Comparing the Bacteria on Captive and Wild Rat Snakes

Blaine Swieder

Tennessee Technological University

Abstract

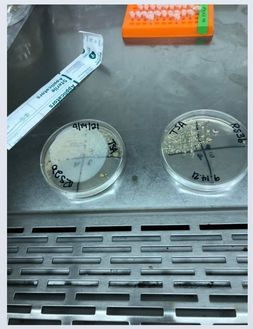
There is a known antifungal bacterium that can be found on the skin of rat snakes, known as *Morganella morganii*, which was found on the skin of wild rat snakes *Pantheropis obsoletus* according to Hill et al. (2018). In this experiment, the goal was to see if rat snakes in captivity share the same bacteria as rat snakes in the wild. I used three different rat snakes in the Tennessee Technological University Herpetology Collection to determine the bacteria. Following gel electrophoresis, successful rounds of bacterial colony PCR were sent to obtain a DNA sequence to determine the species of bacterium found by the microbial identification library. After consulting the microbial identification library, I found the species of bacterium of the three rat snakes sampled from the Tennessee Tech Herpetology Collection. From these findings, we can deduce that rat snakes in captivity may have the possibility to carry the same bacteria as wild rat snakes. With further study, we can see if any other snakes within the Tennessee Technological University Herpetology Collection contain *Morganella morganii* and determine whether these bacteria are harmful or beneficial to captive and wild snakes.

Objectives

- To determine the type of bacteria contained on the skin of the captive rat snakes.
- Use the result of the bacterial colony PCR and Gel Electrophoresis to determine if the bacteria on the Tennessee Tech rat snakes are the same as wild rat snakes.
- Use these results to improve the conditions of all snakes in captivity as pets or for study.

Methods

- At the very beginning of the experiment, I wiped down all equipment with vespine.
- I began my experiment by pouring Tryptic Soy Agar into indicator plates.
- Next, we used sterile water to wash the rat snakes and then swabbed the snakes fifteen times along their left and right flanks.
- Each swab was then placed into a small tube, and then was placed into the freezer.
- I then created the first set of indicator plates called RS1A, RS1B, RS2A, RS2B, RS3A, and RS3B by using the standard microbial streaking technique mentioned in Hill et al. 2018.
- I then let these sit out for a week, and the began an observational period.
- After these indicator plates showed signs of bacterial growth, I created a second set of indicator plates using the same microbial streaking technique (labeled as RS1.A, RS1A.2, etc.)
- I used the secondary isolates RS1A.1, RS3A.2, and RS3B.1 after unsuccessful attempts at PCR using the other secondary isolates.
- In this bacterial colony PCR cocktail, I used the following ingredients: 10 µM 926F internal primer, 10 µM 1492R internal primer, Q5 HiFi 2x MM, sterile, as well as 2.0 µL of diluted PCR product and 23.0 µL of the first set of ingredients to the cocktail into each PCR tube.
- Next, I conducted the bacterial PCR using the program 16s_Baker_INT.
- Then, I used gel electrophoresis to validate the bacterial colony PCR.
- I then sent the PCR Tubes sent off to another lab for the results.



Results

- On the secondary isolate RS3B.1, we found the bacterium named *Psychrobacter ciconiae* which had a NCBI Blast Score of 1092 and had a 99 % identity match.
- On RS3A.2, which had a NCBI Blast Score of 2050 and had a 99 % identity match, we identified the bacterium called *Mammaliicoccus lentus*, which was formerly known as Staphylococcus lentus.
- For the secondary isolate RS1A.1, which had a NCBI Blast Score of 2043 and had a 100 % identity match, we identified the bacterium called *Morganella morganii*. According to Hill et al. 2018, this bacterium is a common symbiont of snakes which has no negative effects upon its host.

Conclusions

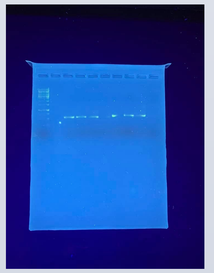
Overall, we can conclude that the *Pantheropis obsoletus* individuals of the Tennessee Technological University Herpetology Collection have the above-mentioned antifungal bacterium. According to Hill et al. 2018, *Morganella morganii* is a common antifungal bacterium on snakes. With further study, we can see if any other snakes within the Tennessee Technological University Herpetology Collection contain *Morganella morganii*. In addition, with further research we can determine how to improve the living conditions in captive environments of all snakes.

References

Hill, Aubree J., et al. “Common Cutaneous Bacteria Isolated from Snakes Inhibit Growth of Ophidiomyces Ophiodiicola.” EcoHealth One Health - Ecology & Health - Public Health Official Journal of EcoHealth Alliance, vol. 15, no. 1, 15 Mar. 2018 https://doi.org/10.1007/s10393-017.

Kämpfer, Peter, et al. “*Psychrobacter ciconiae* Sp. Nov., Isolated from White Storks (*Ciconia ciconia*).” *International Journal of Systematic and Evolutionary Microbiology,* vol. 65, no. 3, 1 Mar. 2015, https://doi.org/10.1099/ijs.0.000013.

Smith, Sierra N., et al. “Venomous Snakes Reveal Ecological and Phylogenetic Factors Influencing Variation in Gut and Oral Microbiomes.” Frontiers in Microbiology, 26 Mar. 2021, https://doi.org/10.3389/fmicb.2021.657754.



Acknowledgements & Contacts

- I give my thanks to both Dr. Panter and Dr. Hill for helping with this research project and their advice on hoe to improve its quality. I should also mention that I appreciate being able to use the rat snakes in the Tennessee Technological University Herpetology Collection.

- bcswieder42@tntech.edu