Bacterial Composition and Diversity During Lignocellulose Decomposition

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Introduction

Soil bacteria are of immense diversity and are key to decomposition of organic materials. As primary decomposers, they break down complex and recalcitrant compounds in organic residues which results into substrates of various complexity for use of other microbes. Lignocellulosic biomass is the most abundant renewable resources and has great potential as source for bioethanol production. Information on the microorganisms involved in lignocellulose decomposition has implications for bioprospecting for potential microbes for biotechnological exploitations. Although degradation of lignocellulose by bacteria has been observed, information on bacterial diversity and dynamics associated with lignocellulose decomposition is very limited. From an ecological standpoint, more detailed look at the bacterial community dynamics during the process could provide meaningful insights on the link between decomposition and bacterial functions.

Objectives

- Our overall goal is to understand bacterial ecology of lignocellulose decomposition.
- Specifically we want to answer the following questions:

1. how bacterial community composition is affected by soil and residue types?
2. how bacterial composition and diversity change throughout decomposition?
3. what are the bacterial communities associated with lignocellulose decomposition?

Methodology

Experimental Set-up

- 2 cm straw mixed to soil @ 0.2 g straw/g soil
- mixture in 1 L canning
- moisture @ -33KPa
- temperature @ ~ 23-25 ºC,
- Experimental design : 2x2x4 factorial in randomized complete block design with 3 replications
- treatments:

2 residues: 1. Rice straw 2. Switchgrass

2 soil types:1. Marietta series 2. Sharkey series

4 incubation periods (days, D): 3D, 18D, 44D, 120D

Sample Preparation

- At termination, each residue was vigorously shaken to remove adhering soil particles and was referred ⟵ **detritusphere**, the hotspot of decomposition.

16S rRNA Clone Library Preparation and Sequence Analyses

DNA Extraction & Amplification of the 16 S rRNA Gene

DNA was extracted 0.25 g detritusphere using the PowerMax Soil DNA isolation kit (MOBIO Labs). Fifteen cycle-PCR reaction was done to apmlify the genes using 27f and 1492r universal primers for prokaryotes.

Construction of Clone Library

The PCR products were cloned using the TOPO TA cloning kit (Invitrogen) Colonies with inserts were then picked into 96-well block containing 2 mL of LB medium & 10 % glycerol. After 1 day of growth, a 0.1 ml aliquot was transferred to a 96-well plate. Both blocks were frozen at -80 C. The plate was then shipped for sequencing.

Sequence Editing & Analyses

Prior to analyses, the sequences were edited using Codon Code Aligner and checked with chimera using Pintail and Mallard programs. Sequences were aligned using Greengenes and subsequent sequence analyses were carried out by following programs: DOTUR, PC-OrD, RDP Query

Table 1. Selected soil properties

Soil	Textural classification	pH	Total C	Total N	Exchangeable Ca	Exchangeable Mg	Exchangeable K	Extractable P
			---------%---------		----------µg g^{-1}----------			
Marietta	fine sandy loam	6.16	2.16	0.17	937	129	111	151
Sharkey	expansive clay	6.31	2.57	0.15	2684	687	195	2

Table 2. Results from multi-response permutation procedures (MRPP)

Treatment	P value
Residue	0.358748
Soil	0.01204078
Incubation	0.0000015

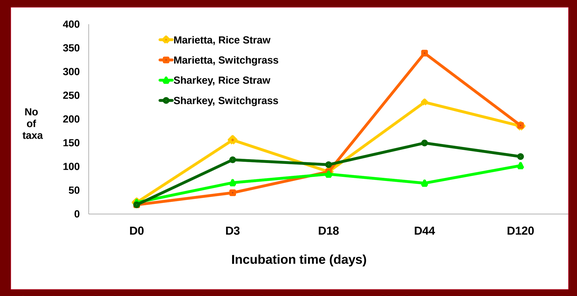

Fig. 1 Bacterial richness throughout lignocellulose decomposition

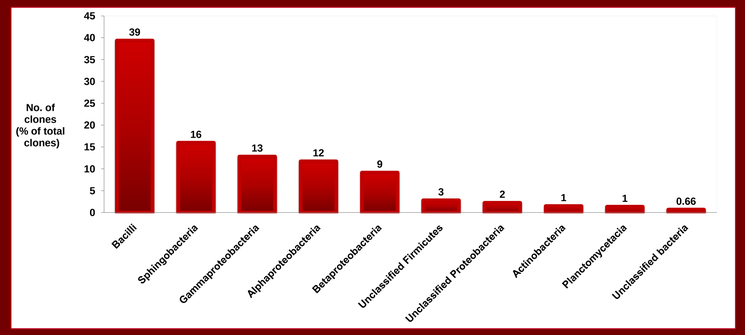

Fig. 2. Broad (Class-level) phylogenetic classification of bacterial communities associated with lignocellulose decomposition

Table 3. Selected key-player species as determined by indicator species analysis

RDP Phylotype and % Similarity	Marietta								Sharkey							
	Rice Straw				Switchgrass				Rice Straw				Switchgrass			
	D3	D18	D44	D120	D3	D18	D44	D120	D3	D18	D44	D120	D3	D18	D44	D120
	----------Average number of clones----------															
Bacillus megaterium (100)	11.67 (±3.5)	6.33 (±1.15)	3.67 (±1.53)	4.67 (±2.89)	15.33 (±4.16)	8.67 (±3.51)	2 (0)	4.33 (±3.22)	9.33 (±1.16)	7.67 (±2.08)	9 (±2)	6.33 (±0.58)	14.33 (±1.15)	4.33 (±2.08)	8.33 (±3.22)	6.33 (±3.51)
Pantoea *sp. (100)*	3.33 (±2.31)	0	0	0	1.67 (±1.53)	0.33 (±0.58)	0	0	2.67 (±1.53)	1.33 (±0.58)	0	1 (±1)	3.67 (±1.56)	0.33 (±0.58)	0.33 (±0.58)	0
Bacillus fumarioli (99.8)	1 (±1)	0	0.33 (±0.58)	0	1.33 (±0.58)	0	0	0.68 (±0.58)	1.68 (±0.58)	1.68 (±1.52)	0.68 (±1.16)	0.68 (±0.58)	1 (±1.73)	0.33 (±0.58)	0.68 (±0.58)	0.68 (±0.58)
Chitinophaga ginsengoli (94)	0	0.33 (±0.58)	1 (±1)	0.33 (±0.58)	0.33 (±58)	2.0 (±1.73)	3 (±1.73)	1.68 (±1.53)	0	1.68 (±1.53)	1.33 (±0.58)	1 (±1)	0	2.67 (±0.58)	4 (0)	1 (±1)
Chitinophaga japonensis (97.7)	0	0.33 (±0.58)	0	0.67 (±1.16)	0	0.33 (±0.58)	1 (±1)	1 (±1)	0	0.67 (±0.58)	0.68 (±0.58)	1 (±1)	0	0.33 (±0.58)	0.68 (±1.16)	1.33 (±1.53)
Bradyrhizobium *sp. (99.7)*	0	0.33 (±0.58)	1.33 (±0.58)	1.33 (±0.58)	0	0.68 (±1.16)	1 (±1)	2.33 (±2.52)	0	0.33 (±0.58)	0	1.33 (±1.16)	0	0	0.33 (±0.58)	1.67 (±1.53)
Niastella yeongjuensis (99.1)	0	1.33 (±0.58)	2.33 (±1.53)	0.67 (±0.58)	0	2.68 (±1.53)	2 (±2)	0.68 (±1.16)	0	0.67 (±1.16)	0.68 (±0.58)	0.68 (±1.16)	0	0.33 (±0.58)	1 (±1.73)	0.67 (±16)
Niastella sp. (99.1)	0	0	0.68 (±1.56)	0	0	0	0.68 (±1.16)	0	0.33 (±58)	0.67 (±58)	2.68 (±1.53)	1.33 (±0.58)	0	3.33 (±1.16)	1.68 (±2.08)	0.67 (±0.58)
Burholderia caribensis (99.9)	0	2.33 (±0.58)	0.68 (±0.58)	0.67 (±1.16)	1 (±1)	0.33 (±0.58)	1.68 (±1.53)	0	0	0	0	0	0	0	0	0
Methylobacterium radiotolerans (100)	0.33 (±0.58)	0.33 (±0.58)	0.68 (±1.56)	0	0	0	0	0	0	1 (±1)	0.68 (±58)	1 (0)	0	0	0.33 (±0.58)	0.33 (±0.58)

Values in parenthesis below the average number of clones are mean standard deviation

Legend: 0 | 0>1 | 1.1 to 5.0 | 5.1 to 10.0 | 10.1 to 15.0

Results

- Selected soil properties revealed that each soil has distinct soil characteristics (Table 1).
- Procedure for testing the hypothesis of no difference between two groups revealed that bacterial communities were significantly different across incubation and soil types while there was no significant difference between residue types (Table 2).
- There was dynamics in bacterial richness observed which generally followed similar trend for all treatments with peak at D44 (Fig 1).
- Of the 1389 clones, Bacilli were the most dominant phylotype associated with lignocellulose decomposition (Fig 2). A diverse set of rare species were also observed; these colonizing communities represent a subset of the soil bacterial diversity (data not shown).
- A varying pattern of species relative abundance and distribution was observed among the key-player species (Table 3). *Bacillus megaterium* was consistently the most abundant species in all the treatments while other species were distributed unevenly between types of soil and residues.

Discussion

- While there are significant differences between the communities in the different soils, the similarities between the communities that come to dominate residue decomposition are very similar in kind and number. There is considerable consistency in the selection of microbiota by the residues from soils.
- The dynamics in bacterial diversity at different stages of decomposition suggests succession of the colonizing communities. This can be attributed to the biochemical transformation of the decomposing residues which results to temporal variation in substrate types available to the colonizers.
- *Bacillus megaterium* is implicated as an important colonizer in the decomposition of lignocellulose as it continues to maintain a strong presence throughout incubation. Such observation warrants further investigation as to how this organism functions in the decomposition process.
- Large majority of the observed phyla associated with lignocellulose decomposition are well-known cultivars implying increased likehood in the culturability of these colonizers which can be useful for biotechnological explorations.

Conclusions

Although the two soils we studied are chemically and physically different, and harbor very distinctive bacterial assemblages, the strikingly similar composition of the dominant microbial communities during residue decomposition suggests a strong selection for specific microbial types. This is in contrast to our expectation that diverse microbial systems would be composed of numerous functionally redundant but taxonomically different species. These results are important because they suggest that diverse microbial systems may be dominated by a few key players that are most important for organic matter decomposition and nutrient cycling.