Diversity of Orchid Fungal Symbionts in Estonian Mine Tailings

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Introduction

The main goal of ecology is to understand the complex relationships along the broad spectrum of conditions which occur in the natural world. Ecosystems are as variable as the organisms that inhabit them. They have varying amounts of resources and varying species diversity which can cause competition and specialization. Ecologists strive to better understand these complex relationships so that their concepts can be applied to real world problems such as land reclamation or ecosystem stabilization. Ecosystems with limited resources are among the most informative study sites because of the inferences we may make in regards to restoring decimated environments.

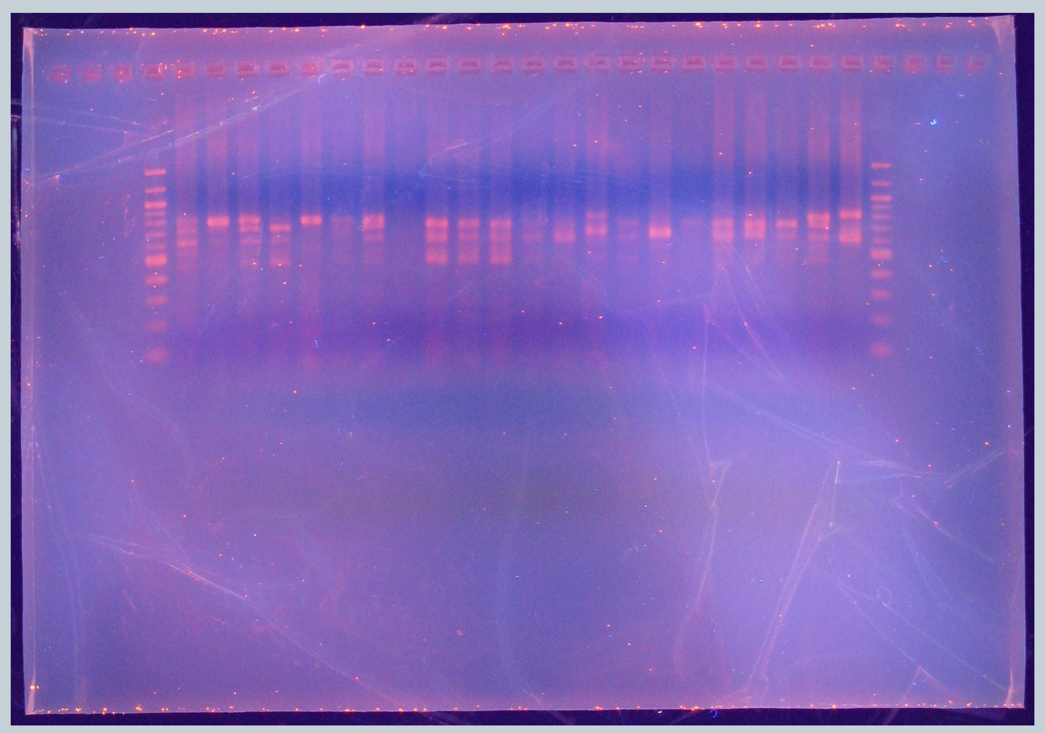
The term mycorrhiza comes from the Greek word “myco” or pertaining to fungi and the Greek word “rhiza” or roots. This term refers to the relationship between plants and fungi (Whitman 2009). It is believed that the mycorrhizal fungus supplies the orchid with carbohydrates and minerals throughout the orchid’s lifespan in its symbiosis (Smith & Read 1997). The mycorrhizal fungi can supply more minerals to the orchid than it can procure alone because the fungi are capable of breaking down and taking up organic matter that the plant cannot and passing it along to the plant (Whitman 2009). Thus, they are key to establishing a solid plant community in areas with little resources.

The site which the orchid root samples and mycorrhizal soil samples were taken is on a barren hill that was created when Estonia burned oil soaked shale to produce electricity and dumped the waste. We are particularly interested in these orchid mycorrhizae because they enable the orchids to colonize the highly toxic soil. Thus, their presence begs the question of how the fungi are utilizing the nutrients from the burnt oil and shale to support the orchids (Shefferson et al. 2008).

The presence of rare orchid species in these mine tailings suggests we might be able to use damaged areas for endangered plant conservation (Shefferson et al. 2008). This could be true if the environment that we are examining is conducive to colonization by at least some endangered plant species and if the area is missing certain competitive or predatory relationships that might have lead to the rare species’ endangered status. The damaged areas could act to replace plants’ natural habitats which may have been lost due to habitat destruction.

Though mycorrhizal fungi are obviously present in the soil of this area, their ability to colonize must be limited due to the scarcity of nutrients and toxicity of the soil. Thus, I believe there will be little diversity in the mycorrhizal fungi living in the sampled mine tailing areas.

Estonia Map indicating Johvi-Kose area. Courtesy of Google Images



RFLP gel of several samples.

Methods

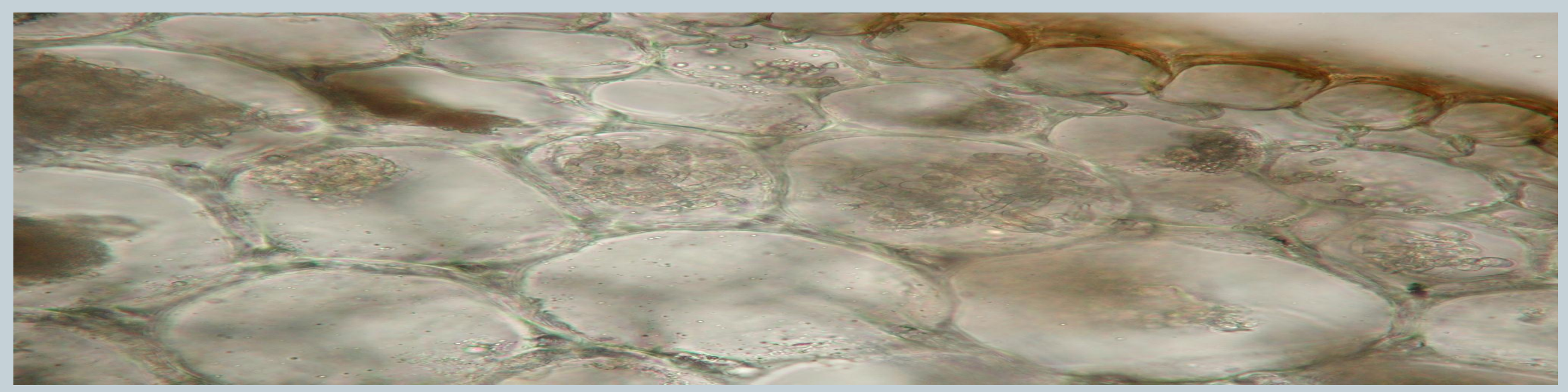
Soil samples were collected from semicoke or ash hill areas near Johvi-Kose in Estonia, approximately around Latitude 59º N and Longitude 27º E (Shefferson et al. 2008). Climate data for the area suggests that, during the fall and summer, temperatures are quite moderate, ranging from 14 to 26 degrees Celsius in the summer and from 14 to 23 degrees Celsius during the fall (Weather). Precipitation records for the area are apparently nonexistent. Two samples were taken from each site ranging from sites A through L during the summer. Fall samples were taken from sites M through FF, but five samples were taken from each with four being proximal to each other and one being distant from the four within the sample site.

We extracted DNA from the soil samples gathered in the summer and September of 2009 using the MoBio UltraClean Soil DNA Isolation Kit. We then did PCR on the samples using GoTaqGreen PCR master mix (Promega) and various primer pairs, including ITS1F and ITS4, ML5 and ML6, and ITS10F and ITS40F. We ran gel electrophoresis on the PCR products to determine the presence of DNA and the purity of the sample using a 1.5 percent agarose gel at one hundred volts for thirty minutes, and we stained the gel using Ethidium Bromide.
We ran restriction enzyme digests using the enzymes Alu1 and Hinf1, ran gel electrophoresis in a 2.2 percent agarose gel for ninety minutes at eighty volts, stained the gel using ethidium bromide and developed a RFLP profile for the samples using the NEB 100 base pair ladders (New England Biolabs). We documented and analyzed all of the gels using a UVP gel photo documentation program.

We sent the samples which appeared to have only one species of fungal DNA for sequencing and edited the sequencing data using ChromasPro (Technelysium), BioEdit (Bioedit) and Clustal X (Science Foundation of Ireland) for sequence alignment, and conducted BLAST searches using the NCBI database. For those RFLP profiles which appeared to have DNA from multiple fungal species, we cleaned and polished the PCR products using Stratagene PCR ScriptAmp Cloning Kit. We introduced the plasmids containing the target DNA strands into XL10-GoldKan ultracompetent cells and used a Blue-White Screening technique to determine which samples took up the plasmids containing the target DNA. After cloning, we used the MoBio ultraclear Microbial DNA Extraction Kit to extract the DNA, amplified with the appropriate primers, and sequenced the PCR product.

Abstract

Nutrient and water availability are two key requirements of plant survival. Mycorrhizal symbioses help orchids subsist in areas with apparently low soil nutrients and water availability, in this case, in Estonian mine tailings that contain little more than burnt oil soaked shale and ash. We extracted all DNA in soil samples taken from multiple sites in the mine tailings in both the summer and the fall. The sample size for summer was 25 and the sample size for the fall was 62. We carried out Polymerase Chain Reaction (PCR) on purified DNA samples, and performed gel electrophoresis and Restriction Fragment Length Polymorphism (RFLP) pattern determination. We then sequenced non-identical RFLP patterns and carried out Basic Local Alignment Search Tool (BLAST) searches to determine the organismal composition of the areas. We found 20 unique RFLP patterns for the fall samples and 12 unique RFLP patterns for the summer samples. The dominant organisms found were mycorrhizal fungi in families Thelephoraceae and Cortinariaceae. The overall diversity of mycorrhizal fungi determined by Shannon’s Diversity Index shows that for the fall, H=2.33, and for the summer, H=1.84. This is higher than the mycorrhizal diversity in the Brazilian rainforest where H ranges from 1.2 to .87. This is important because although the fungi in the mine tailings only associate with orchids, it shows that mycorrhizal fungi in general may assist in reclaiming and restoring environmentally decimated locations such as mine tailings, landfills, and other nutrient poor areas.



Mycorrhizal fungi symbiosis with Orchid root cells. Image courtesy of Richard Shefferson

Tables

Table 1: Species Estimates for Summer 2009

RFLP Pattern	Closest Match	Percent Match	Sequence ID	% of Samples
A	Thelepheroid Mycorrhizal(SYM)	97	AJ509802	4
B	Cortinarioid Mycorrhizal(SYM)	96	AJ509800	4
C	Thelepheroid Mycorrhizal(SYM)	91	AJ509798	4
D	-	-	-	8
E	-	-	-	4
F	Thelepheroid Mycorrhizal(SYM)	97	AJ509802	4
G	Thelepheroid Mycorrhizal(SYM)	97	AJ509802	8
H	-	-	-	8
I	Cortinarioid Mycorrhizal(SYM)	97	AJ509800	4
J	-	-	-	4
K	*Tomentella lateritia*(SYM)	98	AD00165 0	4
L	*Tomentella lateritia*(SYM)	98	AD00165 0	8

Table 2: Species Estimates for Fall 2009

RFLP Pattern	Closest Match	Percent Match	Sequence ID	% of Samples
M	Thelepheroid Mycorrhizal(SYM)	97	AJ509802	1.61
N	Thelepheroid Mycorrhizal(SYM)	97	AJ509802	6.45
O	-	-	-	3.23
P	Thelepheroid Mycorrhizal(SYM)	96	AJ509802	1.61
Q	Thelepheroid Mycorrhizal(SYM)	97	AJ509802	3.23
R	Cortinarioid Mycorrhizal(SYM)	97	AJ509800	3.23
S	-	-	-	1.61
T	*Tomentella lateritia*(SYM)	98	AD0016501	4.84
U	Cortinarioid Mycorrhizal(SYM)	97	AJ509800	6.45
V	Cortinarioid Mycorrhizal(SYM)	97	AJ509800	1.61
W	Cortinarioid Mycorrhizal(SYM)	97	AJ509800	3.23
X	-	-	-	1.61
Y	-	-	-	6.45
Z	*Tomentella lateritia*(SYM)	98	AD001650	8.06
AA	*Tomentella lateritia*(SYM)	98	AD001650	4.84
BB	*Tomentella lateritia*(SYM)	98	AD001650	3.23
CC	*Tomentella lateritia*(SYM)	98	AD001650	3.23
DD	*Tomentella lateritia*(SYM)	98	TSU86859	1.61
EE	Cortinarioid Mycorrhizal(SYM)	97	AJ509800	6.45
FF	Cortinarioid Mycorrhizal(SYM)	97	AJ509800	1.61

Results and Discussion

We estimated the Species Richness for the summer and fall samples as the number of unique RFLP patterns obtained from the samples (Tables 1 and 2) since each pattern can represent different species. The Richness for the summer samples is 12 and the species Richness for the fall samples is 20. To estimate diversity, we used Shannon’s diversity index which is determined by the formula:

$$H = -\sum_{i=1}^{s} p_i \ln p_i$$

In which, s is the total number of species and p_i is the proportion of a species to the whole. The percentage of each species to the whole can be found in Tables 1 and 2. The diversity of the summer samples is H = 1.84. The diversity of the fall samples is H = 2.33.

We were able to obtain PCR product from 64% of the summer soil samples and from 74.19% of the fall soil samples. Many samples with strong PCR products contain multiple fungal species, this was expected as environmental samples of fungal communities often show high diversity even in very small soil volumes (Anderson et al. 2004). Fungi from all of the sequenced samples were found to be symbiotic; this might indicate that symbiotic species have an easier time surviving in the harsh, nutrient scarce environment of the mine tailings areas.

As shown in Tables 1 and 2, there are many RFLP patterns that returned similar BLAST results. This could be because they are the same species, but it could also be because the BLAST search, in some instances, matched only at higher taxonomic levels higher than Cortinarioid or Thelepheroid fungi. This is permissible because the aim of this project was to establish a basic view of the diversity of the samples taken.

The percentage of samples matching a particular RFLP pattern as found in the last column of Tables 1 and 2 does not add up to one hundred percent. This is due to the fact that some samples were not successfully amplified and others did not produce informative RFLP patterns; of all of the samples, 36% of the summer samples and 25.81% of the fall samples were unsuccessful for one of these reasons.

The evenness and overall diversity for the fall samples (E = 20; H = 2.33) is greater than that for the summer samples (E = 12; H = 1.84). This could be due to fewer samples taken during the summer (25) than during the fall (62). However, it could also indicate that the diversity increases during the fall due to some change in the conditions of the environment, such as more hospitable temperatures for the fungi or more rainfall. Temperature has been shown to affect germination and colonization rates in some mycorrhizal fungi, but the effect varies by species and genetic adaptations to their environment (Smith & Read 1997).

When the soil fungal diversity of the mine tailings areas are compared to another environment with mycorrhizal fungi such as a Brazilian forest with an estimated H value for mycorrhizal fungi ranging from 1.2 to .87 depending on the season and the level of disturbance, one can see that the diversity of these mine tailings areas is quite high (2.3 to 1.8) by comparison (Moreira et al. 2007). We must be cautious with our interpretation of the site diversity since we likely overestimated diversity based on the RFLP patterns. However, our interpretation is acceptable for the aim of this project.

A perceivably barren area can support mycorrhizal fungi and their symbionts. Perhaps the goal of land reclamation for areas which have been overlooked in the past, due to the belief that it would be too costly or not time effective enough to reclaim, would appear more worthwhile and economically feasible due to the findings of this research. If an area as barren as an ash and coal pile can harbor such diversity, then surely other areas such as landfills and waste dumps can as well.

The next step in this project is to gain a better picture of the species diversity of this area through extensive cloning and sequencing procedures that would yield more precise species identification throughout our samples. With a better picture of what exact species are resent in this area, we can better estimate the diversity and thus the ecological stability of this area. Other projects that might be interesting would include testing soil composition and recording rainfall. Also, we could study other reclaimable areas such as landfills or waste dumps to search for similar results.

Conclusions

The diversity of the Johvi-Kose mine tailings area does not seem to be very limited by the area’s apparent scarcity of nutrients. Life is quite resilient and its ability to adapt and survive is fundamental in its nature. Without adaptation, diversity would not exist and indeed many places on Earth would be inhospitable to life. However, life can adapt to harsh environments and, for this reason, even a barren environment can support a diverse array of organisms.

Johvi-Kose mine tailings area. Courtesy of Richard Shefferson

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