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# A First Look at the Microbial Community of Rabidosa rabida, a Wolf Spider in Searcy, Arkansas

Patricia Rivera
*Harding University*, privera@harding.edu

Ryan Stork
*Harding University*, rjstork@harding.edu

Amber Hug
*Harding University*, ahug1@harding.edu

# A First Look into the Microbial Community of *Rabidosa rabida*, a Wolf Spider in Searcy, Arkansas

P. Rivera*, R.J. Stork, and A.G. Hug

*Department of Biology, Harding University, Searcy, Arkansas 72149, USA*

*Correspondence: patty.rivera001@gmail.com

Running Title: Microbial Community of *R. rabida*

## Abstract

Many diverse animal models have been used to explore the interactions between host organisms and their microbiota. Increased understanding of microbe-host interactions could lead to improved healthcare and drug development. Spiders have venom, digestive fluid, and body fluid components that have been suggested to possess antimicrobial properties that could lead to new and interesting host-microbe interactions. While studies have been published on interactions between bacteria affecting the immune function and behavior of spiders, the spider microbiome has not been established to date. Excreta and body swabs were collected from *Rabidosa rabida*, a wolf spider typically found on tall grass or low vegetation. Bacteria were cultured on tryptic soy agar, an all-purpose media known to grow most common bacterial strains, plates and 53 bacterial samples were Gram stained, catalase, and coagulase tested using aseptic technique. *Staphylococcus aureus*, *Staphylococcus* sp., and a Gram-positive bacillus were found on the excreta samples while *Staphylococcus sp*., Gram-negative bacilli, and Gram-negative cocci were found on the body swabs. Most of the excreta samples had little to no growth. The body swabs had multiple types of microorganisms that were limited to body location. A better understanding of this relatively simple host-microbe interaction can provide an understanding of the factors affecting these interactions allowing us to then understand more complex interactions such as those found in humans.

## Introduction

In recent years, the symbiotic relationships between humans and microbes have become an area of focus for researchers (Li et al. 2008). With this growing interest on the microbiome, researchers have decided to focus on identifying members of the microbe community in hosts, to obtain insight into the ecological and evolutionary host-microbiota interactions in nature (Chow et al. 2010). The identification of microbial members in a host can lead to an understanding of the complex host-microbiota interactions which can eventually lead to personalized healthcare and to new targets for drug development for numerous systemic infections in humans (Kinross et al. 2011).

In this paper, microbiome is defined as the vast collection of aggregated symbiotic microorganisms harbored internally and externally by a host (Kinross et al. 2011). Numerous studies have suggested that the microbiome especially that found in the gut, has been the culprit for major health issues (DiBaise et al. 2008; Vrieze et al. 2010). Insect models in microbiome studies vary greatly in morphology and physio-chemical properties from humans, but can help researchers by providing answers to basic interactions between hosts and their microbial symbionts (Engel and Moran 2013).

Charroux and Royet (2012) studied Drosophila, a widely-used model for the study of developmental diseases, to determine advantages of gut microbiota. This led to the discovery that a very specific microorganism had a role in maintaining intestinal homeostasis. Researchers found that bumblebees' microbiota provided protection against the Trypanosome gut parasite *Crithidia bombi*. Koch and Schmid-Hempel (2011) also found that social contact between bees was necessary for the establishment of the protective microbiota in the gut. Researchers also found that bacterial communities in the gut of closely related species of the genus *Nasonia* assisted in the speciation and evolution of this genus (Flintoft 2013). Studies such as these and many more have given researchers a better understanding of these relationships (Potrikus and Breznak 1981; Dillon et al. 2000).

In addition to bees and wasps, spiders have also been studied to determine behavior and immune function as a result of infection with bacteria. (Gilbert et al. 2016; Keiser et al. 2016). However, little to no research has been conducted focusing solely on the microbiome of the spider. A few studies have been completed on spider venom and its components

including lycotoxins. (Yan and Adams 1998; Kuhn-Nentwig et al. 2002). Assays by Yan and Adams (1998) demonstrated some pore-forming activity against bacterial and yeast cell membranes that potentially makes these proteins from the venom of spiders antimicrobial in nature. Due to the antimicrobial potential of venom, researchers were curious to determine if spiders carry bacteria near their fangs.

*Rabidosa rabida* is a large wolf spider found across eastern North America that prefers tall grass and low vegetation (Brady and McKinley 1994). Spiders, like *R. rabida*, have a complete and relatively simple gut (Foelix 1996). *R. rabida*'s uses extra-oral digestion, where digestive fluid is expelled onto the prey and the liquefied contents are suctioned with a muscular pump called the sucking stomach initiating the catabolism of food. (Zibaee et al. 2012). Food remnants are then held in a pocket lined with cuticle before secretion occurs (Foelix 1996). In this study, we analyzed, for the first time, some of the microorganisms living on and in *R. rabida* with the use of standard microbiology methods. We hypothesized that there would be no microbial growth due to the antimicrobial properties of various spider body fluids.

## Methods

Adult or nearly mature *R. rabida* were taken from tall grasses and low vegetation along the biking trail North of Berry Hill Park (35.261, -91.719) in Searcy, White County Arkansas after dark. The spiders were collected beginning in late June through early July of 2016. Maturation generally occurs in late July and August. The spotlight technique described by Wallace (1937) was used to locate and collect spiders. Captured spiders were immediately placed in sterilized collecting tubes and taken to the lab. In our first trial, excreta was collected using UV-sterilized plastic bags. Thirty spiders were placed in plastic bags where they were rearranged so that posterior end of abdomen faced an uncontaminated or sterile surface of the bag. Spiders were kept in that position until they excreted contents. Excreta was collected immediately to prevent contamination due to spider movement. Spiders (N=7) that did not excrete were excluded from this study. The plastic around the excreta sample was cut enough for the inoculation loop to reach the sample to prevent contamination.

In our second trial autoclaved microcentrifuge tubes were placed on the posterior end of the abdomen of 30 spiders until excretion occurred. Spiders were taped to a sterile surface. Microcentrifuge tubes were placed so that the excreta could be collected making sure that the spider's exoskeleton did not come in contact with the sample. The excreta were transferred onto tryptic soy agar (TSA) using a sterile inoculating loop via aseptic technique. Plates were incubated for 48 hours at 25ºC. The 25ºC incubator was used because in preliminary experiments fungi growth occurred at higher temperatures within hours, before analysis of bacteria could be performed. After this time, the plates were checked for growth and recorded. Each morphologically different colony was plated separately by streak plate method and incubated for another 48 hours. Gram stains, coagulase, and catalase tests were performed on pure cultures.

Sterile cotton swabs, moistened with sterile water were used to collect samples from the body surface of 3 spiders at five different locations. The body swab samples were transferred into tryptic soy broth (TSB) and incubated at 25ºC for 48 hours. Colonies were then transferred onto TSA plates and the broth was retained as stock culture. The pedipalps, prosoma, also known as the anterior body segment, abdomen, feet and rear, or posterior end of the abdomen around the anus and spinnerets were sampled (Figure 1). The body swab samples were kept in TSB incubated at 25ºC for 48 hours. Cultures were then transferred onto plates and the TSB was retained as a stock culture.

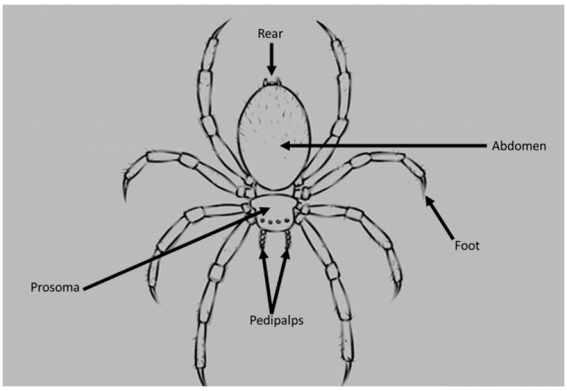


Figure 1. Location of Body swab samples from spider body (drawing adapted from lightofunity.us)

The fresh cultures from both the excreta and body swabs samples were stained with crystal violet to determine morphology and Gram stained to determine cell wall structure. Depending on their stain results, differential biochemical tests were performed. The Gram-positive cocci were tested for catalase and the analysis was recorded. The catalase positive cultures were then tested for coagulase. Bacteria were tested for

staphylocoagulase using a latex agglutination test specific for *S. aureus* surface proteins, a technique used in the further identification from a *Staphylococcus spp*. to *S. aureus* (Idelevich et al. 2014). The data was graphed according to the number of spiders showing each microbial type. Due to time constraints, the gram negative cocci bacteria were not further analyzed. Spiders that did not have bacterial colonies were also included. Data were graphed to show prevalence and variation of individual bacterial types within the spider population. In total 53 excreta samples were collected from both trials.

## Results

Of the 53 total excreta samples, 40 showed no growth, 5 grew a single Gram-negative bacillus, 6 grew *Staphylococcus* sp. and 2 grew *Staphylococcus aureus* (Figure 2). Only one bacteria type was found from each sample.

Body swabs taken from 3 spider bodies made up 15 samples in total. The prosoma, abdomen and feet of each spider grew Gram-positive bacilli. Samples from the posterior end of the abdomen grew *Staphylococcus sp*. The samples from the pedipalps showed no bacterial growth. Fungal spores were found in all body swabs, but were not identified during this study.

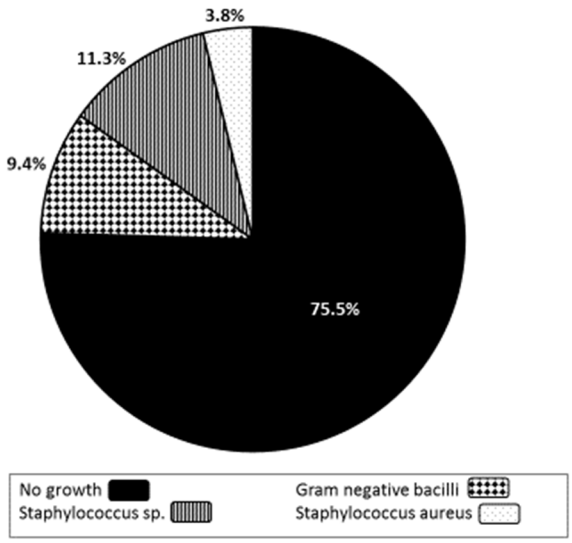


Figure 2. Comparison of the number of microorganism types that grew from the excreta samples from *R. rabida*

## Discussion

Microbial growth was observed from the excreta of the spiders leading us to reject our hypothesis that there would be no microbial organisms in the excreta. Researchers were concerned with potential contamination of excreta samples collected from the inside of the bag the spiders were placed in. We attempted to collect only samples from excreta droplets located in uncontaminated areas. Spiders excrete forcefully and the excreta droplets could be collected further away from the spiders (Seitz 1987).

Excreta samples from both trials did not show significance so the samples were grouped together. The majority of the excreta samples did not grow any observable microbes and in those that had microbes present only a single type of bacterium was identified per sample. We hypothesize that the number of microbes present may be affected by the antimicrobial properties of venom (Budnik et al. 2004), digestive fluid and other body fluids.

In contrast to the excreta, the majority of the body swab samples, except the pedipalps, grew one or more organisms. The pedipalps, located in close proximity to the mouth, may be in contact with venom which is proposed to contain antimicrobial proteins (Yan and Adams 1998). In future tests, we plan to look at the effect of venom and other body fluids on the survival and growth of microbes.

Due to time constraints, we were not able to identify the majority of the microbes to species level. However, we did identify *Staphylococcus aureus*, which was found only in the excreta. *S. aureus* is a firmicute bacterium commonly found in the environment known to cause staph infection in humans (Foster 1996). More research is needed to see if this spider is a carrier for this potential pathogen.

*S. aureus* was only cultured from the excreta samples. The prosoma, abdomen and feet of each spider grew Gram-positive bacilli, while samples from the posterior end of the abdomen grew *Staphylococcus sp*. Pedipalp samples showed no bacterial growth. These differences show a spatial ecology that should be explored. This is not an exhaustive look of the microorganisms found in and on the spider. Different types of culture media with different pH levels, oxygen levels and nutrient content could be used to obtain a better understanding of the scope of the spider microbial community. In addition, researchers plan to obtain 16S rRNA sequencing to identify the bacteria living within and on the wolf spiders. Identifying the bacteria to species level will aid in determining if spiders are carriers of potential pathogenic bacteria as well as providing information related to spider habitat and movement patterns. With a better understanding of this relatively unknown spider-bacterial relationship, we can

better understand behavioral and social effects bacterial communities have on their host organisms. From symbiotic relationships to harmful parasitic relationships, bacteria may control more aspects of spider physiology and behavior, (Gilbert et al. 2016; Keiser et al. 2016), than is currently realized and thus allow us to broaden our understanding of more complex bacterial-host relationships.

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