Multilocus sequence typing of *Pseudomonas* from zebra finch gut is determined to be *P. putida*

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BIOLOGY

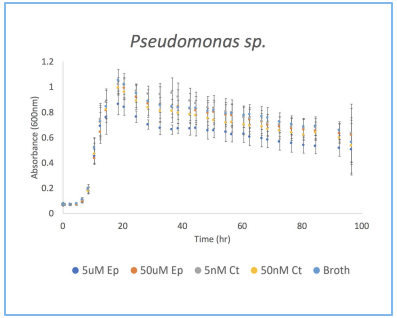
ABSTRACT

The growth of gut bacteria is affected by signaling molecules of a host, especially stress hormones. In previous studies, a bacterium isolated from the zebra finch gut presumptively identified as a *Pseudomonas* showed a decreased growth rate in the presence of epinephrine. As the species of *Pseudomonas* was unknown, a multilocus sequence typing (MLST) scheme was used to identify the specific bacterial strain. Sequencing data classified the bacteria as *Pseudomonas putida*, a gram-negative, rod shaped bacterium. This information provides a more complete understanding of bacteria involved in the communication within the brain-gut axis.

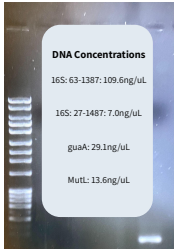
INTRODUCTION

Animal digestive tracts are inhabited by billions of microorganisms, mostly bacteria. These naturally-occurring gut microbes are affected by the changes in environment, in this case the host. Because of this close contact, some bacteria respond to signaling molecules secreted by the host. Zebra finches contain a natural gut flora that interacts with hormones secreted during the stress response to various stimuli. Little is known about this brain-gut axis: growth rates of specific bacterial species when subjected to stress hormones, such as epinephrine, are yet to be studied. In order to better understand these interactions, previous student researchers isolated bacteria from the GI tract of zebra finches. Bacteria of the genus *Pseudomonas* were found to be present within these birds and showed a response to stress hormones epinephrine and corticosterone. To better understand the brain-gut axis at work, it is important to identify the bacteria to species level and to better understand how their individual morphology and biochemical processing may be affected by secreted stress hormones. Determination of species in *Pseudomonas* can be performed using a multilocus sequence typing (MLST) scheme composed of several housekeeping genes from various members of this bacterial genus (Curran et al., 2004). This process includes using several PCR primers for the selected genes within the MLST scheme to determine the genetic makeup of the organisms of interest. A better understanding between the relationship of these native gut bacteria and the secreted signal molecules of the zebra finches may lead to a better understanding of this specific species-species relationship, as well as to serve as a model to further explore this brain-gut axis in other organisms, such as humans.

RESULTS

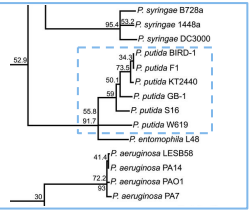


Caption: Most drug treatments were associated with decreased growth rates compared to the control. Growth was inhibited most by the 5uM Ep treatment, with less of an effect by the 50nM Ct condition.



Caption: Gel electrophoresis was completed after PCR analysis to determine if the gene was correctly amplified. The image above shows the molecular weight markers in lane 1 and a successful amplification in lane 6.

Sequence #	Gene	Strain	Percentage Match
3	guaA	putida NX-1	93.59
		putida PC2	93.59
4	guaA	putida PC2	94.24
		putida NX-1	94.11
11	guaA	putida PC2	94.2
		piutida NX-1	94.08
12	guaA	putida PC2	94.41
		putida NX-1	94.28



Caption: Finch TV software and BLAST analysis was used to analyze sequence data sent from McLab. The guaA gene showed the strongest sequencing results. BLAST analysis identified *Pseudomonas putida* as having the highest gene similarity, with ~93-94 percent DNA match. *Pseudomonas* phylogenetic tree image from Duan et al., 2013, March 13, *PLOS One, 8*, pg 11.

METHODS

Pseudomonas sp. was previously isolated from fecal pellets of male and female zebra finches and streaked onto Luria broth plates and incubated at 37°C. Individual colonies were taken from each plate and placed in a 5mL nutrient agar test tube. Test tubes were agitated at 130RPM at 37°C for 24 hours. Each test tube solution was then diluted 1:1000 and followed by 24 hour incubation. Solution of 500nM and 50nM corticosterone were prepared with 100% EtOH, diluted with dH2O, and filter sterilized. Likewise, solutions of 500uM and 50uM epinephrine were prepared with 1N HCL. Using 96-well plates, drug stock solutions and bacteria were mixed in a 1:10 dilution and incubated at 37°C. Absorbance readings were taken over the course of 96 hours at a wavelength of 600nm using a Thermo Fisher Scientific Original Multiskan Ex Microplate Reader and Ascent Software Version 2.6 (Sippel, Peck & Schneider, 2019).

To determine the species of *Pseudomonas* samples, a multilocus sequence typing (MLST) experiment was conducted. The *Pseudomonas* bacteria was cultured in liquid media at 37°C. Chromosomal DNA extraction was performed using lysis buffer and proteinase K and intubated overnight at 55°C*. Spin column genomic extraction was used to isolated the bacteria DNA from other cell contents*. Concentration of extracted DNA was measured with the NanoDrop 1000 Spectrophotometer. The sample was then diluted in nuclease-free water in preparation for polymerase chain reaction (PCR). Nince primer sets designed to identify *Pseudomonas aeruginosa* (16S: 63-1387, 16S: 27-1487, ascA, aroE, guaA, mutL, nuoD, ppsA, trpE) were used to amplify several genes found within most *Pseudomonas sp.* After PCR, agarose gel electrophoresis was used to determine if amplification had occurred. Positive samples were then subjected to spin column DNA purification and NanoDrop quantification*. Samples containing a sufficient DNA concentration were prepared and sent to MCLAB for sequencing. Sequencing data was reviewed with FinchTV software and a BLAST search was performed.

*Protocol was taken from *Qiagen DNeasy Blood & Tissue Kit*

FUTURE DIRECTIONS

As expected, *Pseudomonas sp.* had a decreased growth rate in the presence of stress hormones epinephrine and corticosterone. The pattern of bacterial growth mirrored the results of prior work.

Multilocus sequence typing identified *Pseudomonas putida* as the species extracted from the zebra finch gut. *P. putida* is a gram-negative, rod shaped, saprotrophic soil bacterium.

Attempts to further narrow down the specific substrain could be accomplished. This would require using more specific PCR primers that would allow distinguishing between *P. putida NX-1* and *P. putida PC2.*

REFERENCES

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2. Sippel, H., Peck, H., & Schneider, J. (2019, April). *Differential concentrations of corticosterone and epinephrine on the growth rates of bacteria from zebra finches (Taeniopygia guttata).* Presented at SNC Undergraduate Research Forum, De Pere, WI.

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