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Habitat preferences, distribution, and temporal persistence of a novel fungal taxon in Alaskan boreal forest soils

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ABSTRACT

Prior work in the boreal forest unearthed a novel ITS-LSU sequence (NS1) that falls outside known fungal phyla. Here we performed a targeted PCR survey to investigate the ecology of NS1. NS1 was found in three out of 99 soil cores at one black spruce (*Picea mariana*) site, and two cores were from nearby subplots, but clumping could not be demonstrated statistically. However, NS1 was detected 6 yr later in the same subplot, and in an adjacent subplot, with a join count probability of 0.0073. NS1 was not found in other lowland black spruce sites, but was detected in several upland mixed hardwood/white spruce (*Picea glauca*) sites and was correlated with presence of white spruce ($p = 0.0011$). It was also found in the same upland sites sampled in consecutive years. Our results provide clues concerning the ecology of NS1 and suggest that rare, divergent sequences should not necessarily be discarded from environmental sequence datasets.

Introduction

Fungi fulfill many crucial ecological functions as decomposers and plant symbionts (O'Brien et al., 2005). They play prominent roles in boreal forests due to their ability to function at low temperatures, low pH, and in nutrient poor environments (Taylor et al., 2010). Nevertheless, until relatively recently our understanding of boreal forest microbial ecological processes has largely been focused on functionality with little regard given to which species were fulfilling these functions (Taylor et al., 2010).

Beginning in 2003, a large-scale project to characterize soil fungal communities in the major stages of boreal forest succession using molecular methods was undertaken at the Bonanza Creek Long Term Ecological Research Site (BNZ-LTER) near Fairbanks, Alaska, USA (Taylor et al., 2010). These

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sites are representative of the boreal forests of Interior Alaska; several lowland sites were dominated by 100–300 yr old black spruce (*Picea mariana*), while upland sites contained white spruce (*Picea glauca*) and either trembling aspen (*Populus tremuloides*) or Alaskan paper birch (*Betula neoalaskana*) ranging from 25 to ~300 yr old. These sites have been extensively studied as part of the BNZ-LTER program over the last 40 yr. Tens of thousands of soil clone sequences were analyzed and the majority of sequences appeared to be representatives of previously described fungal species, genera or families (Taylor et al., 2014). Of those that did not fall into known fungal clades, one sequence in particular, hereafter referred to as novel sequence one (NS1), appeared particularly divergent. Both BLAST (Altshul et al., 1997) and phylogenetic analyses place the sequence within the kingdom Eumycota but do not place it within any known phylum (Glass et al., 2013).

We conducted a variety of analyses to assess whether the NS1 sequence might be some sort of artifact such as a chimera or pseudogene. We found no evidence that it is a chimera using both Chimera Checker (Nilsson et al., 2010) and Uchime (Edgar et al., 2011). We then modeled the rRNA secondary structure of the NS1 sequence and compared its folding to that of other fungi to evaluate the presence of conserved motifs, domains, and compensatory base changes. The results of these analyses were consistent with NS1 representing functional rRNA rather than a biological or lab artifact (Glass et al., 2013). The original NS1 sequence was found in a soil clone library originating from a floodplain black spruce (*P. mariana*) stand within the BNZ-LTER (site code FP5C). A highly similar ~1 200 bp sequence, varying at only eight sites, was found in another clone library from a mid-successional upland site (mixed white spruce & paper birch, UP2A) amplified using different primers. These independent detections further support the authenticity of the sequence and suggest that it might occur in multiple boreal forest habitats in Interior Alaska.

Patterns of occurrence of DNA sequences in environmental samples can reveal valuable information regarding the ecologies of both well-known and unknown or novel species. The discovery of novel archael and bacterial 16S sequences in thermal hot springs ushered in the era of environmental genomics (Pace et al., 1985). Much can be learned about the habitat preferences of unseen organisms by tracking their DNA. Subsequently, focused efforts at visualization and culturing can be mounted. These efforts have been particularly successful, for example, with marine bacterioplankton (Rappé et al., 2002; Stingl et al., 2007), but have also been applied to fungi (Collado et al., 2007). Within the fungi, a clade of Ascomycota initially called Schadt clade 1 dominated soil clone libraries above the treeline in a study site in Colorado (Schadt, 2003). Subsequent environmental sequencing efforts demonstrated that members of this lineage are diverse and widely distributed across the globe (Porter et al., 2008). Most recently, a member of this clade, now named the Archaeorhizomyces, was brought into cultivation and characterized in much more detail (Rosling et al., 2011).

With respect to the ecologies of unseen and uncultured organisms, analyses of spatial autocorrelation can reveal clumped, random or over-dispersed distributions, each of which provides unique insights into the natural history of the organism. For example, over-dispersion is often taken as circumstantial evidence for the role of intra-specific competition. Occurrence of a sequence across seasons can illuminate seasonal dynamics (Schadt, 2003; Taylor et al., 2010), while occurrences across forest types (Geml et al., 2009), soil horizons (Dickie et al., 2002; Taylor et al., 2014), pH gradients (Toljander et al., 2006), edaphic variation (Schechter and Bruns, 2008) etc. reveal additional aspects of habitat preferences. Hence, even for organisms that have never been seen, molecular ecology can provide important clues about their ecology and evolution.

Here we utilize PCR surveys to gain further insight into the ecology of NS1. In particular, we assess its: (1) within-site distribution; (2) occurrence among forest and habitat types; (3) soil horizon preferences; and (4) temporal persistence.

Materials and methods

Initial detection of NS1

As described in previous publications, we collected soil cores from several BNZ-LTER sites near Fairbanks, AK, USA between 2003 and 2005. Large-scale PCR, cloning and clone library sequencing were conducted to characterize the fungal communities present. Site descriptions, DNA extraction and amplification methods have been described previously (Geml et al., 2010, 2009; Taylor et al., 2014, 2010). Briefly, the gene-region (~1 200 bp) encompassing the ribosomal internal transcribed spacers (ITS) and a portion (~700 bp) of the ribosomal large subunit (LSU) were amplified from soil extracts using the fungal-specific PCR primers ITS1-F (Gardes and Bruns, 1993) and TW13 (Taylor and Bruns, 1999). Amplicons were cloned using a TOPO-TA pCR 4.0 kit (Invitrogen, Carlsbad, CA, USA) and libraries were sent to the Broad Institute of MIT and Harvard where transformations, automated clone-picking, and sequencing of clone libraries took place. We refer to this as our "non-targeted" survey. The NS1 sequence comprised one out of 384 clones from the site FP5C sampled in 2003, and a closely related sequence comprised one out of 931 clones from site UP2A sampled in 2004 (Glass et al., 2013). FP5C is a black spruce site located adjacent to the Tanana river in the floodplain, while UP2A is a mid-successional upland site located approximately 2 miles from the Tanana River and is dominated by paper birch and white spruce.

Sites and samples

Two different sets of soil samples, which have been described and analyzed previously, were used to explore the ecology of NS1. The first set of samples was collected at FP5C, the site in which NS1 was originally discovered.

A 200 × 200 m study area was established within BNZ-LTER site FP5C. Within this area, nine square plots 3 m × 3 m were arrayed in a stratified-random spatial design using randomly generated coordinates (Fig 1). Corners of plots were marked with PVC pipe. Each plot was divided into nine square subplots of 1 m × 1 m. In 2003, three soil cores (1.8 cm in diameter × 30 cm deep) were collected from each subplot within plot 1 (27 cores in total), while a single core was