



# Analysis of the Zebra Finch Gut Microbiota

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## ABSTRACT

The gut-brain axis refers to the bidirectional communication between the microbes present in the gut and the host's nervous system. Zebra finches are a useful model in the study of the gut-brain axis due to the prevalent interactions between the hormones and the gut bacteria. We have identified some of the bacteria in zebra finch fecal samples in order to select organisms for further analysis as candidates for interactions with the nervous and endocrine systems of the host. Using 16S rRNA gene sequencing, we have identified several bacterial species from two separate groups of birds (one group from Kentucky and one from Illinois). Identified species include *Staphylococcus gallinarum*, *Staphylococcus aureus*, *Pseudomonas oryzihabitans*, *Rothia sp.* and *Bacillus sp.* There were both common and unique species isolated between the two bird populations. These bacterial species may be used in the future to test their growth in the presence of certain hormones. The common bacterial species can also be used as a control in an analysis of the effect of stress on the presence of certain species of gut bacteria.

## INTRODUCTION

The zebra finch (*Taeniopygia guttata*) is widely-used model system in neuroscience and endocrinological studies. Their song-learning abilities and sexual dimorphisms make them especially useful in studies of learning and memory. A significant body of literature examines the effects of hormones on memory and learning in these birds. Like all animals, zebra finches also serve as the host to numerous microorganisms.

Bacteria play vital roles within avian hosts through multiple pathways including the gut-brain axis. Previous studies have shown bacterial pathogens playing significant roles in mortality, while others have displayed importance in nutrition and protection from infection. A growing body of evidence suggests that these gut bacteria may be sensitive to host stress hormones, among other signals. Given the relative ease of manipulating neuroendocrine profiles in the zebra finch, this songbird represents a potentially powerful model system to examine this potential interaction. The first step to developing such a system is the characterization of the gut flora in zebra finch. A recent publication reports a number of bacteria present over much of the zebra finch life cycle (Benskin *et al.*, 2010).

Bacteria are sensitive to molecules that serve as hormones and neurotransmitters in animals. The term "microbial endocrinology" has been used to describe the study of potential interactions between bacteria and host hormones and neurotransmitters (Lyte and Freestone, 2009). Our long-term goal is to assess the effect of specific hormones on gut bacteria, and the effect of specific gut bacteria on endocrine function. This study aims to isolate and identify specific bacteria present in zebra finch guts using molecular techniques. Once bacterial species are identified we aim to determine whether there are bacterial responses to specific stress hormones.

## METHODS

**Sample Collection and Bacterial Isolation:** Fecal pellets were collected into 1.0 mL of sterile distilled water and vortexed for 30 sec, followed by incubation at 37°C for 20-24 hr. Dilutions of 10<sup>-2</sup>, 10<sup>-3</sup> and 10<sup>-4</sup> were plated on nutrient agar and incubated for 24 hr at 37°C. Representative colonies were then streaked for isolation on tryptic soy agar. Unique colonies were collected for PCR, gel electrophoresis, and sequencing.

### PCR and Gel Electrophoresis:

Using the 16s rRNA 63F primer and 1387R primer, a mastermix was made with sterile water, a 10X buffer, free dNTPs, and Taq polymerase (Table 1). A portion of each unique colony was transferred into a PCR tube with 50 µL of the master mix. The PCR tubes were then placed into a thermocycler that used a 16s appropriate program to amplify the DNA. Each sample of DNA was then compared with a 10,000 bp DNA ladder in the gel electrophoresis with 0.7% agarose gel. The successful samples with a band at 1.2 kb were finally prepared for sequencing.

### Sequence Analysis:

Each sample was purified using the Promega PCR Clean-Up System. After multiple rounds of centrifugation, the DNA samples had both a forward and reverse primer added and were sent to the Molecular Cloning Laboratories in San Francisco, California. The forward and reverse sequences were then examined and analyzed using the National Center for Biotechnology Information 16s ribosomal RNA database. The species with the highest sequence similarity was then identified. If no distinction could be made between the species identified, then only the corresponding genus was noted.

Name	Volume	Concentration
63F primer	40 µl	10 µM
1387R primer	40 µl	10 µM
Sterile H2O	350 µl	
10X buffer	50 µl	
dNTP	20 µl	2.5 µM each
Taq	5 µl	

Table 1: Concentrations used for PCR master mix.

## RESULTS

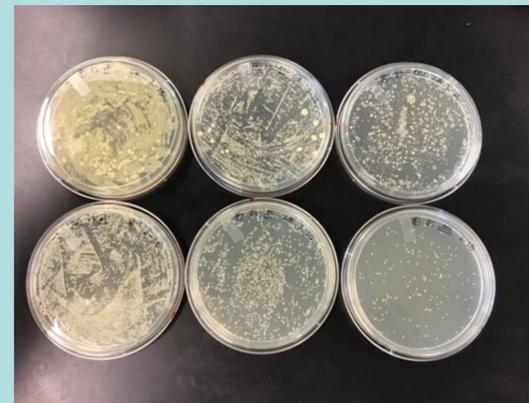


Figure 1: Plates of zebra finch fecal sample dilutions. Note the varied colony types.

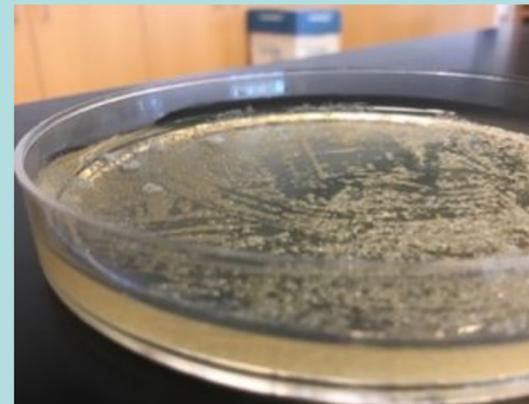


Figure 2: Fecal sample plate with a rhizoid crateriform colony. Later identified as a species belonging to Bacillus genus.

Sequence Length	Species with best similarity	Percent Identity
618	Rothia sp.	97.58%
660	Staphylococcus gallinarum	100.00%
626	Lactococcus garvieae	100.00%
604	Citrobacter freundii	100.00%
668	Enterobacter soli	99.25%
639	Bacillus sp.	100.00%

Table 2: Species identified from sequence in first zebra finch population from Illinois.

Sequence Length	Species with best similarity	Percent Identity
629	Staphylococcus aureus	99.84%
650	Lactococcus garvieae	99.85%
636	Pseudomonas oryzihabitans	99.84%
647	Bacillus sp.	99.85%

Table 3: Species identified from sequence in second zebra finch population from Kentucky.

## CONCLUSIONS

- 1) Zebra Finch gut bacteria are successfully isolated and identified using this technique
- 2) The bacterial species listed in Tables 2 and 3 are present in the gut microbiota of the zebra finch
- 3) *Lactococcus garvieae* and *Bacillus sp.* are common identified species between the two bird populations

## FUTURE PATHS

### Effects of hormones on bacterial species:

This study has provided a list of bacterial species found in the gut microbiota of the zebra finch. This list can be used for further research on the effects of various hormones on the growth of these bacterial species. This could give further insight into the microbial endocrinology and the gut-brain axis of the zebra finch.

### Effects of stress on gut microbiota:

The results of this study can also be used as a control group for the bacterial species present under normal conditions. Therefore, we could introduce certain stressors on the birds to test whether there is a new prevalent bacterial species.

## REFERENCES

- Lyte, M and Freestone, P (2009). Microbial endocrinology comes of age. *Microbe*, 4(4): 169-175.
- Benskin, CM, Rhodes, G, Pickup, RW, Wilson, K, and Hartley, IR (2002) Diversity and temporal stability of bacterial communities in a model passerine bird, the zebra finch. *Molecular Ecology* 19(24):5531-44.

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