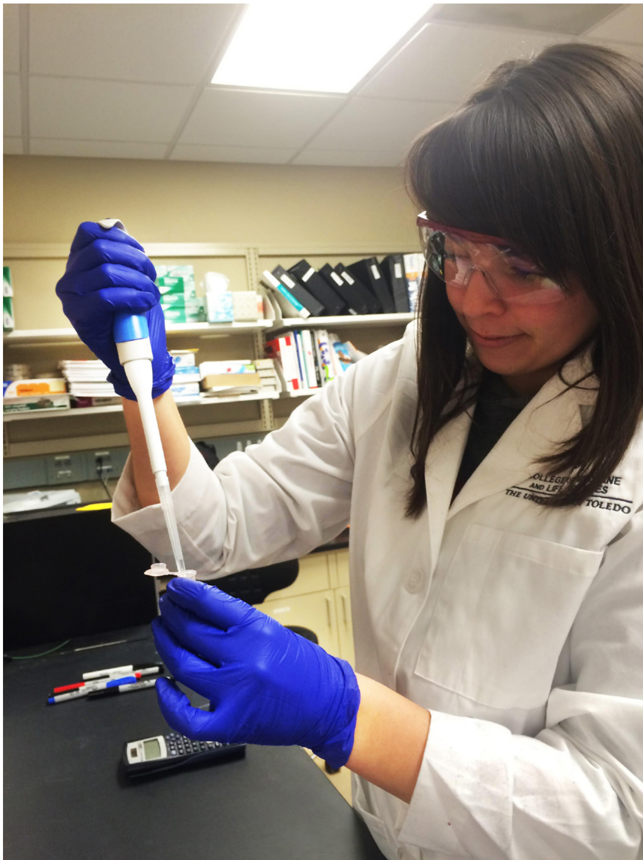
Featured Author: Alison Brandel

Coming into school at Ohio State, I had every intention of getting my bachelor's degree and going to veterinary school. Half way through my junior year, I realized that my childhood dream of being a vet wasn't what I wanted anymore. I was inspired by a professor I had in one of my biology classes to consider a career in research. The lecture that day was on a gut bacteria which may be responsible for causing Crohn's disease, which is near to my heart as two of my best friends suffer from it. After meeting with this professor to discuss what a research career would entail, I began looking into possible labs I could join to begin garnering experience. I chose to apply to Zakee Sabree's lab because his work dealt with gut bacteria (in cockroaches, but I figured some gut bacteria was better than none at all!) I started working in his lab March 2014.



Working in the lab was a good experience for me. I learned lots of techniques that were only glanced upon in my microbiology classes, which enabled me to become much more proficient in a laboratory setting. I also learned that I liked the work, which is important as I was starting a new career path. Being in a laboratory also helped me to better my skills in regards to reading research papers. I learned the expectations that come with working in a lab, and that tests and procedures probably aren't going to work the first time around. Failure is okay, and almost expected at some points.

I graduated from The Ohio State University in the spring of 2015 with a Bachelor of Science in Biology. Presently I'm a first year student in the Master of Science in Biomedical Sciences program at the University of Toledo, studying microbiology. I'm working in the lab of Jason F. Huntley PhD, studying bacteria from Lake Erie and trying to determine if there are natural lake microbes with the capability to degrade the toxin which is released by the blue-green bacteria during the summer algae blooms. The skills I learned during my undergraduate research have been most beneficial in my new lab, and allowed me to easily start working on a new project.

Isolation and characterization of nitrogen-fixing bacteria from American cockroach *Periplaneta americana*

Alison Brandel

ABSTRACT Nitrogen fixation is the mechanism of taking atmospheric nitrogen (N2) and transforming it into ammonium (NH4+). This process is crucial for organisms, especially those which grow in anaerobic environments, as it provides the essential nutrients required to synthesize the basic building blocks of life. Building blocks include such necessities as nucleotides for DNA and RNA, amino acids for proteins, and formation of fatty acids. Converting atmospheric nitrogen to ammonium is done by the nitrogenase enzyme, which is encoded by the nif gene. The aim of this study was to determine whether nitrogen-fixing bacteria were detected in the bacteria residing in the gut of the American cockroach Periplaneta americana. This was done by isolating bacterial cultures from the gut (foregut, midgut, and hindgut) and surrounding fat body. Confirmation of the presence of the nif genes was to be done using Polymerase Chain Reaction (PCR) detection, along with liquid growth culture experiment and acetylene reduction assays. The project did not move past the PCR detection and gel electrophoresis analysis as nif genes were not detected in any of the isolates obtained from the cockroach. This may have been due to the media used, or because the primers were for a different nif gene that was present in the bacterium. Further testing could be done using different primers, or growing them on different media.

INTRODUCTION

Nitrogen (N) is a key component of cellular materials that include nucleic acids, proteins and fatty acids. Lifeforms on Earth must obtain N to survive and they employ a variety of methods to accomplish this task. Given that N comprises nearly 80% of atmospheric air, organisms equipped to take advantage of this largess stand to benefit. Biological fixation of nitrogen (BFN) is known to be the sole province of a few microbial species that employ enzymes called nitrogenases that can cleave highly stable N2 alkynes that comprise N gas (Kästner, 2004). Specifically, nitrogenases catalyze the reduction of atmospheric nitrogen (N2) to ammonia (NH4) and is found only in some members of Bacteria and Archaea; no eukaryotes have been shown to produce these enzymes de novo. Currently, there are three known nitrogenase types: those that contain molybdenum (Mo), vanadium, and those that are heterometal-independent (McGlynn et al., 2012).

The reduction of one mole of N2 by Mo-nitrogenases is energetically-expensive in that it requires 16 moles of ATP, 8 moles of protons, and 8 moles of electrons and yields 2 moles of ammonia, one mole of hydrogen (H2), 16 moles of ADP, and 16 moles of phosphate (Dixon and Kahn 2004). BNF is an anaerobic process as nitrogenases are inactivated by O2 (Wong and Burris 1972). The *nifHDK* operon encodes the structural proteins of the more common Mo-nitrogenase, and its expression is down-regulated by ammonia, a prime product of nitrogen fixation (Hubner et al., 1993).

Nitrogen-fixing bacteria are known to form beneficial symbiotic relationships with a few insects including termites, which share a recent common ancestor with cockroaches (Bell et al., 2007). However, research on nitrogen-fixing bacteria from the gut of cockroaches has not been a large focus of investigation in the past. To date there has only been one nitrogen-fixing bacterium (Klebsiella oxytoca) isolated from cockroach (Periplaneta americana) (Cruden and Markovetz, 1987). This research aims to investigate nitrogen-fixing gut bacteria that live symbiotically with cockroaches and provide essential, assimilable N for their host.

Methods

A cockroach that was fed on a low-nitrogen (LN) diet was dissected to obtain its gut and the surrounding fat body tissue. The gut was separated into three sections (foregut, midgut and hindgut) and each was ground up and diluted in the defined liquid medium NNUA-G, which lacked a fixed source of nitrogen (NH4+). The medium NNUA-G consists of: 0.025% MgSO4 7H2O; 0.0015% CaCl2 2H(2)O; 1 ml/liter trace element solution (Wahlund et al., 1991); 20 mM potassium phosphate, pH 6.7; 1 ml/liter vitamin mixture (Pfennig, 1978); 20 mM D-glucose; 10 mM NaHCO3

; 2 mM Na2S2O3; 0.01% yeast extract . The aforementioned dilution was spread onto the NNUA-G agar plate and anaerobically incubated at 30° C. When colonies were visible, they were patched on the NNUA-G plates and the ANUA-G plates, which contained NH4+ as a fixed source of nitrogen. ANUA-G is composed of the same components as NNUA-G, with the addition of 10 mM NH4Cl. The patched plates were prepared in pairs so that one was incubated under anaerobic conditions, while the other was incubated under aerobic conditions. This step was taken (i) to pre-confirm nitrogen fixing capability of the isolates and (ii) to determine whether the isolates were facultatively or obligately anaerobic. Isolations were carried out on the ANUA-G plates. A colony of each isolate was inoculated in the liquid ANUA-G tube. The fresh culture was isolated and stored in 10% (final concentration) dimethylsulfoxide at -80° C.

Genomic DNA was isolated to be used as a template for 16S rRNA gene amplification by PCR. The PCR products were then run on a 0.8% agarose gel to give definitive proof the RNA was present. When bands were present in the gel between the 1.65 and 2.0 Kb markers the products were then purified, sequenced and bioinformatically analyzed to identify the isolates' taxonomic position. A PCR amplification was also used to look for the presence of the *nif* gene, using the primers described by Pinto- Tomás et al.(2009). The products were also viewed on a 0.8% agarose gel, along with being purified, sequenced and bioinformatically analyzed. To confirm growth in the absence of a fixed nitrogen source, isolates were to be grown under gaseous N2 headspace in the closed tubes. Nitrogenase activity by cells is tested by acetylene (HC=CH) reductions assay using gas chromatography. The detection of ethylene ($H_2C=CH_2$) by the assay indicates the activity of nitrogenase.

Results

Confirmation of 16S rRNA presence

In order to resolve the taxonomic position of the of possible nitrogen-fixing bacteria from the gut of P. americana, cultures were obtained from the four gut segments and isolated into pure colonies. The DNA was isolated and used as template for 16S rRNA. The PCR products were successfully run on the gel, as seen in Figure 1. The purified 16S was then sent in for sequencing. Results indicated that the isolated species belonged to the families of Enterobacteriaceae and Shimwellia, both of which are enteric microbes.

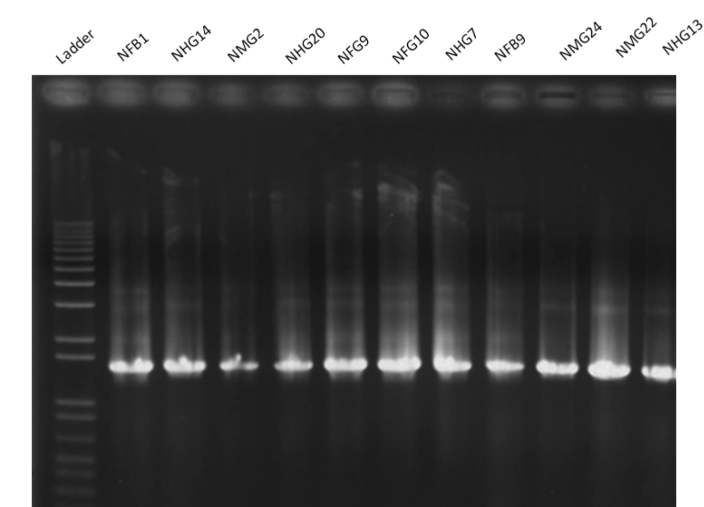


Figure 1: A 0.8% agarose gel on which 16S rRNA from the different isolates were run. PCR products from the fat body (NFB), hindgut (NHG), midgut (NMG) and foregut (NFG) were run and then purified and sent for sequencing in order to be bioinformatically analyzed.

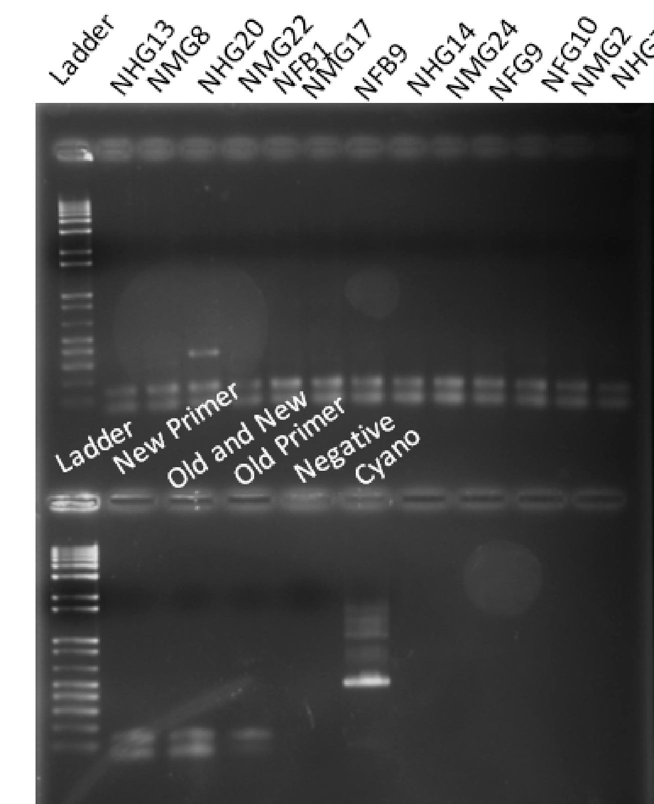


Figure 2: A 1.5% agarose gel on which nif amplicons were run. The NHG20 has a band between the 200-300 base pairs. The Cyano bacterium, which has a nif gene, has a band around 350 base pairs. Water was used as a negative control. Old and New Primers were also included for primer controls

No Detection of *nif* genes

PCR amplification was also done to test for presence of the *nif* gene. Cyanobacterium was used as a positive control as it is known to possess the *nif* gene (Muligan, 1989). One of the samples (NHG20) yielded a band that was similar in size to the *nif* gene, but further analysis via sequencing yielded negative results (Figure 2). Acetylene reduction assays were not performed, as *nif* genes were not found in any of the isolates.

Conclusion

The *nif* gene was not found in the cockroach studied. This does not necessarily mean that a nitrogenase enzyme is not coded for in the insect. There are many other tests that could be done to further this study. Some of these tests include: expanding the number of test subjects, using a growth media that does not contain any traces of nitrogen, and using primers that amplify different *nif* genes. Using a larger range of test subjects would benefit the study as the same bacteria are not going to be found in every cockroach gut. The use of a media that does not contain any traces of nitrogen would be beneficial as it would select only bacteria that can fix nitrogen. Primers that amplify different *nif* genes would also be a possible area to test further, as the bacteria present in the cockroach gut may have coded for a different nitrogenase than the one which was tested.

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