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Comparing the Gut Microbiomes of Arachnids that Differ in Feeding Ecology

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ABSTRACT

Different gut microbial communities yield the ability to decompose different types of materials, and thus are expected to vary between organisms that have different diets. *Arachnids* in the order *Opiliones* (e.g. harvestmen) consume both decaying organic matter and live insects. While other arachnids, like spiders (Order *Arachnea*) are strict predators, only consuming live prey. Given these differences in diet, in this study, we investigate the gut micro biomes of *Opilionides* and wolf spiders with regards to the presence of bacteria that have the ability to decompose cello-biose. Upon collection, we inoculated samples onto agar plates, where the sole organic energy source was, Cello-biose. All microbes were grown at exact same temperatures and given same nutrients and growth time. After the third inoculation was performed to generate pure colonies, the samples were sent for identification. Both organisms contained microbes that were able to decompose the agar plates. Wolf-spiders had a uniform bacteria found throughout their plates that was able to break down the cello-biose, *Opilionides* gut microbes demonstrated a high degree of variation in the dominant bacteria that breaks down plant material. Interestingly, some of the microbes isolated belong to the bacterial genus of *Bacillus,* which are linked with causing food poisoning. From this same genus another microbe was found that is known as an important insect pathogen. The most interesting finding was that of *Bacillus Anthracis* which is the etiologic agent of Anthrax.

Keywords: Opiliones, Lycosidae, cellobiose, bacteria

INTRODUCTION

Many organisms throughout the world have an array of characteristics that differentiate them from each other. Some organism posses better physical traits in order to hunt down their prey. Some organisms have a large physical stature and require a large amount of food, for example pandas, which require about 20-40lbs of bamboo daily. Many organisms vary on their characteristics depending on the type of environment they are found in, these differences are typically results of adaptations that enhance fitness given their habitat or niche. One key characteristic that helps in understanding the eating habits of certain organisms is in understanding the microbial community that resides within their gut. A micro-biome is defined as "the entire habitat, including micro-organisms, their genomes (i.e., genes) and the surrounding environmental conditions. This definition is based on that of "biome" the biotic and abiotic factors of a given environments."(Ravel, 2013) Certain microbes aid in the digestion of only certain types of food, which in turn determine what that organism can and can't digest once consumed. For example termites have bacteria, archaea, and protozoa microbes residing in their gut. All these microbes work in unison in order to break down the complex sugars that make up wood. Termites have Trychonympha cells in their guts, which have the ability to engulf wood particles but need the bacteria they harbor to break down the wood particles. Also in this process, these bacteria release by-products that are used as nutrients for the termite. (Noll)

Organisms in the order *Opiliones* (i.e. harvestmen or daddy longlegs; Class (Arachnida) are widely distributed, occurring in most terrestrial habitats and on all continents except Antarctica. (http://www.museunacional.ufrj.br/mndi/Aracnologia/opiliones.html) *Opiliones* have a unique diet, as they will eat a variety of different things such as aphids, caterpillars, beetles, flies, mites, small slugs, snails, earthworms, spiders, other harvestmen, decaying plant and animal matter, bird droppings and fungi(Conrad). The generality of harvestmen's diet is particularly interesting being that they are arachnids, and most arachnids are seen as exclusive predators. (Pinto-da-Rocha 2007).

The broad range of foods that *Opiliones* can digest sparks curiosity to the microbial community that resides within them and enables them to digest certain parts of their diet. The most interesting thing about their diet is their ability to consume and digest both organic matter and other insects. This is because organic matter is composed of "lignocellulose which represents 90% of the dry weight of all plant materials is primary composed of the sugar polymers cellulose (35-50%) and hemicellulose (20-35%) together with lignin (5-30%) that provides structural support for the plant" (Bashir,). Through the process of hydrolysis cellulose can be broken down into a disaccharide which consist of two D- glucosepyranoses joined by a 1,4,-beta-glycoside bond.(Chem.ox.ac.uk) In order to breakdown these different types of foods an

Opiliones must have different types of gut microbes, that are able to perform this breakdown, or have the ability to release the enzyme cellulase which has the ability to break down cellulose, as most organisms cannot by themselves.

The gut micro-biome of *Opiliones* will be compared to that of the wolf spider. They will be compared to one another, due to the fact that both organisms are found in the same environment and because they are both from the Class *Arachnida*. Wolf spiders can be either idle predators or they can be actively hunting their prey on the substrate surface of their environment. While both harvestmen and wolf spiders both have the tendency of preying on the same type of insects (e.g. crickets and houseflies), wolf spiders are strict predators and will not consume decaying organic material. This difference in feeding ecology could potentially result in a different gut micro biome when compared to harvestmen.

The main goals of this experiment are to show the difference in microbial community between *Opiliones* and the wolf spiders. Both organisms are found in the same environment, but have vastly different diets. In order to compare the gut microbes, Microbes will be isolated once they have been obtained from *Opiliones* and wolf spiders; and screened for the presence of bacteria that can potentially process/use cellulose as an energy/carbon source. For this experiment the disaccharide cello-biose was used in place of cellulose; due to the fact that cello-biose would be an easier organic material to be broken down by microbes. Thus far, all research has been conducted on ants, termites, and other insects, which only digest organic matter. Little research is known about *Opiliones* microbial community and the exact types of bacteria that reside in their guts. Due to the variance *in* Opiliones diet, they should have microbes in their guts, which are able to digest cello-biose; while wolf spiders shouldn't.

MATERIALS AND METHODS

Both the *Opiliones* and wolf spiders were collected in a wooded area, at McPherson State Fishing Lake in Kansas. Individuals were collected by looking through surface areas that were covered with leaf litter. Many of the *Opiliones* were found just roaming the surface of these settings and were captured in tubes kept alive and I.D. The majority of the *Opiliones* were captured during the day. On the other hand, the wolf spiders were obtained using the eye shining technique. Wolf spiders were found throughout the surface of the woods in locations similar to the *Opiliones*, yet some were found in less covered areas of the ground surface. After both spiders and *Opiliones* were obtained they were left in their tubes and taken back to the lab. They were not allowed to leave their containers in order to avoid any contamination from the lab setting. Once in the lab both specimens were put in test tubes that were labeled in accordance to their I.D

From there, the *Opiliones* and wolf spiders needed to be prepared, before they were squeezed, in order to put in nutrient broth tubes containing cello-biose. A picture of each *Opilione* and wolf spider was taken before drowning. Two beakers of 500mL one containing autoclaved D.I water, and the other containing Alcohol. The specimens were submerged in the beaker with alcohol, to kill any microbes on its surface, and to also kill the specimens. Once the subject is dead it can be transferred to the beaker with the D.I water to rinse off the alcohol. Once rinsed the test subject was placed in the nutrient broth tubes containing cello-biose. There the test subjects were squished using tongs in order to release any microbes that could be residing within their gut.

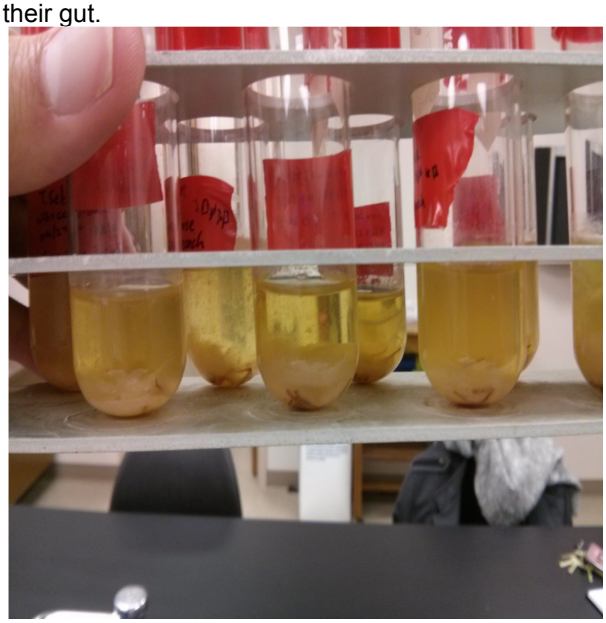

Figure 1. Shows the tubes pertaining to the wolf spiders and how they looked after being allotted 24-hour growth period.

In order to prepare the Nutrient-cello-biose tubes, 125mL were measured out into an, Erlenmeyer flask, of D.I. water than 1.625g of Nutrient Broth were also mixed into the flask. 1.25g of Cello-biose was also put into the Erlenmeyer flask and mixed. The Erlenmeyer flask had a magnetic stirrer to allow the solution to completely be dissolved and look clear and not cloudy or with any signs of having particles in it. The solution was then separated into 15 tubes and put in racks to be put into autoclave and be sterilized.

The agar plates were made from the following ingredients: 500mL of D.I. water, 6.5g of Nutrient broth, 5g of cello-biose, and 7.5g of Agar. After the solution came to a boil, the labeled Erlenmeyer flask was sterilized in the autoclave. After solution was

sterilized and allowed to cool down, solution was poured into Petri dishes in a hooded system, to avoid any contamination.

NB tubes with cello-biose were incubated in the 30-degree Celsius incubator for a period of 12-15 hours in order to allow any microbes in the broth to be allowed to grow. After the allotted time had passed the tubes were inoculated onto Nutrient Broth Agar plates containing cello-biose. The streak plate method was used in order to isolate colonies and have pure colonies of a single type of bacteria. The streaked plates were then incubated at 30-degree Celsius and allotted about 12-15 hours for growth to occur. After that, another inoculation was performed using an isolated colony on the plate to a new agar plate in order to try and isolate that bacterial colony. This same technique was performed three times to make sure that the last set of microbes that are sent to get sequenced belongs to one set of bacteria and not get a different array of microbes. Plates were then stored in refrigerator to slow down growth of bacteria on plates.

A total of thirty-nine tubes were made, eighteen tubes for wolf spiders labeled by their number followed by the letter B. For the *Opiliones* 21 subjects were collected and put in nutrient broth tubes, the first 15 specimens collected were just given a number in order to identify; six more *Opiliones* were later collected and ran through the same experiment. Each test tube was inoculated onto an agar plate and streaked; this process was done three times for each. Lastly, six plates were chosen form the *Opiliones* and four plates from the wolf spiders were randomly selected and inoculated into an agar test tube. From these, ten tubes were sent for sequencing and identification at the Molecular Epidemiology, Inc.

RESULTS

The broth tubes containing the guts and microbes of *Opiliones* showed many differences between each tube. Tube 3A for example, changed into a dark brown colored solution. While tube number 6A was a mixture of brown and yellow solution. Differences ranged from color of solution, to by-products that began to form on the surface of the solutions. Tube number 2A displayed growth of what appeared to be fungal spores on surface of solution. All tubes that contained the wolf spiders were uniform throughout, and were a light yellow colored solution after being inoculated.

From the data collected all the specimens' demonstrated growth once they were plated. The four test tubes (4B, 9B, 13B, 15B) from the wolf spider groups demonstrated a uniform type of genus for microbes; also test tube eight from the *Opiliones* showed the same results as the wolf spiders. The genus is from the family *Bacillus* and has species ranging from *Anthracis* to *thuringiensis*. (See Table 1)

The *Opiliones* results showed a higher degree of variance. Each sample varied in the genus and species that were sequenced from the agar tubes. (See Table 1)

Figure 2. Shows an *Opilione (2A)* whose gut micro-biome has been released to interact with the Nutrient Cello-biose broth and shows growth of spores forming on surface of solution.

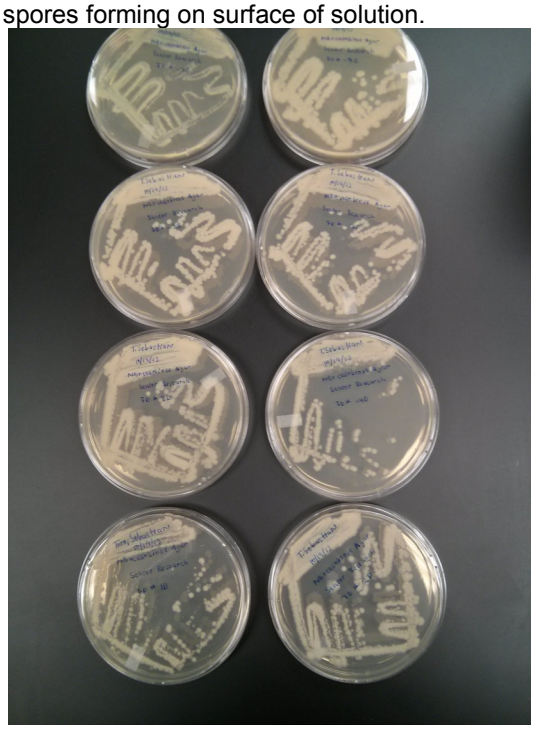

Figure 3. Shows the microbial growth of the Wolf Spiders once inoculated onto the cello-biose agar, and given a 24-hour growth period.

Table 1. Number of arachnid gut microbe plates that were identified to *Enterobacter*[a]

	Total # Plates	EA	EK	EL	ES	ECA	ECL	EAR	EAM	EAE
Lycosidae	4	0	0	0	0	0	0	0	0	0
Opiliones	5	2	2	1	1	4	2	1	2	1

a Species Abbreviations: EA= *Enterobacter asburiae,* EK= *Enterobacter kobei*, EL, *Enterobacter ludwigii*, ES= *Enterobacter soli*, ECA= *Enterobacter cancerogenus*, ECL= *Enterobacter cloacae*, EAR= *Enterobacter aracihdis*, EAM= *Enterobacter amnigenus*, EAE= *Enterobacter aerogenes*

Table 1 cont. Number of arachnid gut microbe plates that were identified to *Bacillusa*

	Total # Plates	BA	BC	BP	BT	BM	BW	BCY	BG	BPA	BTE
Lycosidae	4	4	4	4	4	4	4	4	2	1	1
Opiliones	5	1	1	1	1	1	1	1	1	0	0

a Species Abbreviations'= *Bacillus anthracis, BC*= *Bacillus cereus*, BP= *Bacillus pseudomycoides*, BT= *Bacillus thurigiensis*, BM= *Bacillus mycoides*, BW= *Bacillus weihenstephanensis*, BCY= Bacillus cytotoxicus, BG= *Bacillus galliciensis*, BPA= *Bacillus panaciterrae*, BTE= *Bacillus tequilensis*

Table 1 cont. Number of arachnid gut microbe plates that were identified to other bacterial species*a*

	Total # Plates	RO	RP	CC	RT	KM	YR	SM	SN	SU	PC
Lycosidae	4	0	0	0	0	0	0	0	0	0	0
Opiliones	5	1	1	1	1	3	2	2	2	2	1

a Species Abbreviations: RO= *Raoultella ornithnolytica*, RP= *Raoultella planticola*, CC= *Cronobacter condimenti* RT= *Raoultella terrigena*, KM= *Klebsiella michiganensis*, YR= *Yokonella regensburgei, SM*= *Serratia marcescens,* SN= *Serratia nematodiphila*, SU= *Serratia ureilytica*, PC= *Pectobacterium carotovorum*

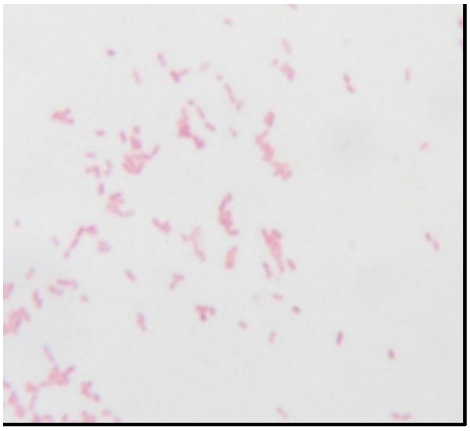

Figure 4A. Microbes found in test tube 5A. From the results these microbes were a combination from three Genus. *Serratia, Pectobacterium*, *Enterobacter*, and *Klebsiella***.**

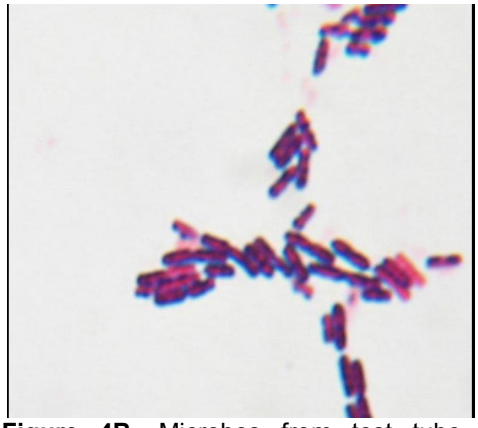

Figure 4B. Microbes from test tube 4B, these microbes all belonged to the genus *Bacillus*, and the species with the closest genetic markers were *Thuringiensis, Anthracis*, and *Cereus*.

DISCUSSION

Both *Opiliones* and wolf spiders showed microbes in their guts that were able to break down the cello-biose. *Opiliones* demonstrated a broader array of different bacteria from the cultures that were sequenced. Wolf spiders on the other hand, demonstrated more of a uniform type of bacterium that was able to break down the cello-biose. Of the species the wolf spiders were harboring, two were very intriguing, *Anthracis* is the etiologic agent behind *Anthrax* while *thuringiensis* is a unique bacterium in that it shares a common place with a number of chemical compounds which are used commercially to control insects important to agriculture and public health" (Ibrahim, 2010). The genus *Bacillus* is commonly known for being "rod shaped, endospore-forming aerobic or facultatively anaerobic, gram-positive bacteria, the many species in this genus exhibit a wide range of physiologic abilities that allow them to live in every natural environment. The spores are resistant to heat, cold radiation, desiccation, and disinfectants"(Turnbull).

Opiliones had both gram positive and negative bacterium's, having a comparison to the wolf spiders microbes of gram positive. This could be due to the fact that *Opiliones* have the ability to digest organisms that the wolf spider also preys on, and because many species from the genus *Bacillus* are found in the soil, this might be a point source where exposure occurs to both wolf spiders and *Opiliones.* Being how both types of test subjects were found in the same environment they also ran a high risk of being exposed to the same factors. If this is the case wolf spiders only had the *Bacillus* bacteria in their gut because they probably picked it up while they scavenged looking for food and were exposed to it; this could also be the case for the *Opiliones* thus explaining the similarity between microbes between them and the wolf spiders.

Majority of the *Opiliones* tubes displayed a vast difference in microbial community to that of the wolf spider. This could be in turn to the ability *Opiliones* being able to digest decomposing organic material like leaves and decaying plants. Which require different microbes than those found in the gut of wolf spiders. The difference in bacteria obtained from the *Opiliones* in comparison to that of the wolf spiders, demonstrates there's a difference in bacterium in the guts of both wolf spiders and *Opiliones*. This information can hopefully one day help us identify certain bacteria that can aid in the digestion of cello-biose, which in turn can be introduced into the guts of humans. Also the microbes of this study can better be ran through more research in order to find a resourceful way in which they can be harnessed and used towards a renewable energy source.

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LITERATURE CITED

Bashir, Zeenat, Vamsi K. Kondapali, Nidihi Adlakha, Anil Sharma, Raj K. Bhatnagar, Girish Chandel, and Syed Shams Yazdani. "Diversity and functional significance of cellulolytic microbes living in termite, pill-bug and stem-borer guts." *Scientific Reports* (2013). Web.

Classification of Opiliones. Ed. Adriano B. Kury. Museonacional, N.d. *google*. Web. 28 Sept. 2013

Conrad, Jim. "Harvestmen." *Back Yard Nature*. N.p., 11 Dec.. 2011. *Google,* Web. 3 Mar. 2014

Harrison, Karl. *Cellobiose.* N.p., 1996. *Google.* Web. 28 Sept. 2013. <www.Chem.ox.ac.uk>.

"Harvestman." *Columbia Electronic Encyclopedia.* 6th. 28 Sept. 2013. ed. 2012. *Infoplease*. Web. 28 Sept. 2013.

Ibrahim, Mohamed A., Natalyka Griko, Matthew Junker, and Lee A. Bulla. "Bacillus thuringiensis." *BioengineeredBugs.* Ncbi,Jan 2010. *Google Scholar.* Web. 30 Mar. 2014

Noll, Kenneth. N.p., n.d. *Google*. Wen. 29 Mar. 2014.

Ravel, Jacques. "all things genomics." *allthingsG*. N.p, 13 Jan. 2013. *Google*. Web 29 Sept. 2013.

Ritter, Stephen K. "To Make Better Biofuels, Scientist Mine Microbiomes. "*Chemical and Engineering News.* N.p., 3 Dec. 2012. Web. 22 Jan. 2014

Rocha, Ricardo Pinto d., Glauco Machado, and Gonzalo Giribet, *Harvestmen: The Biology of opiliones*. N.p.: Harvard University Press, 2007. 9. Web 1 Oct. 2013.

Smithsonian National Zoological Park. N.p., n.d. Web. 29 Mar.2014

Todar, Kenneth. "Bacillus anthracis and Anthrax." . N.p., 2008. Web. 13 Mar. 2014.

Turnbull, Peter C. *Bacillus*. N.p., 1996. *Google.* Web. 30 Mar. 2014.