Metaproteome Analysis of a Termite Hindgut Microbial Community: Relevant to Biofuel Development

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Costa Rican termite’s (*Nasutitermes corniger*) relevance to biofuel development

- Currently there is great interest in understanding the diversity of biological mechanisms employed during lignocellulose degradation

(photo by DOE Joint Genome Institute)
Costa Rican termite’s (*Nasutitermes corniger*) relevance to biofuel development

- Termites harbor a symbiotic gut microbial community believed to be responsible for their ability to thrive on wood
- Metaproteomic analysis of the anaerobic bacterial community resident in the hindgut of the wood-feeding “higher” Costa Rican termite is being used to gain insight into potential symbiotic mechanisms, including:
  - Cellulose and Hemicellulose degradation
  - Fixation of atmospheric $N_2$
Metagenomic and metaproteomics analyses

- Analyses focused on the P3 compartment containing ~ 1 µL of gut fluid
- LC-MS/MS based metaproteomic analyses
- Metagenomic sequencing

(Top) Costa Rican Termite (Middle) Termite gut optical image; P3, proctodeal hindgut segment. Photos by DOE Joint Genome Institute. (Bottom) Nanoorange stain of the microbial consortia. Photo by Catherine L. Reardon, PNNL.
Metagenomic analysis results

- 16S rRNA gene sequencing identified a broad diversity of bacteria representing at least 12 phyla and 216 phylotypes

- Metagenome contains 72,401 predicted proteins that can be categorized into 2,050 protein families

- \textit{In silico} digestion of metagenome generated 5,681,415 fully tryptic peptides

*Results underscore how complex even a 1-µL environment can be*

\textit{Warnecke et al. Nature 2007 (DOE Joint Genome Institute / Lawrence Berkeley National Laboratory)*}
Metaproteomic samples were prepared using two complementary methods

(a) Sample Preparation 1

- Lysed Bacteria in Gut Fluid
- Centrifuged
- P3 hindgut contents from 100 termites (~100 μL)
- Global Tryptic Digestion 1 Fraction
- Insoluble (Pellet)
- Soluble (Supernatant)
- Tryptic Digestion SCX fractionation
- 25 Fractions

(b) Sample Preparation 2

- Centrifuged
- P3 hindgut contents from 100 termites (~100 μL)
- CL8 Cleanup Tryptic Digestion SCX fractionation
- Pellet Fraction (Bacteria)
- Supernatant (Gut Fluid)
- Washed
- Pellet (Bacteria)
- Lysed bacteria Tryptic Digest SCX fractionation
- 10 Fractions

(c) Identification Results from Tandem Mass Spectrometry (LTQ) Analyses of the 5 Samples of Interest

<table>
<thead>
<tr>
<th></th>
<th>Global</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>Supernatant</th>
<th>Pellet</th>
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<tbody>
<tr>
<td>Unique Peptides</td>
<td>1,295</td>
<td>1,877</td>
<td>399</td>
<td>2,267</td>
<td>3,582</td>
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<td>Proteins (≥ 2 peptides)</td>
<td>226</td>
<td>322</td>
<td>71</td>
<td>402</td>
<td>626</td>
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</table>
A total of 870 unique proteins (≥ 2 peptides were identified

Filtering Criteria:

• XCorr:  
  ≥ 1.9 (+1), 2.2 (+2), & 3.75 (+3)

• Tryptic & Partial-Tryptic peptides

• Minimum peptide length of 6 residues

• ≥ 2 peptides / protein

Sample Preparation 1
- bacteria were lysed in the gut fluid (455 total proteins)

Sample Preparation 2
- bacteria were isolated from the gut fluid prior to being lysed (740 total proteins)

Proteins found in Sample Preparation 1 & 2 (325 proteins)
Metaproteomic analysis identified every enzyme need for sugar transport and metabolism.
Wood-feeding termites thrive only on nitrogen-deficient food (i.e. lignocelluloses)

The gut microbial community must be responsible for both fixation of atmospheric dinitrogen and supply of all the essential nitrogenous compounds that termites cannot synthesize.

Metaproteomic analysis identified every enzyme need for nitrogen fixation and assimilation
Summary

• Metaproteomic analysis identified enzymes involved in:
  – Carbohydrate (Xylose / Sugar) Transport
  – Hydrolysis of β-linked disaccharides
  – Glycogen Cycle and Glycolysis
  – Fumarate Respiration
  – Lipopolysaccharide (LPS) Synthesis
  – Nitrogen Fixation
  – Amino Acid Synthesis
  – Hydrogen Metabolism

• Every enzyme was identified in pathways essential to termite survival
  – Sugar Fermentation to Acetate
  – Nitrogen Fixation and Assimilation

• In this analysis we only identified 11.5% of all the protein families in the metagenome; Additional optimization in sample preparation methods should improve our recovery
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