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Project Summary

Project Title

What role do gut bacteria play in termite digestion?

Introduction

Termites are well known for their ability to digest cellulose and turn the cellulose into glucose (i.e., release glucose from cellulose) to support their daily energy expenses. Generally speaking, there are three factors contribute to this digestion process. These three factors are bacteria, protozoa, and the host (termites) itself, which form a "digestion triangle". Traditionally, protozoa have been thought to dominate this process, and much strong evidence has been found to support a role for protozoa in cellulose digestion. Recently, the Scharf lab at Purdue has found new genetic level evidence to support the idea that the host enzymes play an important role in this process as well. But, the role that bacteria play to help termites degrade the cellulose is remaining unknown. In this project, by removing one component (bacteria) in the digestion triangle, I found termite cellulose degradation ability cannot reach the same level as when all three factors present. My findings therefore provide new evidences to suggest that bacteria are indispensible in the digestion process.

Materials and Methods

Bioassay

Termites from the WSLR, B1 and B2 colonies at Dr. Scharf's lab were used for this project. They were allowed to feed and grow in the incubator under 37℃ for 7 days before I collected the data. A mixture of sawdust and paper was used as food during the feeding process. 60 termites were used each time, and they were equally divided into control and treatment groups. Only water was added to the diet of control groups. They received 200µl on the first day, and 100µl on the 3rd and 6th day. In contrast, the diets of treatment groups were treated with the following antibiotics: Kanamycin, Ampicillin, Tetracycline and Metronidazole (Chart 1). They were replenished with water on the same day and at the same amount as controls.

Termite colony	Kanamycin	Ampicillin	Tetracycline	Metronidazole
WSLR	5%	5%	2.5% (died under 5%)	5%
B1	5%	5%	2.5%	5%
B2	5%	5%	2.5%	5%

Chart 1. Summary of antibiotic treatments used in this study. Percentage (%) values indicated weight of antibiotics prepared per volume of water.

Gut Media Preparation

Guts were removed on the 8th assay day under the dissection microscope. The control and treatment guts were preserved separately in centrifuge tubes filled with 750µl PBS in ice. The guts were ground into small particles before centrifuging, using a Teflon-glass grinder. Then I centrifuged the mixture 15min at 15,000g and used the remaining supernatants (liquid portions) in sawdust assay.

BHI Media Plates Examination

BHI approximate formula per liter purified water

Brain heart, infusion form (solid) 6.0g

Peptic digest of animal tissue 6.0g

Sodium chloride 5.0g

Dextrose 3.0g

Pancreatic digest of gelatin 14.5g

Disodium phosphate 2.5g

Three replications were made for each kind of antibiotic. Control groups were also available to pair with treatments. Each BHI (Brain Heart Infusion) media plate had 100µl gut mixtures applied on the surface. The control plates were applied with control gut media, while treatment plates bore treatment gut media on their top. The plates were placed in an incubator upside-down at 37℃. The number of bacterial colonies was counted and recorded after 20h.

Sawdust Assay

The antibiotic treatment and control groups were further examined in sawdust assay tests, each with three replications. These "sawdust assays" were conducted to measure the ability of antibiotic-treated and control guts to release glucose from pine sawdust, and ultimately, to determine the relative roles of termite gut bacteria in cellulose digestion. Methods described by Scharf et al. (2011) were used for sawdust assays. 0.015g sawdust was deposited on the bottom of each centrifuge tube with and 600µl sodium acetate buffer (0.1M, pH 7.0) and 150µl gut mixtures (controls and treatments were marked based on the gut mixtures added, and negative controls were treated with 150µl buffer instead). Thus, nine tubes in total were assayed, and each with a hole drilled on the top.

These tubes were then placed in the incubator at 37℃ and shaking speed at 220rpm. Twenty-hours later, 15µl EDTA was added to each tube to stop the chemical reaction. The mixture was centrifuged 15min at 15,000g and the remaining supernatant used for glucose detection in 96-well microplates.

To set up the 96-well microplates, I divided each replicate into three 50µl fractions. Each well was filled with one 50µl fraction and 200µl of glucose detection reagent. The plate was shaken for 10min in the incubator at 37℃ and speed at 220rpm, and the plate absorbance read at 505nm in the microplate

reader relative to the standard curve made earlier. Results were expressed as µmol glucose released per min per termite gut equivalent.

Results

Culture Plate Count (fig. 1)

All three treatments (KAN, AMP and TET) significantly reduced the numbers of culturable bacteria by more than 95% compared to the control groups (p=0.0495 for all three antibiotics). TET was especially effective, reducing the culture numbers by over 99%. As further evidence of TET impacts, there were some replicate plates treated with TET that had no bacteria cultures present at all. When examining colony effects, I found that colony B1 contained more bacteria in their guts than the other two colonies that were tested; however, the percentages of reduction are much closer among all three colonies.

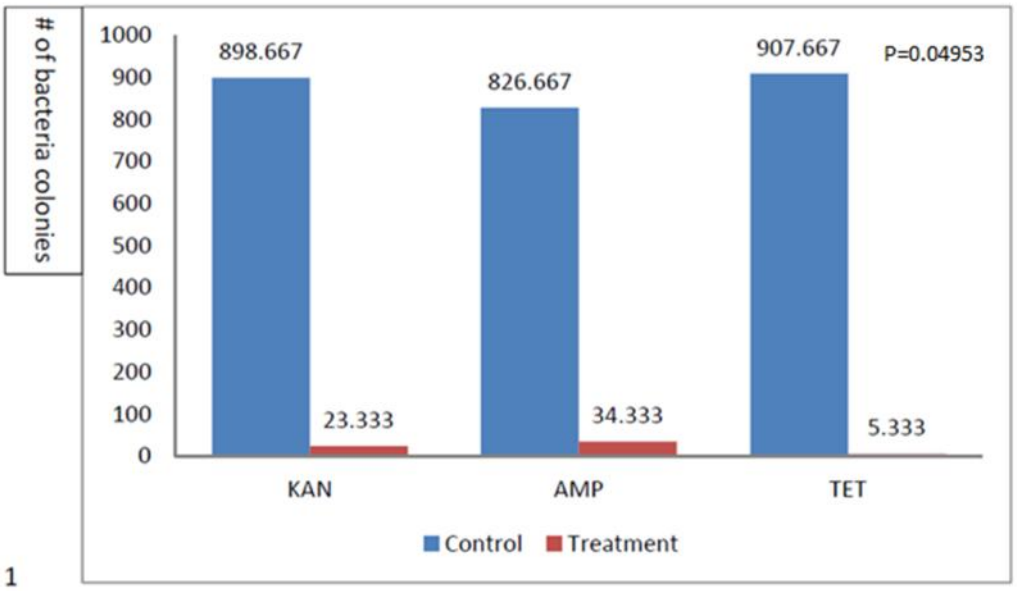

fig. 1

Glucose release (fig. 2)

After treatment with antibiotics, the abilities of termites to convert cellulose into glucose were reduced by about 50%. However, only AMP caused a significant reduction in glucose release (p=0.0495). The KAN and TET results were not significant (p=0.1266 and 0.1212, respectively), and more replicates may need to be done before I reach significant results on KAN and TET. Although there were some variances, the colony factor does not seem to affect the glucose release very much.

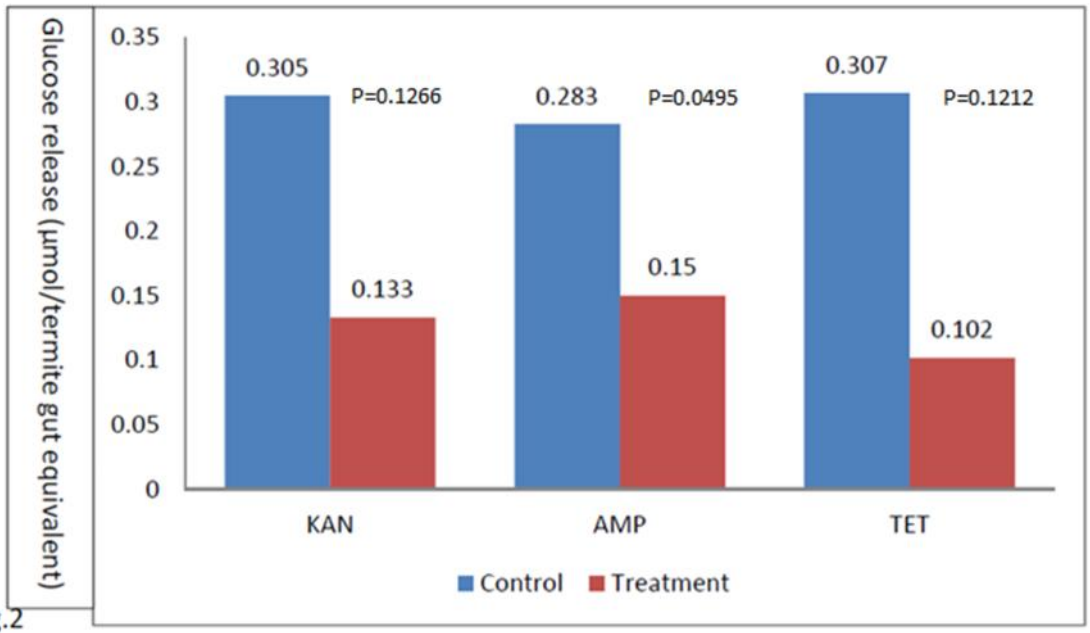

fig.2

Discussion

The termite gut can be roughly divided into the foregut, midgut and hindgut. The hindgut, which is the habitat of microorganisms, can be identified as an enlarged bag behind where the Malpighian tubules join the midgut. Host enzymes are considered to be released at the salivary gland before the foregut. In this project, I removed all three gut regions along with the salivary gland out of the termites as a whole. Thus, the digestion triangle consisting of host gut, symbiotic protists and symbiotic bacteria remained intact in my experimental control group. The treatments groups, which received antibiotics in their food, were missing the bacterial component of the digestion triangle.

My data from culture plate counts showed less than 3% bacteria on average remained after feeding on the various antibiotics as compared to controls. Statistical analysis indicated these culture reductions were significant at the $p<0.05$ level. Although not completely eliminated, the 3% of bacteria that remained likely could not do a lot in the way of digestion. So, here I assume, the bacteria had been removed from the digestion triangle. Thus, all glucose release from the gut media after antibiotic treatment could only be explained by the collaboration of protozoa and host enzymes.

The glucose release data suggested an average drop of 56% when comparing treatments with controls. That is not to say that bacteria alone contribute 56% of termite's digestion ability, because the mechanisms behind the digestion triangle are not clear. Maybe, the host enzymes need some coenzymes provided by bacteria to work properly and the elimination of bacteria limits activity by the host enzymes. Or maybe, protozoa need to work with bacteria to achieve a high efficiency. There are a lot of assumptions can be put here. But without the knowledge of triangle mechanisms, it's hard to tell if bacteria can degrade 56% of cellulose alone. What is clear, however, is that removing bacteria from the symbiosis triangle leads to reduced cellulose digestion capabilities.

The triangle mechanisms may be different among different species of termites. So, bacteria may contribute more or less in digestion process in different termites. For example, higher termites lack protozoa completely and thus bacterial probably play a greater role in cellulose digestion in higher termites.

In conclusion, bacteria do contribute to the termite digestion process although it remains mystery how important they are in the digestion triangle. Further research may provide a better explanation by target at the collaboration mechanisms among the three components (protozoa, bacteria and host enzyme). My results are important because they help to advance termite digestion science by providing a new experimental approach and some of the first results to suggest how much gut bacteria might contribute to termite digestion.

References

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