

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.

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).

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> | |

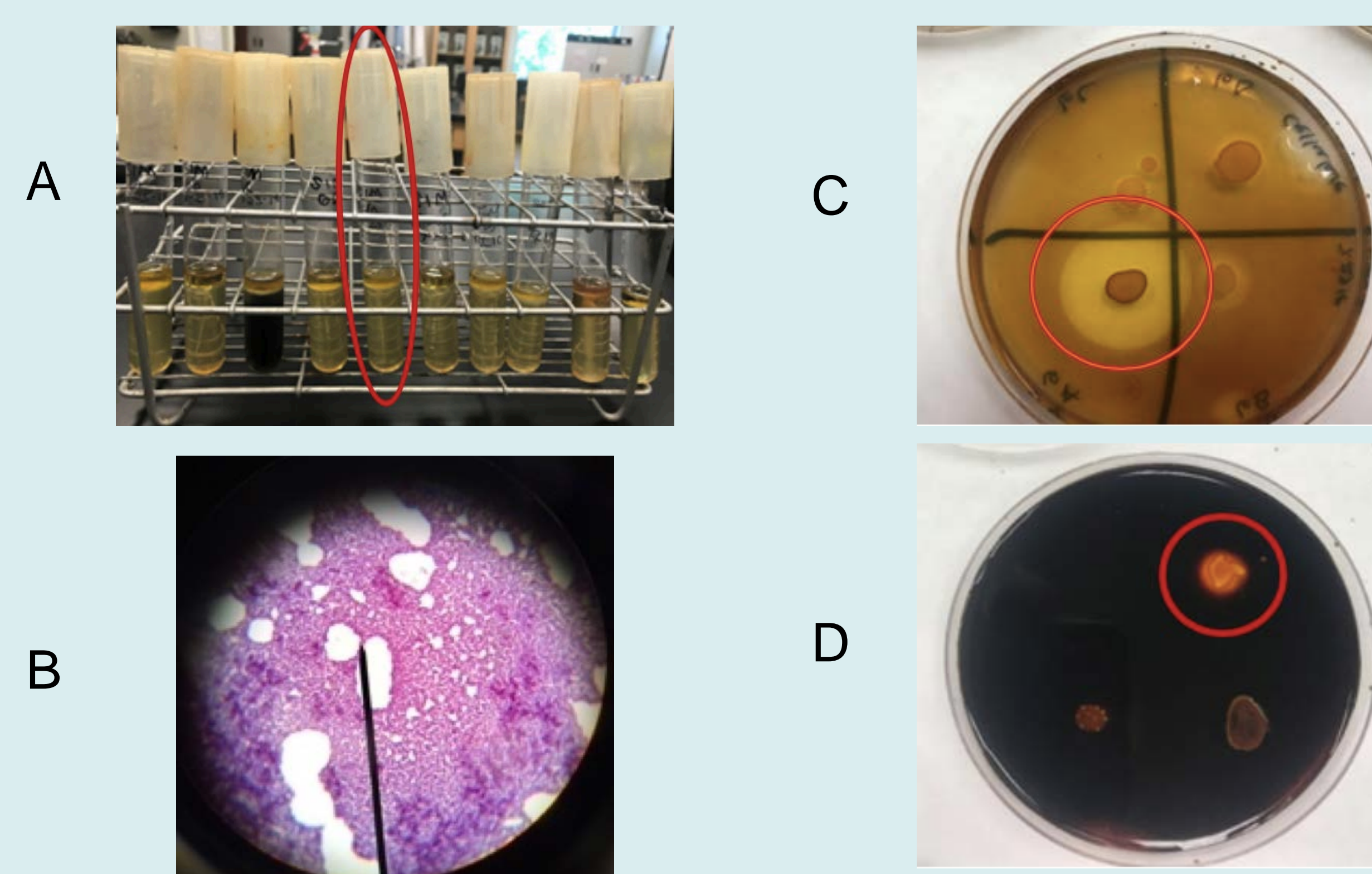
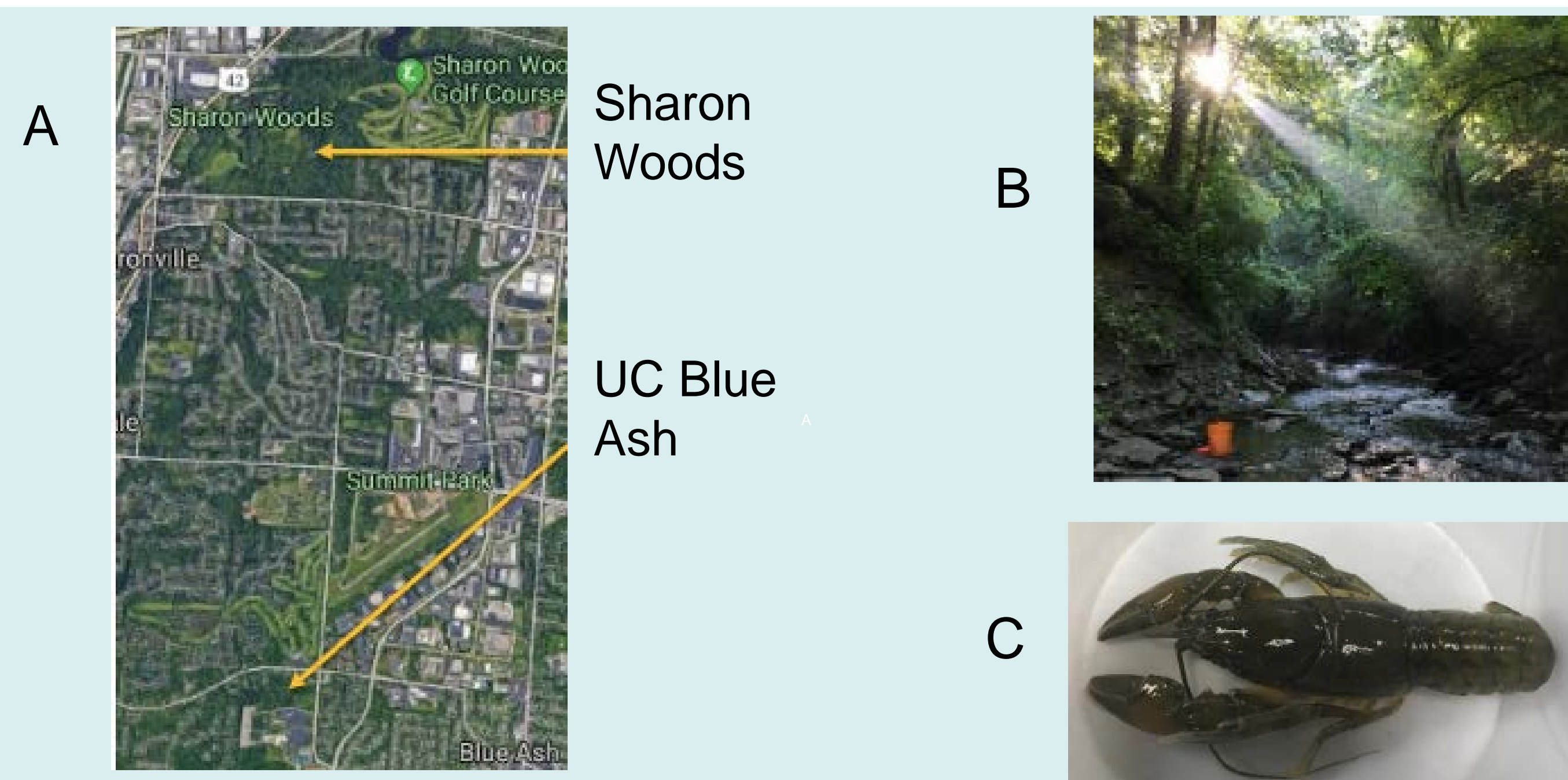


Figure 2. Various SIM tests done on the samples (A), gram stain (B), catalase hydrolysis test (C), and amylase hydrolysis test (D) for culture 6A.

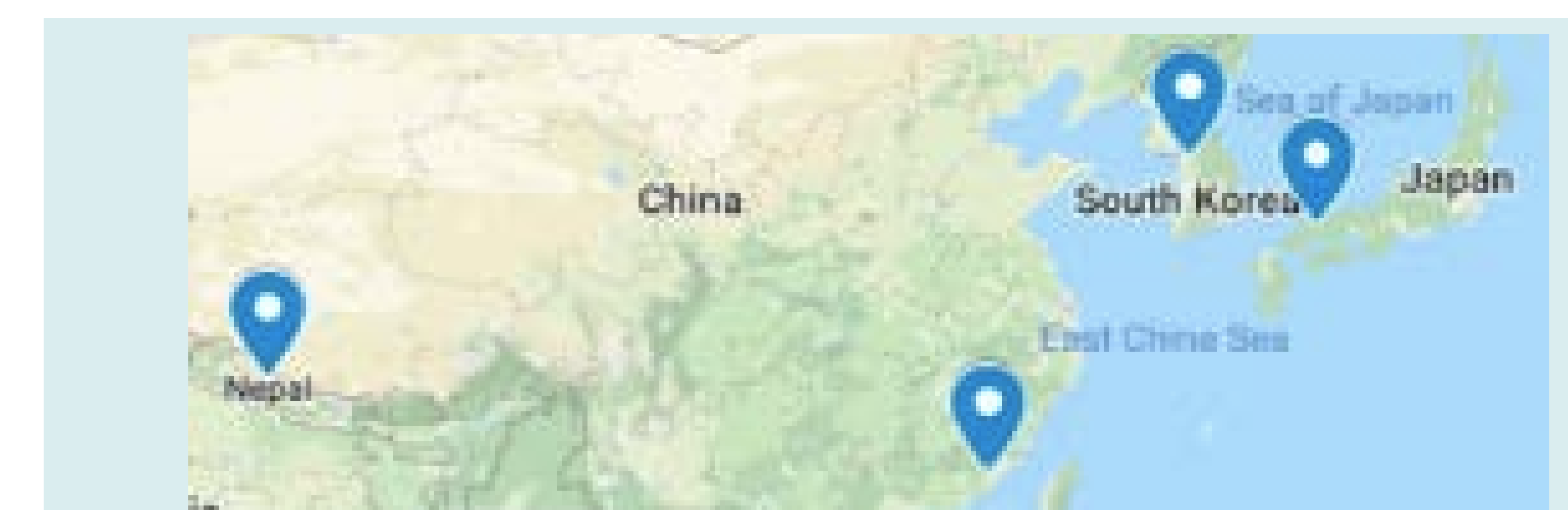


Figure 3. Map indicating the various known origins of bacterial species found in the gut contents of locally collected crayfish.

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.

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.

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>.