

## ECOLOGY OF MICROBES PRESENT IN THE ARTHROPODS ALIMENTARY CANALS

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Microbial ecology within animals is a developing field of study. Researchers have primarily focused their studies on the large microbial communities found in the digestive tract of ruminants. Many of the bacteria they isolate are able to ferment sugars and degrade cellulose. Arthropods are another species believed to host large populations of bacteria and benefit from their nutritional capabilities. In this experiment, we liberated bacteria from the alimentary canals of *Periplaneta americana*, *Acheta domesticus*, and *Zophobus morio* using an EDTA solution (0.04mM) and sonication. CfU counts were greater than  $5.0 \times 10^5$  for all three arthropods. Bacteria were isolated, purified, and cultured. From the cultures, PR broths, lactate broths and cellulose broths plates were inoculated to test each bacteria's ability to ferment lactose, reduce lactate and degrade cellulose. All bacteria participated in either the fermentation of lactose or the reduction of lactate. The cellulose tests were inconclusive.

**Keywords :** *Acheta domesticus*; Arthropods; *Periplaneta americana*; Ruminants; Sonication; *Zophobus morio*.

### Introduction

Microbes play a crucial role in many ecological systems. Examples are the symbiotic relationship between fungi and the root systems of plants, degradation of organics by bacteria in natural waters and the digestion of cellulose by bacteria in the alimentary canals of animals. Alimentary canals provide a segregated environment for the study of microbial ecology<sup>1</sup>.

By determining the ratio of cfu/biomass and the characteristics of these bacteria, an understanding of the degree to which bacteria play a part in digestion may be gained<sup>2</sup>. Studies of alimentary tract ecology have been performed most often using ruminants. Many of the isolated bacteria are believed to assist ruminants with the degradation of cellulose and participate in nutritional pathways such as glycolysis. Bacteria aid in breaking down cellulose into simple sugars (6C). Simple sugars from cellulose degradation and other sources may then be fermented, and their by products reduced. Lactose, for example, is fermented into lactate. Lactate is then reduced to lactic acid, pyruvic acid and ethanol. The host may then use these short chain end products for nutrition<sup>3</sup>.

The nutritional benefits seem especially crucial in the development of juveniles. Mucosal development in the rumen of calves is stimulated by the availability of

fatty acids, such as butyric, propionic and lactic acid produced by bacteria<sup>3</sup>. The strength and density of lamb's rumen musculature has also been shown to be associated with the amount of bacteria residing in the rumen.

Significant numbers of microorganisms have been isolated from arthropods. The high ratio of microbes to biomass leads to theories that bacteria are also aiding arthropods in digestion<sup>4</sup>. The bacteria's attachment to the lining of the alimentary tract further supports this assumption<sup>5</sup>.

Extensive 'normal flora' inhibits the alimentary canals of arthropods. One hundred species of bacteria have been identified as normal flora of cockroach alimentary canals<sup>1</sup>. Twenty five species, many members of the genera *Citrobacter*, *Klebsiella* and *Yersinia*, have been isolated from crickets<sup>5</sup>. Microbes have also been found in desert millipedes and scarab beetles<sup>4</sup>. Despite similar nutrition between many organisms, the diversity and density of bacteria is quite unique to every species<sup>5</sup>.

Arthropods are believed to utilize enzymes produced by microbes. Cellulose, produced by arthropods and bacteria, breaks down cellulose found in plants they consume. In the species, *Tibula*, researchers estimate 20-25% of nutritional uptake is derived from the enzymatic activity of microbes. Products of microbial fermentation,

Table 1. Result of the glycolytic tests.

Table 1:

**The Glycolytic Pathway**  
Lactose → Lactate →  
Pyruvate/Acetic/Ethanol

v	Cockroach		
	- Species 1	no	<input type="checkbox"/>
	- Species 2	proteolytic	<input type="checkbox"/>
	- Species 3	<input type="checkbox"/>	<input type="checkbox"/>
	- Species 4	<input type="checkbox"/>	<input type="checkbox"/>
	- Species 5	<input type="checkbox"/>	<input type="checkbox"/>
v	Mealworm		
	- Species 6	<input type="checkbox"/>	<input type="checkbox"/>
	- Species 7	<input type="checkbox"/>	<input type="checkbox"/>
v	Cricket		
	- Species 8	<input type="checkbox"/>	<input type="checkbox"/>
	- Species 9	proteolytic	<input type="checkbox"/>

such as acetate and lactate, may supply arthropods with yet another energy source.

Similar to ruminants, increased nutrition appears to be most benefited during the juvenile stage of development. The administration of the drug metronidazole, which kills obligate anaerobes, resulted in the stunted growth of cockroach nymphs<sup>3</sup>.

Analyzing the diversity and total populations of bacteria found within the digestive systems of arthropods is often difficult. Bacteria adhere either to the epithelium or the dense mucous layer of the gut<sup>6</sup>.

Our hypothesis is that bacteria will be isolated from the digestive canals of each arthropod species. Equal populations, however, will not be found, and the diversity of isolated microbes will be limited. Growth will be observed in some but not all of the cellulose, lactate and acetate tests.

*American cockroach (Periplaneta americana)* (Fig. 1): A large species of cockroach winged, and growing to a length of 1" to 1½" (2.5 cm to 4 cm). It is very common in the southern United States, and in tropical climates, and can be found in many locations throughout the world, due to its travels *via* shipping and commerce between locations. In the southern U.S., it is often called a Palmetto Bug or a Waterbug. Sightings have been reported in the northeast U.S., such as in New York City, and in southeast Canada, such as in Montreal, where it is mostly found near human habitations due to its lack of cold tolerance. The American cockroach can also be found near various ports throughout the world. They are the largest species of common cockroach.

The insect is believed to have originated in Africa,

but had become established in the southern U.S. by the time that it was given its name.

The insect can travel quickly, often darting out of sight when someone enters a room, and can fit into small cracks and under doors despite its fairly large size. It is known to be very mobile, and it also has wings which allow it to be quite a capable flier.

The insect is often considered a pest since it invades living quarters for sanctuary and food.

*Acheta domesticus* (House cricket) (Fig. 2.): House crickets are closely related to the Grasshoppers and locusts, and like them they have the hind legs which are modified for jumping (In crickets, the tympanum (ear) is located on the tibia of the hind legs). The adults are about 2 cm long, and pale brown with a black pattern on the head and thorax. They have two pairs of wings of which only the back pair are for flying. At one time house crickets were associated with bakeries, but this is no longer so. Nowadays they are more likely to be found in warm ducts and in paneling behind heating installations, quite frequently in breweries. Another way that house crickets get into domestic premises is when the occupants keep exotic pets such as tarantulas and lizards. These types of pets have to be fed live food and the poor old house cricket is the answer, however, if the owner isn't careful the live foods tend to make a getaway.....and disappear over the horizon, not really, they usually get under the floorboards and drive the occupants mad with their chirping. House crickets often occur in new buildings and this is probably because such places provide good shelter and food, and half finished houses are easy to enter. It is also possible that these insects may, in some cases, be brought in with the building materials or packaging.

In northern Europe house crickets do not normally survive outside during the winter and most of them come indoors at this time. However, they can survive throughout the year, and will sometimes multiply in enormous numbers on refuse tips where decomposing waste is producing quite high amounts of heat. The abdomen of female crickets ends in a long narrow structure, the ovipositor, which allows them to lay eggs in the ground.

House crickets take two to three months to complete their life cycle when reared at 80 to 90° F. Eggs are deposited in whatever damp substrate is provided- for example, sand or peat moss. Juveniles resemble the adults except for being smaller and wingless. Each female will lay between 50 to 100 eggs that hatch in about two to three weeks (Incomplete metamorphism). Newly hatched nymphs are of the same size as the eggs, and blend in with



Fig. 1. *Periplaneta americana*



Fig. 3. *Zophobus morio*

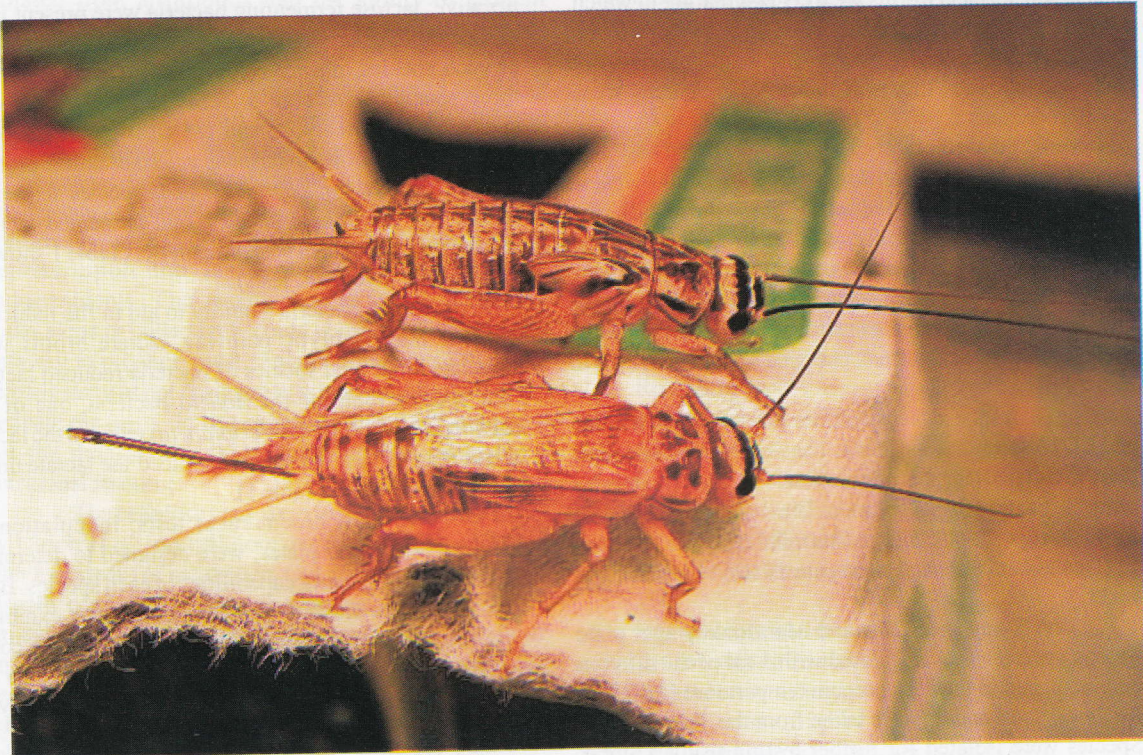


Fig.2. *Acheta domesticus*

their surroundings. Adult crickets will eat their own young (cannibalism). Also, it is normal for some adults to die naturally after mating. The remaining eggs will continue to hatch for 10 to 15 days. As is the case with other orthopterans, when crickets first hatch they already look much like adults (nymphs), except that their wings and genital organs are not yet developed. It takes these tiny crickets eight to twelve weeks to reach full maturity. Adult crickets generally live two to three months.

Crickets need warm temperatures of at least 80°F. Nymphs held at 80°F require up to 60 to 65 days to mature, while those held at 90°F require only 30 to 35 days to complete development.

Crickets feed on almost any kind of organic matter. They prefer soft plant matter, but will also eat other insects and carrion.

*Zophobus morio* (Superworms) (Fig. 3): Commonly known as Superworms, are the hard-bodied larvae of the darling beetle. Easy to pupate and very inexpensive to buy and keep, superworms make a great simple study for students. Larvae do not spin but simply shed their hard casing, only to pupate, sit and wait for beetle development. Superworms are truly fascinating to watch. Unlike hornworms, silkworms and butterworms, superworms do not possess the same nutritional value. Nonetheless; they still make great crunchy treats for most animals.

#### Materials and Methods

*Periplaneta americana*, *Zophobas morio* and *Acheta domesticus* were sacrificed using chloroform kill jars, sterilized with 70% ethanol and rinsed in sterile deionized water. Using dissection probes, the digestive canals were exposed for removal by separating the head from the abdomen. The digestive canals from ten individuals of each species were measured, chopped up and collected in test tubes.

To release all bacteria from the gut, 9ml of a saline and EDTA solution was added. The digestive canals were then incubated for 15 minutes at 4°C and treated by ultrasonication for 45 seconds. The solution was shaken for 30 sec using a vortex mixer. One milliliter of the sonicated EDTA and guts was used to prepare a 1:10,000 dilution in water.

A T-soy agar plate and MacConkey agar plate for each species was inoculated with 1 ml of the dilution and incubated in the dark at room temperature (22-25°C) for 24 hr. Total counts were taken from the T-soy plates. Species were isolated for further testing using a streak plate inoculated with each 1:10,000 dilution. For each isolated species a pure culture was obtained using an

agar slant. T-soy broths were then inoculated. The broths were used for the inoculation of phenol red broths, lactate broths, cellulose broths and cellulose enriched plates. The cellulose enriched plates were created by overlying T-soy agar with a small piece of filter paper. All plates and broths were inoculated and incubated at room temperature (22-25°C) in the dark for 24-48 hr.

#### Results and Discussion

Bacteria were isolated from all three species. On the *Periplaneta* T-soy plate, approximately ten species of bacteria grew, and five were isolated. They differed in color and appearance; an upraised white species, upraised yellow species, a large mucosal species, an orange species and a red species were isolated. The total cfu count was  $\sim 2.8 \times 10^6$ . Only two species grew on the *Zophobas* T-soy plate. Both were mucosal in appearance; one had a whiter sheen. The total cfu count was  $\sim 6.48 \times 10^5$ . The *Acheta* plate revealed the presence of only two bacterial species as well. Again, the species were mucosal in nature, one having a whiter sheen than the other. The total cfu count on the *Acheta* plate was  $\sim 1.0 \times 10^6$ .

The MacConkey plates indicated that gram negative; lactose fermenting bacteria were present in all of species. To determine the lactose fermenting capabilities of each species, phenol red broths. From *Periplaneta*, three species were able to ferment lactose. A fourth species was proteolytic, and the fifth species showed neither the capability to ferment lactose or utilize proteins for nutrition. In the *Zophobas*, both species fermented lactose. Of the *Acheta* species, one fermented lactose, and the second species was proteolytic. (Table 1 showing result of the glycolytic tests).

To test bacteria's ability to aid in the reduction stage of the glycolytic pathway, lactate broths were inoculated. All nine species of bacteria reduced lactate. Four species (both of the *Acheta* bacteria, one of the *Zophobas*, and one of the *Periplaneta*) reduced the lactate in less than 12 hrs. All species reduced the lactate under 15 hrs of incubation (Table 1).

The final biochemical tests, inoculation of cellulose broths and plates, were designed to determine the cellulose degrading capabilities of each species. Both tests were inconclusive. The cellulose broths exhibited turbidity, but there was cellulose in suspension. We could not visually determine if bacteria were present, so we viewed broth samples microscopically. No bacteria were viewed on the slides.

Bacterial growth was present on the T-soy agar overlaid with filter paper, but visual evidence of filter paper degradation was never seen.

The results of our study confirm the results of other researches. Cazemier<sup>4</sup> and his research team found bacterial counts proportionately equal to the cfu number collected. In both studies, the highest bacterial counts were from the *Acheta* plates, then *Zophobus* and finally, relatively low counts were noted on the *Periplaneta*. Overall, the Cazemier team reported higher numbers, in the range of  $1.0 \times 10^9$ . They, however, used an SEM to view the bacteria within the arthropod alimentary tract. As discussed earlier, bacteria do adhere to the endothelium. While use of the EDTA and sonication in present study helped to liberate bacteria from the lining, this method did not ensure that all bacteria would be released<sup>7</sup>. Our results confirmed the presence of high densities of bacteria. This data helps to support the assumption that bacteria are permanent residents of the alimentary canals.

While scientists discuss the possibility of microbes aiding arthropods nutritionally, virtually no experiments have been conducted to test this theory. Previous assumptions have been based on studies conducted in ruminants<sup>3</sup>. The second goal of our experiment, therefore, was to determine if the bacteria isolated from arthropods had the ability to ferment compounds, reduce the by products of fermentation, or degrade cellulose.

The glycolytic experiments demonstrated the ability of every bacterial species to contribute to the glycolytic pathway. Arthropods and many bacteria have the ability to ferment lactose, but bacteria are primarily responsible for the conversion of lactose to lactate<sup>1</sup>. Animals and presumably arthropods that feed on "incomplete diets" depend upon the metabolic activities of their endosymbionts<sup>5</sup>. Our results showed that each bacteria could alone or collectively could complete the glycolytic pathway to produce short chain end products (Table 1). Experiment was not designed to provide results that would indicate the hosts do use or rely on these end products, but the data proves that bacteria could serve as a nutritional resource.

The cellulose tests were inconclusive. In a study by Ulrich *et al.*, no cellulose degradation was observed after four weeks of incubation. Our plates, which we could visually confirm, had bacteria growing on them, had only ten days of incubation before data had to be collected. If time had not been a limiting factor, we believe cellulose degradation would have been observed. This assumption is based on the previous identification of large numbers of cellulolytic bacteria within the guts of termites<sup>6</sup>. Similarly,

the cellulose broths showed no increase in turbidity, and samples viewed microscopically did not reveal any bacteria.

The success of our study in proving that high densities of microbes, which have nutritionally beneficial metabolic activities, inhabit arthropods leads to many ideas for additional research. Clearly, more experiments to determine cellulose degrading capabilities would be appropriate. Tests could also be run to determine if other sugars or other compounds may be fermented. Treating arthropods with antibiotics during various stages of their life cycle may also provide valuable information. These studies will help to increase our understanding of mutualistic relationships and microbial ecology.

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