Root-associated fungal communities of a threatened Illinois conifer, *Larix laricina*

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Abstract

Tamarack (*Larix laricina*) is a conifer that is an important bog pioneer, yet it is threatened by anthropogenic and climate change in its southern distribution. Our study considered the ectomycorrhizal communities associated with the southern range of bog tamarack. The results of our research suggest that this ectomycorrhizal community is taxonomically diverse, but more sampling of this community and others in the distribution range of tamarack will be needed to characterize the community in detail.

Introduction

Tamarack (*Larix laricina*), a member of the Pinaceae family, is a deciduous conifer that favors wet, acidic soil in cool climates. Already threatened or endangered in its southern range, *L. laricina* is vulnerable to anthropogenic and climate change as warmer temperatures and drier soils encroach on its northern range. As a pioneer in bogs, *L. laricina* is often the first tree to colonize bogs and create a firm peat mat hospitable (Waterman 1926).

USDA

Tamarack is a known host of ectomycorrhizal fungi (Brundrett *et al.* 1989). This is a mutualistic relationship in which soil nutrients and carbohydrate from photosynthesis are exhanged between fungi ar the plant roots they colonize.

Bogs function as carbon sinks and habitats for endangered species (Gorham 1991). Lack of water drainage makes bogs acidic, resulting in unique plant communities that are tolerant of acidity (Waterman 1926). Research shows that the ectomycorrhizae associated with bog plants are likewise unique (Wurzburger 2004). However, the ectomycorrhizal community profile for *Larix laricina* in bogs has not yet been characterized. A full description of the diversity of ectomycorrhizal fungi associated with tamarack would benefit conservation efforts of the threatened conifer and its bog habitat.

Methods

Field Methods

We took 25 soil cores from three sites in Lake County, IL.

- Wauconda Bog Nature Preserve (12 cores)
- Volo Bog State Natural Area (6 cores)
- Gavin Bog and Prairie Nature Preserve (7 cores)


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Samples were taken near live *L. laricina* to avoid sampling other species or dead roots.

Molecular Methods

8-10 root tips that approximated the morphological diversity of the sample were isolated from each soil core and pooled into a single DNA extraction using a Quiagen Dneasy Kit.

The ITS region of fungal DNA was PCR amplified with primers ITS1 & ITS4; rbcL was used to amplify plant chloroplast DNA to confirm that the sample roots were from *L. laricina*.

The amplified ITS regions from the pooled extractions were cloned with Invitrogen TOPO® One-Shot Cells to isolate DNA from individual species. The colonies were sampled and subjected to PCR to amplify the individual species with primer M13.

Restriction fragment length polymorphism (RFLP) analyses used restriction enzymes to digest the amplifications of cloned colonies. RFLP digests produced banding patterns in agarose gel that indicated unique species.

Each unique banding pattern from the RFLP analyses was sequenced. Sequences were analyzed using CodonCode Aligner and then subjected to BLAST searches on GenBank (http://www.ncbi.nlm.nih.gov/GenBank) and UNITE (http://www.unite.ut.ee) databases. Sequences were aligned and a phylogenetic tree was constructed on the MAFFT server (http://www.align.bmr.kyushu-u.ac.jp/mafft/software).

Objectives

- Describe the diversity of ectomycorrhizal fungi that associate with tamarack in bogs.
- Approximate the level of endemism and specificity of ectomycorrhizal species to tamarack.
- Contribute to bog conservation efforts by providing information about a bog pioneer.

Results and Conclusions

Sequencing of plant DNA from Gavin Bog root tip extractions confirmed that samples were from *Larix* roots.

Ectomycorrhizal root tips isolated from Gavin Bog samples.

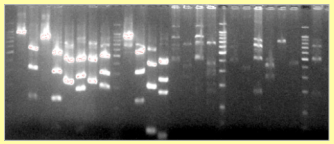

Figure 1: Agarose gel of an RFLP digest of Wauconda Bog samples to be sequenced, showing the potential diversity of the site. Each unique banding pattern suggests a unique sequence.

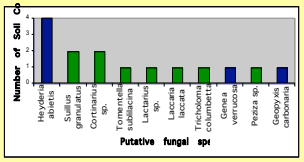

Figure 2: Rank abundance chart of putative identifications of fungal species found in Volo and Gavin Bogs. Green bars indicate known ectomycorrhizal species or groups while blue bars indicate saprotrophs.

	Volo Bog	Gavin Bog
Cortinarius sp.	VB1	GB5
Genea verrucosa	VB6	
Geopyxis carbonaria	VB1	
Heyderia abietis	VB1, VB6	
Laccaria laccata	VB1	
Lactarius sp.		GB7
Peziza sp.		GB3
Suillus granulatus	VB1	GB6
Tomentella sublilacina		GB6
Tricholoma columbetta	VB2	

Figure 3: Putative identifications of fungal species sequenced from soil core extractions from Volo and Gavin Bogs based on BLAST searches of GenBank and UNITE databases.

BLAST searches identified a diversity of fungi, both ectomycorrhizal and saprotrophic, associated with the roots of *Larix laricina*. While we found ectomycorrhizal root tips in the soil cores taken from each bog, many of the roots in these cores were dead. Decomposition of these dead roots is the most likely source of the saprotrophic fungi identified by BLAST searches. Future research will involve recognizing dead roots before isolating root tips from them.

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Results and Conclusions

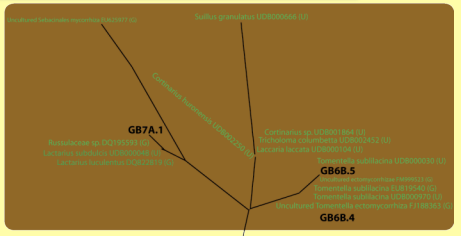

Figure 4: Star tree diagram of selected sequences from cloned colonies of Volo and Gavin bogs (bold) and results of BLAST searches of UNITE and GenBank databases. Brown box indicates Basidiomycete taxa; blue box indicates Ascomycete taxa. Green taxa are GenBank/UNITE sequences known to be ectomycorrhizal while blue taxa are GenBank/UNITE sequences known to be saprotrophic.

While our data set is too small to approximate the level of endemism and specificity of ectomycorrhizal species to *L. laricina*, the data do describe the diverse fungal community that associates with the conifer in Illinois bog ecosystems.

Future Research

•Continued sampling from Illinois bogs will be accompanied by sampling from other bogs in North America. Sampling will continue over an extended time period in order to study temporal variation in ectomycorrhizal communities.

•Soil cores will also be taken from upland tamarack to assess the differences that drier, less acidic soil makes in mycorrhizal community composition.

•These data will contribute to a larger study of fungal nutritional modes.

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