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The Isolation and Identification of Various Bacteria Found in the Gut Contents of Local Crayfish (*Orconectes rusticus*, and *Cambarus bartonii*)

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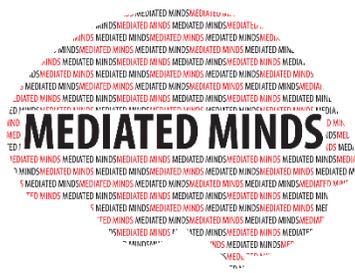
Abstract — *In this presentation, we will report our study and identification of the bacterial intestinal content of crayfish through the process of gram staining, biochemical tests, and genetic sequence analysis.*

I. INTRODUCTION

The major focus of this experiment was to study the bacterial intestinal contents of local crayfish. The goal was to determine the phenotypic characteristics and genetic sequences of the isolated bacterial colonies in order to identify the various species of bacteria in the gut contents of local crayfish. By determining the phenotypic characteristics and genetic sequences of the bacteria, the species and their origins were identified. This provided information about what bacteria live in local waterways in the Blue Ash area, and what bacteria live in the guts of the crayfish in those waterways.

II. FINDINGS

The major findings of this research were the isolation of intestinal bacteria from local crayfish and the identification of the bacteria through microbiological and molecular genetics techniques. When the species were identified and researched, it was discovered that these bacteria had surprising origins from around the world.



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REFERENCES

Skelton, James & Geyer, Kevin & Lennon, Jay & P. Creed, Robert & Brown, Bryan;
Multi-scale ecological filters shape the crayfish microbiome, *Symbiosis*, Volume 72,
Issue 3, 14 December 2016, <https://doi.org/10.1007/s13199-016-0469-9>.

The Isolation and Identification of Various Bacteria Found in the Intestinal Contents of Local Crayfish

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BACKGROUND

- Rusty Crayfish (*Orconectes rusticus*) are a commonly known species of crayfish that are native to the Ohio river.
- Very little has been found about the microbiome of the crayfish. A report in 2016 provided some characterization of the crayfish microbiome, but it did not involve isolation and culturing of individual microbial species (Skelton et al. 2016).
- This research was done to gain more information about the microbiome of the crayfish with the resources that we had available.

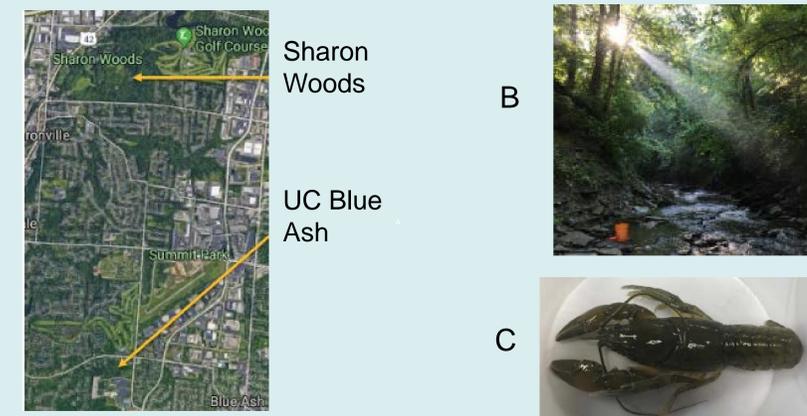


Figure 1. Sample locations (A), typical habitat sampled (B), and a crayfish collected from the UCBA creeks (*Cambarus Bartonii*) (C).

METHODS

- We performed traditional microbiology tests such as gram staining and biochemical tests such as SIM, and various hydrolysis tests to determine the phenotypic characteristics of the bacteria isolated from the guts of both *Cambarus bartonii* and *Orconectes rusticus*.
- After the microbiology tests, the most common cultures found within the crayfish were resuspended, had their DNA isolated using primers that were modified from the universal 16S primers and sequenced.
- The microbes were genetically identified based upon the comparison of known sequences archived in NCBI and the morphological characteristics of the colonies.

RESULTS

- The biochemical tests provided phenotypic data of the isolated bacterial colonies. The results showed whether the colonies were positive or negative for tests analyzing characteristics such as cellulase, amylase, indole, or hydrogen sulfide production, motility, and gram staining. Tests from culture 6A can be observed in the circled areas of the photos (Fig. 2).
- After analyzing the ten sequences from the samples and comparing known morphological characteristics with those that were observed, we were able to identify the species of microbes found in the gut content (Table 1).

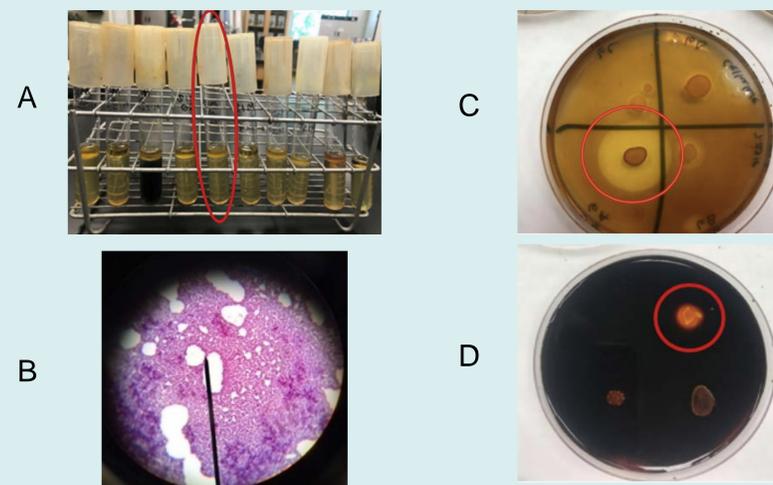


Figure 2. Various SIM tests done on the samples (A), gram stain (B), catalase hydrolysis test (C), and amylase hydrolysis test (D) for culture 6A.

CONCLUSIONS

- The bacteria isolated were identified by comparing the sequenced data to known bacterial strains on NCBI. The isolated culture sequences were matched to the known sequences (Table 1.) and their known origins of isolation.
- These species represent organisms typically found in soil and water, and they originate from around the world.
- Biochemical tests showed that some of these bacteria produce enzymes to aid in the digestion of various materials. Future studies will involve determining whether these microorganisms are permanent residents of the crayfish intestine or if they are transient.

Table 1. The strains of bacteria found in the gut contents of rusty crayfish (*Orconectes rusticus*) and common crayfish (*Cambarus bartonii*) samples. Known origins of the species are listed.

Crayfish Species	Culture Name	Species Identification	Species Origin
<i>Cambarus Bartonii</i>	6A	<i>Exiguobacterium sp.</i>	Han River, South Korea
	6B	<i>Microbacterium aurum</i>	Corn Steep Liquor in Japan
	6C	<i>Shewanella putrefaciens*</i>	
	6D	<i>Pseudomonas fluorescens</i>	
<i>Orconectes Rusticus</i>	RC1A	<i>Bacillus pumilus</i>	
	RC1B	<i>Agromyces salentinus</i>	
	RC1C	<i>Bacillus zhangzhouensis</i>	Zhangzhou, China
	RC1D	<i>Bacillus cecembensis</i>	Pindari glacier of the Indian Himalayas
	RC2A	<i>Aeromonas veronii</i>	
	RC2C	<i>Bacillus idriensis</i>	



Figure 3. Map indicating the various known origins of bacterial species found in the gut contents of locally collected crayfish.

ACKNOWLEDGEMENTS

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REFERENCES

- Skelton, James & Geyer, Kevin & Lennon, Jay & P. Creed, Robert & Brown, Bryan; Multi-scale ecological filters shape the crayfish microbiome, *Symbiosis*, Volume 72, Issue 3, 14 December 2016, <https://doi.org/10.1007/s13199-016-0469-9>.