Proceedings IRG Annual Meeting (ISSN 2000-8953)

IRG/WP 17-10882

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 1 **Biology**

Analysis of hindgut bacterial phyla frequency and diversity in subterranean termites exposed to chitosan-treated wood

Olanrewaju Raji[1], Juliet D. Tang[2], Telmah Telmadarrehei[3], Dragica Jeremic[4]

[1, 3, 4] Department of Sustainable Bioproducts, Mississippi State University, Mississippi 39762
Email: oar10@msstate.edu, [3] Email: tt895@msstate.edu, [4] Email: d.jeremic.nikolic@msstate.edu

[2] USDA Forest Service Forest Products Laboratory, Starkville, Mississippi 39759,
[2] Email: julietdtang@fs.fed.us

Paper prepared for the IRG48 Scientific Conference on Wood Protection
Ghent, Belgium
4-8 June 2017

IRG SECRETARIAT
Box 5609
SE-114 86 Stockholm
Sweden
www.irg-wp.com

Analysis of hindgut bacterial phyla frequency and diversity in subterranean termites exposed to chitosan-treated wood

Olanrewaju Raji[1], Juliet D. Tang[2], Telmah Telmadarrehei[3], Dragica Jeremic[4]

[1, 3, 4] Department of Sustainable Bioproducts, Mississippi State University, Mississippi 39762, Email: oar10@msstate.edu, [3] Email: tt895@msstate.edu, [4] Email: d.jeremic.nikolic@msstate.edu

[2] USDA Forest Service Forest Products Laboratory, Starkville, Mississippi 39759, [2] Email: julietdtang@fs.fed.us

ABSTRACT

The termite hindgut contains a microbial community that symbiotically aids in digestion of lignocellulosic materials. For better understanding of the dynamics of the bacteria-termite relationship, a species survey of bacterial hindgut microbes in subterranean termites (*Reticulitermes flavipes*: Kollar) collected from Louisville, Mississippi was performed after exposure to chitosan-treated and control (water-treated) wood samples. Total genomic DNA was isolated from termite hindguts, amplified and 16S ribosomal RNA (rRNA) gene fragments were analyzed using next-generation sequencing techniques. Twenty-six bacteria phyla were identified in both treatment groups, with five bacteria phyla showing significantly differences in abundance between the chitosan-treated and control groups. These results suggest that there was a treatment driven effect on the hindgut bacteria diversity.

Keywords: metagenomics, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae), hindgut, bacteria, chitosan, wood

1. INTRODUCTION

Termites are social cockroaches that primarily feed on cellulosic materials in the form of dead or living wood and plants. Termites harbor a microbial community in their hindgut that includes bacteria, protists and some archaea (Brune, 2014). A defining characteristic of lower termites is the presence of bacteria and protists, while higher termites have some bacteria species, but no protists (Breznak and Brune 1994, Hongoh 2010). The termite hindgut microbial community has been shown to be essential for termites' survival, i.e. responsible for breakdown of food ingested by the termites, although one study has suggested that the presence of endogenous enzymes in the termite midguts may signify the termite's partial responsibility in digestion (Ke *et al*. 2012). The termite-protist collaboration with regards to digestion has been extensively researched, while the purpose of bacteria has been largely ignored (Brune and Dietrich 2015). Diversity and frequency of the hindgut bacterial community of termites have been shown to vary by species (Brune 2014, Raji *et al*. 2015) and diet (Huang *et al*. 2013).

Most of the bacteria species in the termite hindgut remain unidentified and uncultivable, but a significant amount of research has been done to identify the major phyla represented in the termite hindgut. Phylum-level classification of bacterial 16S rRNA genes in the termite hindgut via high throughput sequencing is the most popular and efficient method for termite metagenomics. High throughput sequencing has revealed that the phyla diversity within the hindgut is higher than initially assumed (Köhler *et al*. 2012, He *et al*. 2013). Phylum Spirochaetes is usually the dominating group within the termite hindgut (Brune 2014), but other studies have shown phylum Bacteroidetes as dominant in *Coptotermes formosanus* (Shiraki) (Noda *et al*. 2005), and *R. flavipes* (Raji *et al*. 2015). An increased presence of phylum

Elusimicrobia within *Reticulitermes* spp has also been observed (Noda *et al*. 2005). Arango et al. (2014) found that termite bait toxicants (hexaflumuron and diflubenzuron) effect bacterial communities in the termite gut of *R. flavipes*, but no effect on *Reticulitermes tibialis*.

In this study, the termites were fed with chitosan-treated wood. Chitosan is a derivative of the polysaccharide, chitin, which is a building material of shells of crustaceans, termites' exoskeleton, and fungal cell walls. Chitosan is commercially produced by deacetylation of chitin in excess of alkali sodium hydroxide. It is used in agriculture and horticulture as a bio-pesticide and has shown promise in wood protection studies against fungi (Alfredsen *et al*. 2004). It was also shown to inhibit the growth of several plant and human pathogenic fungi (Stössel and Leuba 1984, Li and Yu 2001, Tsai *et al*. 2002). Chitosan at 0.3–0.4% (w/v) in nutrient media has also shown fungistatic activities against tree pathogens, *Leptographium procerum* (Kendr.), and *Sphaeropsis sapinea* (Fr.) (Torr *et al*. 2005). Chitosan and its derivatives are used for numerous applications in the field of cosmetics, medicine, food, pharmacy and agriculture (Raafat and Sahl 2009). Chitosan has also been shown to improve the mechanical and physical properties of wood (Basturk 2012). Antimicrobial activities of chitosan against both gram positive and gram negative bacteria have also been reported, with contrasting results on which type of bacteria are more susceptible (Kong *et al*. 2010).

The goal of this study is to observe potential effects chitosan has on the diversity and frequency of the termite bacterial hindgut population.

2. EXPERIMENTAL METHODS AND PROCEDURES

2.1 Termites

The termites used in this study were obtained from one population collected at Sam D. Hamilton Noxubee National Wildlife Refuge, Louisville, Mississippi. Decayed logs containing termites were cut into smaller segments and kept in a covered metal bin at room temperature with sufficient moisture until the termites were utilized, which was within 6 months of collection. Termite species identification was performed based on the DNA sequence of the mitochondrial A-T rich region. Genomic DNA was isolated from termite soldier heads using MasterPure Complete DNA and RNA purification kit (Epicentre, Madison, WI). Amplification from the genomic DNA template in a polymerase chain reaction (PCR) was performed using reported forward and reverse primer sequences (Foster *et al*. 2004). The amplified product was cloned into the pGEM T-Easy Vector System (Promega, Madison, WI) and four of the clones were sent to Eurofins Genomics (Louisville, KY) for sequence determination. The DNA sequences obtained were aligned using DNASTAR Lasergene v8 software (Madison, WI). The consensus sequence from the alignment was blast searched against NCBI database and confirmed that the species identity of the collected population was *Reticulitermes flavipes* (Kollar).

2.2 Chitosan Solution Preparation, Wood Treatment and Termite Exposure

Low molecular weight chitosan (50-190 kDa) purchased from Sigma-Aldrich Co. (St. Louis, MO) was suspended in 25% aqueous acetic acid and stirred overnight at room temperature until completely dissolved. Southern yellow pine (*Pinus* spp.) sapwood samples of dimensions 25×25×6 mm (tangential × radial × longitudinal) were dried at 50 °C to constant mass. Five replicates of oven-dried wood samples, with known mass, were treated with an aqueous chitosan solution under vacuum (29.8 mmHg) for 3 hours. Similarly, control samples were treated using distilled water. All treated samples were equilibrated in solutions for approximately 24 hours. Samples were removed from the solutions, gently wiped, dried at room temperature for several hours, then at 50 °C until constant mass was reached

The American Wood Protection Association E1 Standard (AWPA, 2014) was followed for termite exposure to treated-wood samples. Each chitosan-treated, and water-treated wood wafer was placed separately in an autoclaved Qorpak® round-bottom glass jar with 135 g of play sand, wetted with 35 ml of distilled water. One gram of cleaned termites (approximately 355 termite workers and 3 termite soldiers) was added into each jar. After 4 weeks, the jars were disassembled and surviving termites were cleaned and stored at -80°C until processed for DNA isolation.

2.3 DNA Isolation and Library Preparation

From each termite jar, a total of 80 termite guts were used. The termites were dissected following established procedures (Matson *et al*. 2007). Five termite guts were pooled together and macerated with a sterile pestle, then briefly centrifuged to separate hindgut tissue from the gut contents. The supernatant (gut luminal contents) from these 16 extractions was collected and DNA isolated following the MasterPure Complete DNA and RNA purification kit protocols. After extraction, the DNA was combined into 4 subsamples per jar. Thus, each group (treatment and control) contained 5 replicates. Concentration of the subsamples was measured on a NanoDrop™ spectrophotometer (Table 1) and was separated by size using agarose gel electrophoresis. The pooled DNA subsamples were diluted to a final concentration of 5 ng/µl, and the 16S ribosomal RNA gene region was amplified using previously reported primers (Klindworth *et al*. 2013), to which KAPA Hi Fi polymerase mix was added. PCR amplification was performed according to Illumina 16S Metagenomic Sequencing Library Preparation guide (Illumina, Part #15044223 Rev. B).

After amplification, the PCR products were separated on agarose gel electrophoresis for confirmation of the expected amplicon size of approximately 500 bp. Library preparation was performed according to the Illumina guide referenced above. In short, the PCR products were purified with Agencourt Ampure XP Magnetic Beads (Beckman Coulter). PCR products were indexed with Nextera XT DNA library prep kit (Illumina) and then purified with the magnetic beads again. Sequencing was performed at Mississippi State University, Institute for Genomics, Bioinformatics and Biocomputing.

2.4 Analysis of Sequencing Results

The collected sequence data were analyzed and filtered using Illumina BaseSpace 16S Metagenomics App (Illumina) Output files data from the BaseSpace App (Excel format), i.e. abundance (hits) of identified bacterial phyla for each replicate, were analyzed using Statistical Analysis Software (SAS Institute Cary, NC). In order to test for bacterial phyla differences among the samples, MANOVA and Tukey tests were performed. The Shapiro–Wilk test showed normal distribution of the replicate groups' data.

3. RESULTS AND DISCUSSION

3.1 DNA Quality and PCR Amplification

Concentrations of isolated genomic DNA from termite gut and quality are shown in Table 1. Genomic DNA initial concentration was within the range of 2.6 - 140 ng/µl. Electrophoresed PCR products from both treated and control groups are shown in Fig. 1. A consistent and expected band of about 500 bp was observed in all of the samples.

Table 1. Concentration and quality of isolated genomic DNA subsamples from termites fed on control (water treated, **CN**) and chitosan treated (**CT**) wood samples

Sample	Jar	Conc (ng/µl)	260/280	Sample	Jar	Conc (ng/µl)	260/280
CN1	1	71.0	1.94	CT1	1	34.8	1.87
CN2	1	124	1.94	CT2	1	37.6	1.85
CN3	1	88.6	2.00	CT3	1	50.4	1.92
CN4	1	94.2	1.99	CT4	1	44.6	1.91
CN5	2	126	1.95	CT5	2	46.8	1.82
CN6	2	61.2	2.02	CT6	2	38.9	1.74
CN7	2	87.0	2.01	CT7	2	44.3	1.88
CN8	2	41.3	2.11	CT8	2	54.5	1.87
CN9	3	93.9	2.00	CT9	3	45.3	2.07
CN10	3	121	1.99	CT10	3	38.1	2.05
CN11	3	100	1.98	CT11	3	37.5	1.95
CN12	3	89.9	1.93	CT12	3	125	1.54
CN13	4	55.6	2.05	CT13	4	48.8	2.00
CN14	4	52.7	2.05	CT14	4	35.4	2.10
CN15	4	53.4	2.10	CT15	4	2.61	2.24
CN16	4	49.1	2.10	CT16	4	50.7	2.01
CN17	5	125	2.03	CT17	5	33.0	1.99
CN18	5	128	1.95	CT18	5	45.3	2.00
CN19	5	140	1.97	CT19	5	29.2	1.90
CN20	5	119	1.97	CT20	5	29.6	1.90

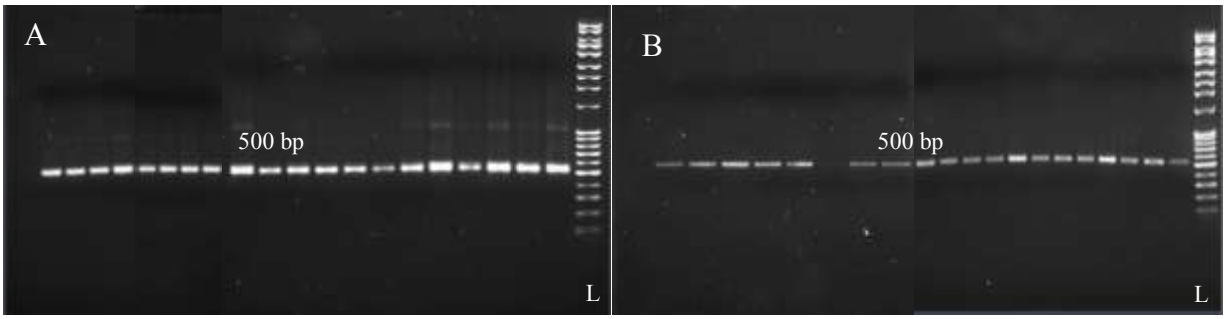

Figure 1. Molecular size estimation of *R. flavipes* gut PCR-amplified 16S rRNA gene subsamples with 1Kb plus Ladder (**L**) through gel electrophoresis (samples from termites exposed to: **A**- control wood samples, **B** - chitosan-treated wood)

3.2 Metagenomic Analysis

3.2.1 Overview

Approximately 1.7 million reads were generated from the control group with a range of 40 – 210 thousand (K) reads per sample while 2.1 million reads were generated from the chitosan-exposed group with a range of 17-284 K reads per sample. The high variation in reads was possibly caused by unequal concentration of DNA samples used for sequencing. It has been also postulated that the low complexity of the 16S rRNA gene may significantly reduce the number and quality of reads generated (Kozich *et al*. 2013). All samples within the control group had approx. 99% of all reads classified to the bacterial kingdom and approx. 93% of all reads were classified to a bacterial phylum (Fig. 2A). For the chitosan-exposed group, approx. 98% of reads from all samples were classified to the bacterial kingdom, and 93-96% of all reads were classified to a bacterial phylum (Fig. 2B). A total of 27 bacterial phyla were identified from all samples. Spirochaeta, Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Endomicrobia are the most abundant bacterial phyla in *R. flavipes* (Fisher et al., 2007). This is also evident in this study, though Spirochaeta is not as abundant compared to results from other studies (Arango et al. 2014, Brune and Friedrich 2000).

There may be between 84 and 1481 putative bacterial and archaeal phyla in the Silva rRNA database depending upon the detection algorithm used (Yarza *et al*. 2014), but only 52 bacterial (including candidate) phyla have been identified so far (Rappé and Giovannoni 2003). Therefore, some of our data may have not been classifiable due to unavailability of information in the database on these uncultured bacteria.

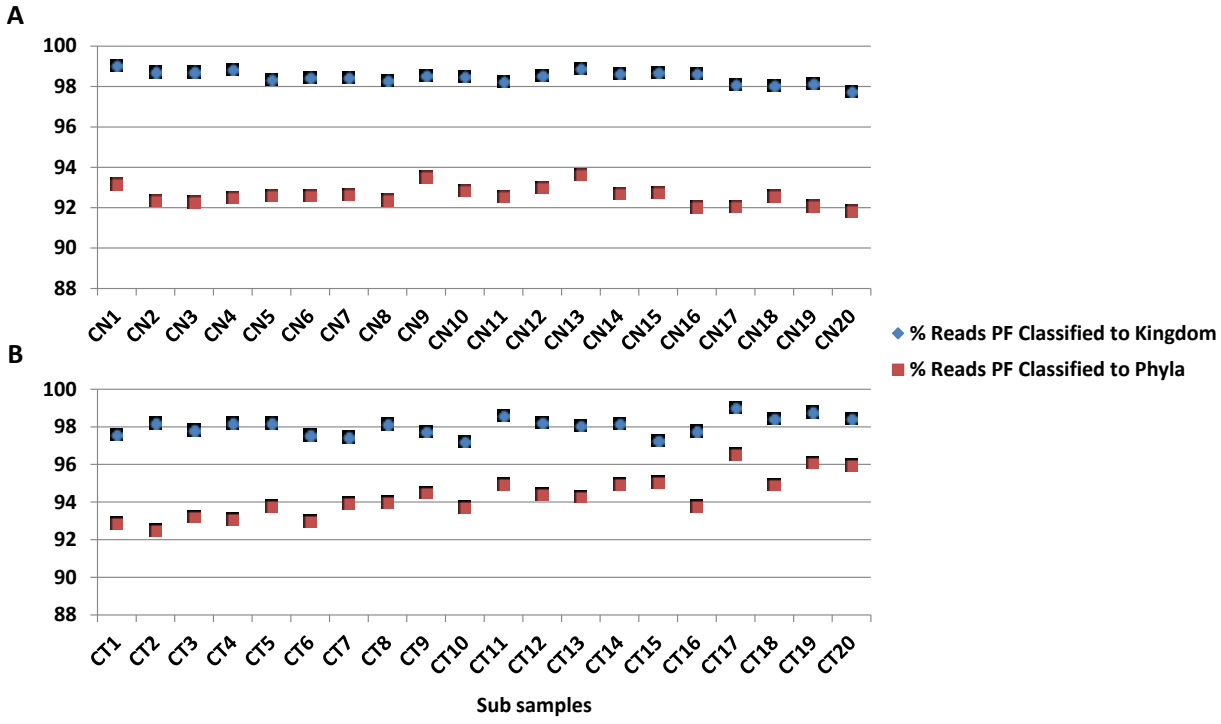

Figure 2. Percentage of subsamples' reads (post-filter) classified to the bacterial kingdom and a bacterial phylum (samples from termites exposed to: **A**- control wood samples, **B** - chitosan-treated wood)

3.2.2 Observed phyla diversity

The BaseSpace program by Illumina revealed a total of 27 bacteria phyla in all samples. Firmicutes, Bacteroidetes and Proteobacteria was consistently the most prevalent phyla within both groups (Fig. 3), and they made up together about 2/3 of the total phyla population. While

Spirochaetes, Thermotogae and Actinobacteria also appeared in all of sample groups, Actinobacteria seemed to be more prevalent in the control group. Verrucomicrobia was also more numerous in the control group, while Synergistetes was more prevalent in termite guts fed on chitosan treated-wood samples.

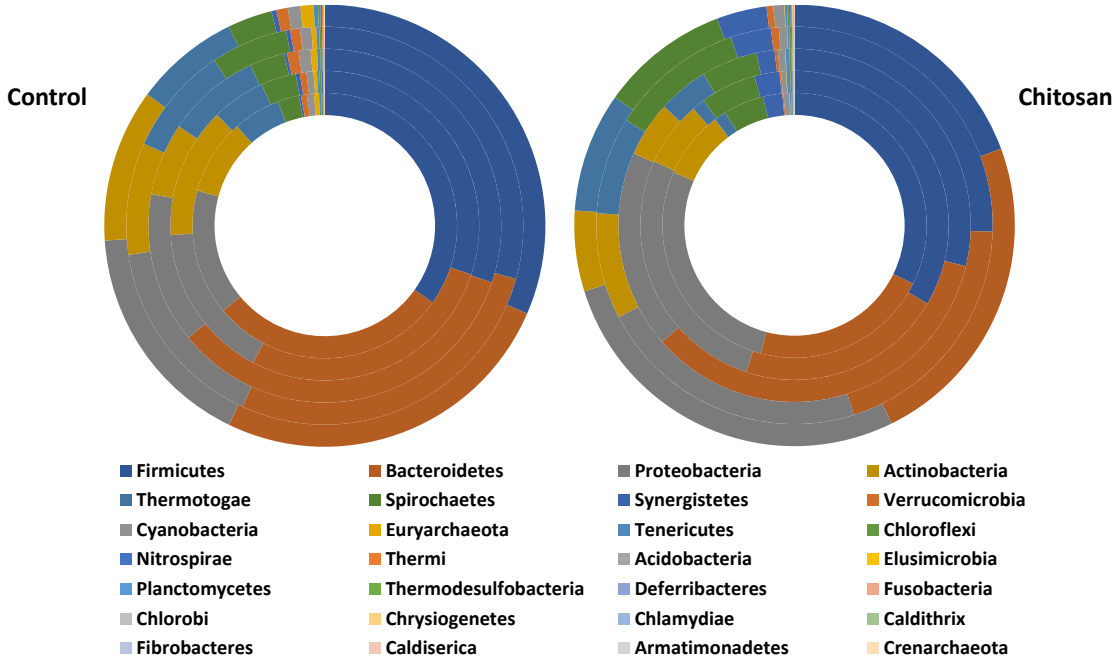

Figure 3. Bacterial phyla diversity within DNA samples of termites fed on control and chitosan-treated wood samples. Rings summarize diversity found for each replicate.

3.2.3 Effect of chitosan on phyla diversity

As evident from Fig. 4, several bacterial phyla were either positively or negative affected by chitosan. Differences between chitosan-treatment and control group are well distinguished in case of some phyla. This suggests that chitosan treatment played a role in the number of bacteria in the termite guts. All DNA metagenomic samples were subjected to MANOVA, and Tukey analysis was performed for differences between sample groups. Four bacterial phyla (Proteobacteria, Synergistetes, Elusimicrobia, and Planctomycetes) showed significantly higher abundance ($p = 0.05$) due to chitosan treatment, while one bacterial phylum, Thermodesulfobacteria, showed significantly lower abundance. The remaining 21 bacterial phyla were not significantly ($p = 0.05$) affected by the chitosan treatment. Interestingly, there were no characteristics of the bacteria that could be correlated to population number as affected by chitosan treatment. For example, some bacterial phyla that were prevalent in chitosan treated group (Proteobacteria, Synergistetes, and Elusimicrobia,) are known to be motile (Bardy *et al*. 2003, Kersters *et al*. 2006, Herlemann *et al*. 2009, Vartoukian *et al*. 2009), but some prevalent in the control group are also known to be motile (Iverson *et al*. 2012). A mixture of gram-positive, gram-negative, aerobic and anaerobic phyla existed within both termite groups. This suggests that chitosan does not specifically target a certain type of bacteria, which is in agreement with a previous study (Kong *et al*. 2010).

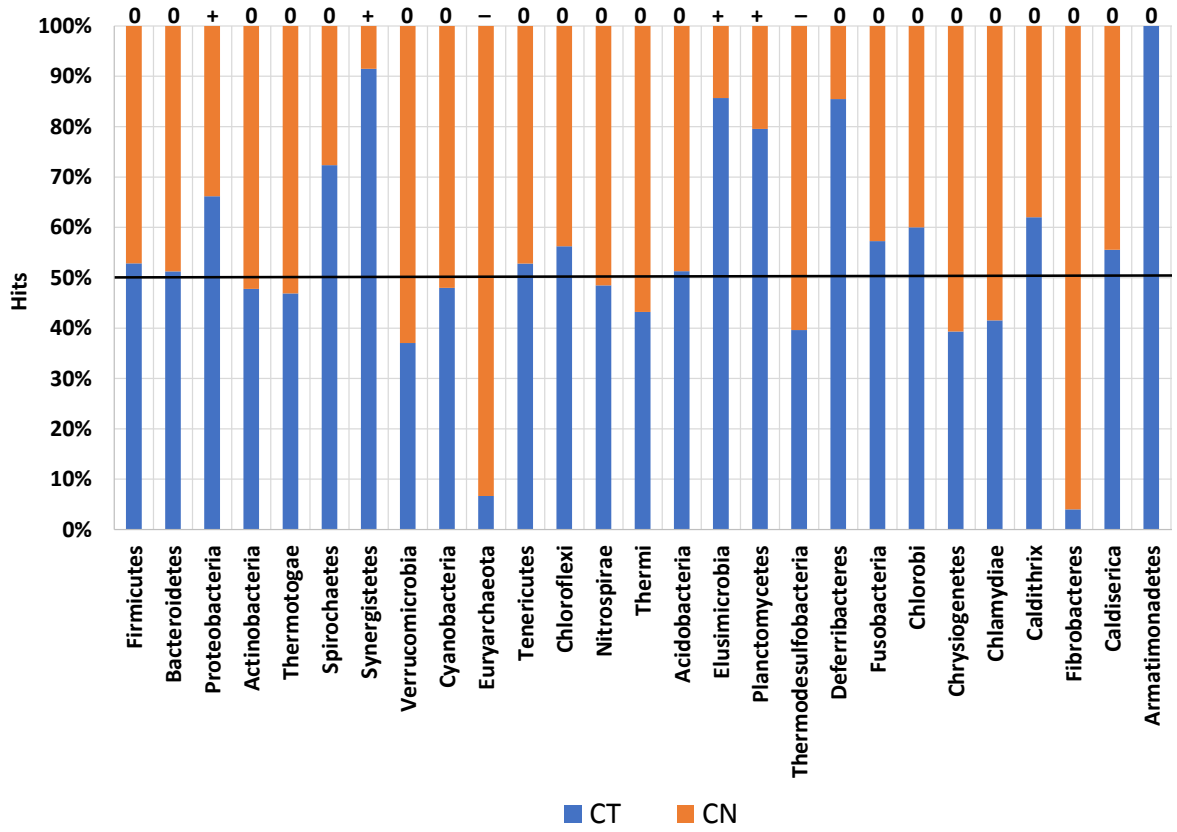

Figure 4. Effect of chitosan treatment on bacterial phyla frequency in termite hindgut sample groups (CT – chitosan-exposed, CN – control); "0" denotes no significant change, "+" denotes increased significant change and "–" denotes decreased significant change in number of abundance ($p = 0.05$)

4. CONCLUSIONS

In this study, sequencing of 16S rRNA amplicons from the gut contents of *R. flavipes* collected from Louisville, MS generated a total of 3.8 million reads. A potential miscalculation in DNA sample concentration used for sequencing may have caused the overall low number of total reads and a high variation between the samples reads. The samples consisted predominately of bacteria from Firmicutes, Bacteroidetes and Proteobacteria phyla, representing almost 66% of all sequence data, which is in agreement with other studies. The remaining sequencing data contained 23 phyla. Five phyla showed significant difference in number between the treatment groups: one phylum was more abundant in the control group, and four phyla were more abundant in the chitosan-treated group. This suggests a treatment effect on some of the bacterial phyla, though further analysis will be needed to assign the effect at the species level.

ACKNOWLEDGEMENTS

The authors would like to show appreciation for Dr. Chuan-Yu Hsu (Institute for Genomics, Bioinformatics and Biocomputing) for her guidance, and valuable input throughout this work, and Ismail Khan (Mississippi State University Sustainable Bioproducts) for his assistance throughout this project. Funds for this research were provided by the USDA Forest Service Forest Products Laboratory Coalition for Advanced Wood Structures and the Mississippi State University Mississippi Agriculture and Forestry Experiment Station/Forest & Wildlife Research Center Directors' Doctoral Fellowship. This manuscript has been approved as Journal Article SB889, Forest & Wildlife Research Center, Mississippi State University.

5. REFERENCES

Alfredsen, G., Eikenes, M., Militz, H., & Solheim, H. (2004). Screening of chitosan against wood-deteriorating fungi. *Scandinavian Journal of Forest Research, 19*, pp. 4-13.

Arango, Rachel A., Frederick Green III, and Kenneth F. Raffa. "Changes in bacterial gut community of *Reticulitermes flavipes* (Kollar) and *Reticulitermes tibialis* Banks after feeding on termiticidal bait material." (2014): 1-10. *Proceedings of the IRG Annual Meeting*, IRG/WP 14-10819.

AWPA. (2014). "Standard method for laboratory evaluation to determine resistance to subterranean termites." E1-13. In: *Annual Book of AWPA Standards*, American Wood Protection Association, Birmingham, Alabama, USA, pp. 412-422.

Bardy, S. L., Ng, S. Y., & Jarrell, K. F. (2003). Prokaryotic motility structures. *Microbiology, 149(2)*, pp. 295-304.

Basturk, M. A. (2012). Heat applied chitosan treatment on hardwood chips to improve physical and mechanical properties of particleboard. *BioResources, 7(4)*, pp. 4858-4866.

Breznak, J. A., & Brune, A. (1994). Role of microorganisms in the digestion of lignocellulose by termites. *Annual Review of Entomology, 39(1)*, pp. 453-487.

Brune, A. (2014). Symbiotic digestion of lignocellulose in termite guts. *Nature Reviews Microbiology, 12(3)*, pp. 168-180.

Brune, A., & Dietrich, C. (2015). The gut microbiota of termites: digesting the diversity in the light of ecology and evolution. *Annual Review of Microbiology, 69*, pp. 145-166.

Brune, A., & Friedrich, M. (2000). Microecology of the termite gut: structure and function on a microscale. *Current Opinion in Microbiology*, *3(3)*, pp. 263-269.

Foster, B. T., Cognato, A. I., & Gold, R. E. (2004). DNA-based identification of the eastern subterranean termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Journal of Economic Entomology, 97(1)*, pp. 95-101.

He, S., Ivanova, N., Kirton, E., Allgaier, M., Bergin, C., Scheffrahn, R. H., Kyrpides, N. C.,Warnecke, F.,Tringe, S. G., & Hugenholtz, P. (2013). Comparative metagenomic and metatranscriptomic analysis of hindgut paunch microbiota in wood- and dung-feeding higher termites. *PLOS ONE, 8(4): e61126.*

Herlemann, D., Geissinger, O., Ikeda-Ohtsubo, W., Kunin, V., Sun, H., Lapidus, A., Hugenholtz, P., & Brune, A. (2009). Genomic analysis of "*Elusimicrobium minutum*," the first cultivated representative of the phylum "Elusimicrobia"(formerly termite group 1). *Applied and Environmental Microbiology, 75(9)*, pp. 2841-2849.

Hongoh, Y. (2010). Diversity and genomes of uncultured microbial symbionts in the termite gut. *Bioscience, Biotechnology, and Biochemistry, 74(6)*, pp. 1145-1151.

Huang, X. F., Bakker, M. G., Vivanco, J. M., Judd, T. M., & Reardon, K. F. (2013). Variations in diversity and richness of gut bacterial communities of termites (*Reticulitermes flavipes*) fed with grassy and woody plant substrates. *Microbial Ecology, 65(3)*, pp. 531-536.

Iverson, V., Morris, R. M., Frazar, C. D., Berthiaume, C. T., Morales, R. L., & Armbrust, E. V. (2012). Untangling genomes from metagenomes: revealing an uncultured class of marine Euryarchaeota. *Science, 335(6068)*, pp. 587-590.

Ke, J., Laskar, D. D., Gao, D., & Chen, S. (2012). Advanced biorefinery in lower termite-effect of combined pretreatment during the chewing process. *Biotechnology for Biofuels, 5:11*

Kersters, K., De Vos, P., Gillis, M., Swings, J., Vandamme, P., & Stackebrandt, E. (2006). "Introduction to the Proteobacteria." In: *The prokaryotes*, Springer, New York, pp. 3-37.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research, 41(1)*, pp. e1-e1.

Köhler, T., Dietrich, C., Scheffrahn, R. H., & Brune, A. (2012). High-resolution analysis of gut environment and bacterial microbiota reveals functional compartmentation of the gut in wood-feeding higher termites (*Nasutitermes* spp.). *Applied and Environmental Microbiology, 78(13)*, pp. 4691-4701.

Kong, M., Chen, X., Xing, K., & Park, H. (2010). Antimicrobial properties of chitosan and mode of action: a state of the art review. *International Journal of Food Microbiology, 144(1)*, pp. 51-63.

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology, 79(17)*, pp. 5112-5120.

Li, H., & Yu, T. (2001). Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit. *Journal of the Science of Food and Agriculture, 81(2)*, pp. 269-274.

Matson, E., Ottesen, E., & Leadbetter, J. (2007). Extracting DNA from the gut microbes of the termite (*Zootermopsis nevadensis*). *Journal of Visualized Experiments, JoVE(4)*, pp. 195.

Noda, S., Iida, T., Kitade, O., Nakajima, H., Kudo, T., & Ohkuma, M. (2005). Endosymbiotic Bacteroidales bacteria of the flagellated protist *Pseudotrichonympha grassii* in the gut of the termite *Coptotermes formosanus*. *Applied and Environmental Microbiology, 71(12)*, pp. 8811-8817.

Raafat, D., & Sahl, H. G. (2009). Chitosan and its antimicrobial potential–a critical literature survey. *Microbial Biotechnology, 2(2)*, pp. 186-201.

Raji, O., Tang, J. D., & Jeremic, D. (2015, April 2015). Diversity of hindgut bacterial population in subterranean termite, *Reticulitermes flavipes. 112th Annual American Wood Protection Association meeting, San Juan, Puerto Rico*, *112*, pp. 48-53.

Rappé, M. S., & Giovannoni, S. J. (2003). The uncultured microbial majority. *Annual Reviews in Microbiology, 57(1)*, pp. 369-394.

Stössel, P., & Leuba, J. L. (1984). Effect of chitosan, chitin and some aminosugars on growth of various soilborne phytopathogenic fungi. *Phytopathologische Zeitschrift, 111(1)*, pp. 82-90.

Torr, K. M., Chittenden, C., Franich, R. A., & Kreber, B. (2005). Advances in understanding bioactivity of chitosan and chitosan oligomers against selected wood-inhabiting fungi. *International Journal of the Biology, Chemistry, Physics and Technology of Wood, 59(5)*, pp. 559-567.

Tsai, G. J., Su, W. H., Chen, H. C., & Pan, C. L. (2002). Antimicrobial activity of shrimp chitin and chitosan from different treatments and applications of fish preservation. *Fisheries Science, 68(1)*, pp. 170-177.

Vartoukian, S., Palmer, R., & Wade, W. (2009). Diversity and morphology of members of the phylum "Synergistetes" in periodontal health and disease. *Applied and Environmental Microbiology, 75(11)*, pp. 3777-3786.

Yarza, P., Yilmaz, P., Pruesse, E., Glockner, F. O., Ludwig, W., Schleifer, K.-H., Whitman, W.B., Euzeby, J., Amann, R., & Rossello-Mora, R. (2014). Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature Reviews Microbiology, 12(9)*, pp. 635-645.