Identification of lignocellullose degrading microbes in a boreal forest soil

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Introduction

Soil carbon
- -Large percentage found in northern boreal soils
- -Tied up in compounds such as lignocellulose

Lignocellulose
- -Found in plant material, main structural component of wood
- -Composed of lignin, celluloses, & hemicelluloses
- -Breakdown of lignocellulose = release of CO_2 and CH_4 in atmosphere

Microbes do the "dirty work"
- -Extracellular enzymes from fungi and bacteria degrade complex organic substances
- -Laccase has completely degrade lignin before

Information limited
- -Don't know what's actively degrading lignocellulose
- -Many microbes = cannot culture in lab
- -Technique: stable isotope probing

Stable isotope probing
- -Incubates sample with 13C substrate
- -13C is "heavier" than 12C
- -After incubation, buoyant density-gradient centrifugation applied, two bands formed
- -DNA retrieved by fractionation
  - -Contains genomes of microbes that absorbed 13C

Purpose of study: to identify what microbes and their associated genes are degrading lignocellulose in boreal forest soils.

Methods

Samples
- -Fractionations from Times 0, 2, 4, 6, 8 (weeks)
- -Fractions 9-14 found to contain labeled DNA
  - -Pooled fractions 9-14, separate samples from fractions

PCR amplification
- -16S ribosomal region for bacteria
  - -Primers 27F & 1392R
- -ITS region for fungi
  - -Primers ITS1F & ITS4

Gel electrophoresis to verify

T-RFLP and ARISA
- -Amplified same regions, but with labeled forward primers (27F-FAM & ITS1F-FAM)
- -T-RFLP: PCR purified (QIAquick kit, Qiagen), restriction digestion (HhaI), incubation
- -ARISA: Suspended in formamide
- -Fragments analyzed using ABI prism 3100 gene analyzer sequencer, results viewed using GeneMapper

Fig. 1

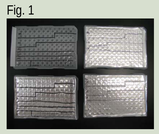


Fig. 2

Fig. 3

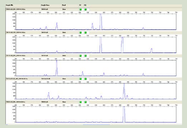


Fig. 1: 96-well plates containing fractionation samples.

Fig. 2: Fractionation of SIP samples after centrifugation

Fig. 3: ARISA results viewed using GeneMapper

Results

- •qPCR results
  - •Bacteria (16S) showed no significant evidence of labeled DNA (See Fig. 4)
  - •Fungi (ITS) showed significant amount of labeled DNA
    - •time 4 and time 6
    - •no sig. amount at time 2 & 8. (See Fig. 5)

- •T-RFLP results were found to be inconclusive due to multiple banding in the positive control. Cloning sequences were not received in time.

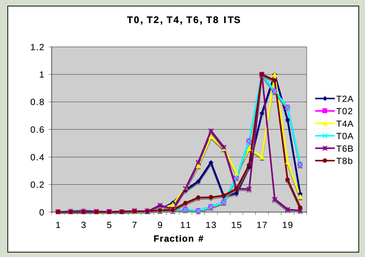
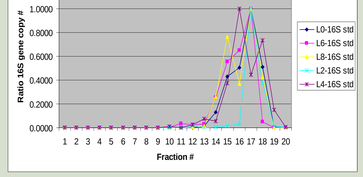


Fig. 4 &5: qPCR results for ITS & 16S samples, respectively. The first peaks represent the ^{13}C-labeled DNA in the ITS graph, and the second peak represents the unlabeled DNA in both ITS & 16S.



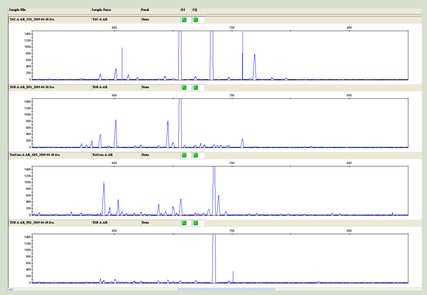


Fig. 6: ARISA results viewed using GeneMapper. Peaks can be seen to line up with others throughout the time points.

- •ARISA results (see Table 1).
  - •588 and 601 bp = larger peaks in total community, time 2, 4, 6, and 8, but not in time 0
  - •656 bp = large peaks in time 4 & 6, medium peak in total community, and low peaks in time 0, 2 and 8.
  - •Magnitude >400: 591, 603, 633, 645, 650, 683, 684, 689, 709, 719, and 980 bp long

T0C	T2C	T4C	T0B	T6B	T8B
				125	
				198	
	227				
	228	177		410	117
					67
	2664	337		892	194
		155			
	140				
	692				
				61	61
		102			
				817	91
	52			137	*
112	70	4190	*	3206	57
	503			68	
		96		53	66
	152				
		3360			
					2989
	215			79	2427
	108				
				54	
	404			273	
		789			
	64		189		*
	827			121	58
				702	
	1496	260*			

Table 1: ARISA results. The left column is fragment length in bp and under each time is the height of the peak present there. A * indicates a peak of magnitude <50 that was present in other fractions, and italics indicate a large peak that was not read by the program but was present in that fraction.

Discussion

- •ARISA fragments with high peaks assumed to be dominant degraders
  - •Higher peak indicates more DNA/PCR product present
- •Some fragments correlate with others
  - •Abundance of 601 bp fragment at time 2; abundance of 656 and 683 bp fragments at time 4; abundance of 656 bp fragment at time 6; abundance of 654 bp fragment at time 8
  - •Suggests microbes (assuming each fragment is single microbe) worked cooperatively over time to degrade the lignocellulose
    - •One microbe starts initial breakdown; after time, another microbe dominates degradation
  - •Suggests microbial crossfeeding
    - •Different taxa degrade different parts of lignocellulose
  - •May be due to different microbial composition of microcosms
- •Fragments present throughout fractions
  - •588, 656, and 601 bp fragments
    - •656 bp fragment peak height increased from time 2 to 4, diminished from time 4 until time 8, suggesting microbe incorporating more of ^{13}C substrate until 4 weeks
      - •After 4 weeks, substrate may be degraded completely, labeled carbon begins shifting through community
- •Fragment peaks in later time points weren't present in earlier
  - •may be microbes that consume or prey on dead lignocellulose degraders

- •Suggestion of fungi domination
  - •16S bacterial qPCR = no evidence of labeled DNA
  - •ITS qPCR = clearly absorbed 13C substrate in biomass

- •T-RFLP
  - •No results due to multiple bands in positive control
  - •Positive control = DNA pure culture of *Pseudomonas stutzeri*
  - •Should only be 1 peak; each peak = one organism
  - •would have been performed again; time constraints prevented

- •Cloned sequences not received in time
  - •Many microbes in heavy fractions could have been identified
  - •T-RFLP and ARISA on individual clones → peaks of dominant lignocellulose degraders identified & assigned to species or taxon
  - •Useful to identify what species of fungi or bacteria involved in degradation, so microbes can be studied thoroughly

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