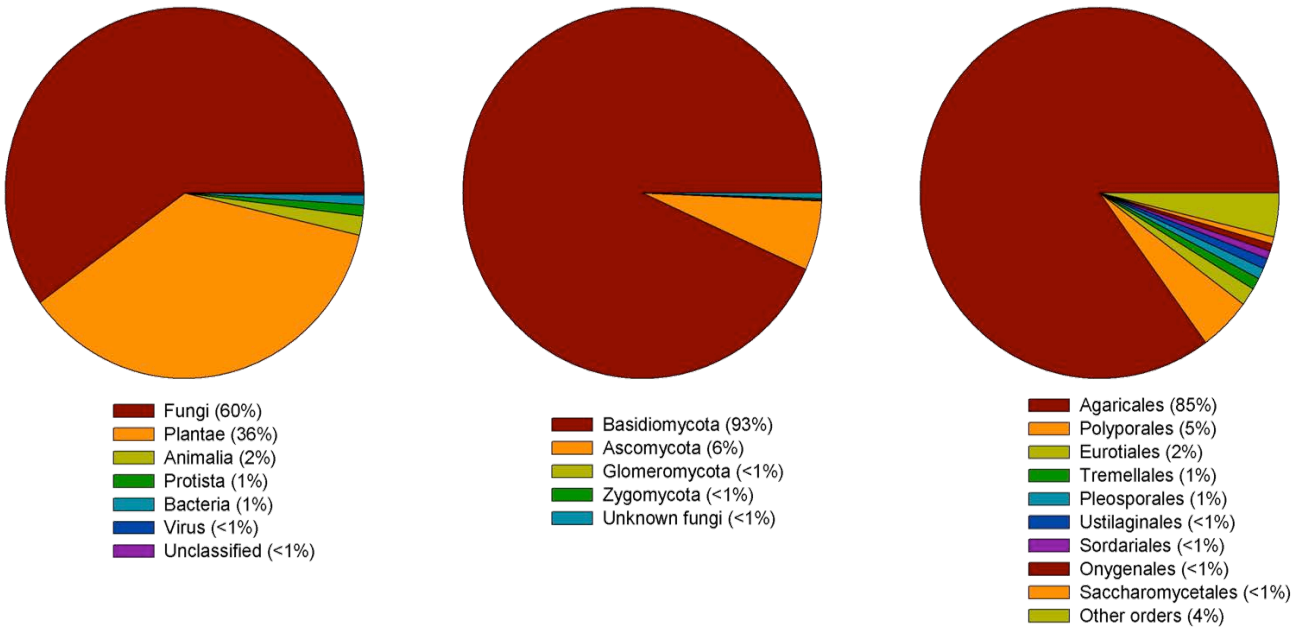


**Figure 2:** Distribution of significant alignments across kingdoms (A) fungal phyla (B) and fungal orders (C).

**Table 2:** Alignments with annotated pathogenicity genes (Oliver and Osbourn 1995; Idnurm and Howlett 2001; Tudzynski and Sharon 2003).

| Class | Function | Hits |
|---|---|---|
| Infection | Host-surface recognition | 1 (FUN34 transmembrane protein) |
| Cell-wall degrading enzymes | Cellulose and hemicellulose degradation | 11 (glucanase and cellobiohydrolase)<br>3 (xylanase)<br>58 (glycoside hydrolase) |
| | Lignin degradation | 26 (peroxidase and laccase) |
| | Pectin degradation | 1 (pectate lyase) |
| Response to host | Toxin efflux | 11 (ABC transporters) |
| Fungal toxins | Toxin biosynthesis | 3 (branched-chain-amino-acid transaminase) |
| Signal cascade components | Change gene expression in response to environment | 2 (MAP kinase)<br>5 (G proteins) |

## RESULTS AND DISCUSSION

***De Novo* Assembly and Transcriptome Characterization.** Of the 39,943 assembled contig sequences, 8,747 had significant alignments when compared against the NCBI NR database using BLASTx ($<1e^{-5}$). Of these, most contigs aligned best with gene sequences characterized from fungi (60%) or from plants (36%; Figure 2a). Of those sequences that best aligned with fungal sequences, 93% of hits fell within the Basidiomycota, 6% within Ascomycota and < 1% within the Glomeromycota and Zygomycota, respectively (Figure 2b). Most of the hits to Basidiomycota were to Agaricales, the order into which *A. solidipes* is placed (Figure 2c). Only 233 of the 8,747 contigs best matched sequences from *Armillaria* species; however, these results largely reflect sequence availability in GenBank.

**Identification of Genes Associated with Pathogenicity.** A total of 19,792 signal peptides were identified from the 39,943 assembled contig sequences. These short peptide chains direct the transport of proteins across membranes; thus, many are likely involved in pathogenicity. Several significant alignments with annotated genes involved in pathogenicity were identified from the transcriptome (Table 2).

Next, we intend to determine the extent (i.e., geographic coverage and infected host species) of this genet through pairing tests with other isolates collected from this well-mapped stand and examine the distribution of genes in the transcriptome assembly assigned to broad gene ontology categories.

## ACKNOWLEDGEMENTS

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## REFERENCES

Burdsall, H.H. Jr, Volk, T..J. 2008. *Armillaria solidipes*, an older name for the fungus called *Armillaria ostoyae*. North American Fungi. 3:261–267.

Cruickshank, M.G., Morrison, D.J., Lalumière, A. 2011. Site, plot, and individual tree yield reduction of interior Douglas-fir associated with non-lethal infection by Armillaria root disease in southern British Columbia. Forest Ecology and Management. 261:297–307.

Idnurm, A., Howlett, B.J. 2001. Pathogenicity genes of phytopathogenic fungi. Molecular Plant Pathology. 2:241-255.

Kile, G.A., McDonald, G.I., Byler, J.W. 1991. Ecology and Disease in Natural Forests. Chapter 8 in: Shaw CG III and Kile GA. *Armillaria* root disease. USDA Forest Service, Agriculture Handbook No. 691. Washington, DC. 233 pp.

Oliver, R., Osbourn, A.E. 1995. Molecular dissection of fungal phytopathogenicity. Microbiology. 141:1-9.

Redhead, S.A., Bérubé, J., Cleary, M.R., and others. 2011. (2033) Proposal to conserve *Armillariella ostoyae* (*Armillaria ostoyae*) against *Agaricus obscurus*, *Agaricus occultans*, and *Armillaria solidipes* (Basidiomycota). Taxon. 60(6):1770-1771.

Tudzynski, P., Sharon, A. 2003. Fungal pathogenicity genes. Applied Mycology and Biotechnology. 3:187-212.

Volk, T.J., Burdsall, H.H. Jr. 1995. A nomenclatural study of *Armillaria* and *Armillariella* species. Synopsis Fungorum. 8:1–121.

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