FY 2006-2007 Report on Progress

(September 1, 2006 – August 31, 2007)

Texas Imported Fire Ant Research And Management Project

Title of project:

Use of genetically modified bacteria to deliver compounds that reduce fire ant fitness.

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Lay Summary of Major Accomplishments:

Ten bacterial species from the fire ant larval midgut were isolated, cultured and identified to species level using microbiological, biochemical and molecular methods. Those species closely related to other known symbionts of insects have been successfully transformed with a red fluorescent marker, demonstrating the ability to introduce foreign genes into the bacterial genomes. The ultimate goal will be to utilize genetically transformed bacteria to deliver compounds that reduce fire ant fitness.

Transformed bacteria were fed to larvae and were subsequently found in the pupal stage up to seven days after the initial feeding. However, the number of bacteria in the pupae was significantly reduced from those found in the meconia which is extruded from the late stage larvae prior to pupation. Due to the importance of the meconia for egg production in the queens, it will be important to study the role of the bacteria in this tissue. Furthermore, meconia containing the transformed bacteria were placed in an uninfected colony and after a few days transformed bacteria were recovered from the naive larvae. Consequently, bacterial transmission from an infected colony to an uninfected colony can be achieved by the natural behavior of the ants.

Antibiotic treatments of the ant colonies were used to assess the importance of the midgut bacteria to fire ant biology. The results of these antibiotic treatments also support the concept that these bacteria are important elements of nutrition for the fire ant colony. Three replicates of antibiotic treatment indicated that after only a few days post-treatment, the fire ant queens had stopped producing eggs and ultimately, the colony was composed of adult ants only, thus having a dramatic negative impact on their fitness. More replicates will be performed to statistically prove that this result is only caused by the lack of the bacteria.

To determine the location of the midgut bacteria, all fire ant stages were embedded in epoxy resin, thin-sectioned and analyzed by Scanning Electron Microscopy and Transmission Electron Microscopy to identify any specialized structures or tissues containing the bacteria. Preliminary results have shown bacteria in the midgut sac of the larvae, in association with the peritrophic membranes. Further investigation on this topic is required, with increased focus on the salivary glands and meconium.

As a component of understanding the bacterial populations in widespread fire ant colonies, bacterial DNA samples have been collected from multiple GPS marked locations in the southeastern United States and frozen for future analysis of the bacterial diversity. In particular, samples were also collected near the site where fire ants first entered the USA. Culture independent methods of species identification will provide a more complete list of bacteria present in the ant.

This project has resulted in two peer reviewed publications and has been presented at multiple national meetings, resulting in the following award: 1st Place Winner of the Poster Section presented at the 54th Annual Meeting of the Entomological Society of America, Indianapolis, IN 2006; and the 2007 Annual Fire Ant National Meeting in Gainesville, FL. The electron microscopy section of the project contains innovative methodology for the visualization of internal structures and microorganisms living inside the insect body. This component of the work was published in the proceedings and will be presented at the National Meeting of the Microscopy Society of America, Ft. Lauderdale FL in August 2007, where it has been nominated for the MSA Raleigh Miller award.

Technical Description of Progress on Individual Objectives:

Objective 1: Introduce recombinant molecules into the fire ant through the use of genetically modified bacteria.

We have genetically transformed a number of bacterial species that are associated with the fire ant, such that they can be used to deliver recombinant molecules *in vivo*. This has been achieved using both transient expression from a plasmid and also through integration into the genome through the use of a transposable element. While our previous studies utilized a red fluorescent marker gene, the proposed studies will focus on candidate genes that are expected to have a negative impact on fire ant fitness. We will initially utilize genes encoding specific compounds that are commercially available and for which the gene sequence is already known. Consequently the rapid determination of their efficacy by direct application to fire ant colonies will facilitate the cloning of the required recombinant gene. There are also a number of different compounds available for testing, including digestive inhibitors, antibacterial peptides, and signaling molecules.

A number of insect-specific toxins have been identified, as well as toxins that can be delivered in a pro-toxin form, such that they will only be activated in the insect gut, or in response to a particular trigger or environmental stimuli. The application of antibacterial peptides will achieve a reduction in the populations of certain classes of bacteria. As an example, gram-positive bacteria could be used to deliver peptides with activity against gram-negative bacteria to determine if they are essential for fire ant survival. Dr. Coates' laboratory staff is currently working on the selection of those specific genes (toxic genes) before proceeding with their introduction into the bacterial genome and finally into the fire ants.

Objective 2: Determine the role of the midgut bacteria.

Treatments with specific antibiotics will help evaluate the importance of the midgut bacteria for fire ant survival, reproduction, development and behavior. Our previously published work shows that the identified bacteria from the fire ant have a wide range of antibiotic susceptibilities and resistances, such that the application of a particular antibiotic to a fire ant diet will only kill a subset of the microbial population in the fire ant gut. Similar parameters as those used for Objective 1 will help determine the impact of the loss of these bacteria on individual fire ants and the colony as a whole. It is possible that this approach will identify bacterial species that are critical to fire ant survival and thus would be ideal candidates for genetic modification and manipulation to detrimentally impact fire ant populations.

It is also important to determine the precise location of the bacterial colonies within the ant body and their possible association with any specific tissue or structure. In particular, fourth instar larvae are like the food processor plant in the fire ant colony from were the food is distributed to the other members, including the queens. Therefore, their importance in colony nutrition is tremendous. These larvae are known for using extra-oral digestion of proteins assisted by secretions from the salivary glands. A set of enzymes are presumably involved in the process, however nothing is known about the potential role of microorganisms. It is our hypothesis that bacteria associated with the fire ant are also involved in this process. The available genetically marked bacterial strains will be used for the finer scale localizations of bacterial colonization and symbiosis within the fire ant body. This objective can be expanded to include additional bacterial species as they are identified in Objective 3 and genetically transformed with the fluorescent marker gene.

The collection of new colonies has been limited due to the recent heavy rainfalls. Therefore, we still need to collect all the colonies needed for the set up of these experiments, which in particular require healthy colonies that are not infected with the microsporidian, *Thelohania*.

Objective 3: Survey the distribution and diversity of bacterial species in fire ants colonies collected from different locations.

With the potential application of genetically modified bacteria and/or compounds that specifically affect certain bacterial populations, it is critical to have a complete understanding of the population levels and diversity of bacterial species that exist in geographically diverse fire ant colonies. It is possible that alternative bacterial species will be more effective in different regions, compared to those that we have already identified. DNA samples have been collected through out the southeastern United States with corresponding GPS information available for each site. The DNA molecules have been isolated, purified and stored for the subsequent PCR analysis using species-specific primers.

Relevance to Achieving the Overarching Goals of the Texas Imported Fire Ant Research and Management Project:

A group of bacteria living inside the midgut were characterized, identified and genetically transformed. Results yielded two articles published in peer reviewed journals and multiple presentations at national conferences. These advances have led us to a comparable standing with other researchers in the field of genetic manipulation of bacteria in insect pests and disease vectors. The next set of experiments will be performed by using the midgut bacteria to express toxic gene products in the fire ants. The proof of principle experiment will utilize the scorpion toxin to demonstrate that delivery of toxic products that detrimentally affect fire ant fitness can be achieved using genetically modified bacteria. Future gene products will be utilized that are ant specific and fire ant specific which will increase the specificity of this biological control approach and thus reduce non-target effects. Similarly, treatments of fire any colonies with multiple antibiotics will reveal the importance of these microbes to fire ant fitness and may provide an alternative control measure, particularly if the most crucial bacterial species (or symbionts) can be identified. Monitoring the negative effects of toxic gene products (to ants and/or bacteria) on fire ant physiology, behavior, development and reproduction will demonstrate their possible use as an effective control method, leading ultimately leading to field tests.

Manuscripts Published/In Press/Submitted:

- Li, H., Medina, F., Vinson, S.B. & Coates, C.J. 2007. Genetic Transformation of Midgut Bacteria from the Red Imported Fire Ant (*Solenopsis invicta*). Current Microbiology, in press.
- Medina, F., Li, H., Vinson, S.B., Coates, C.J. Bacterial microbiology of the red imported fire ant (*Solenopsis invicta* Büren) midgut. Proceedings for the 2007 Annual Imported Fire Ant Conference, April 23-25, Gainesville, FL.
- Medina, F., Ellis, E. A., Pendleton, M. K., Holzenburg, A., Vinson, S. B., and Coates, C. J. Application of SEM and TEM in microbiological studies of the red imported fire ant (*Solenopsis invicta* Büren) midgut. Proceedings of the Microscopy Society of America, Ft. Lauderdale, FL, August 4-9, 2007.

Invited and Submitted Presentations/Posters Presented at Scientific/Technical Meetings/Conferences:

- Student award 1st Place Winner at the 54th Annual Meeting of the Entomological Society of America. 2006. Indianapolis, IN. Poster.
- 2007 Annual Imported Fire Ant Conference, April 23-25, Gainesville, FL. Oral presentation.
- MSA Raleigh Miller award, National Meeting of the Microscopy Society of America. August 4-9, 2007. Ft. Lauderdale, FL. Oral presentation.

PI Signatures:

C.J. Coates	**8/1/07**
Signature	**Date**
S.B. Vinson	**8/1/07**
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