



2015-07 Application of Genetic Sequencing for Comprehensive Analysis of the Bacterial Communities Associated with Mercury-Treated Museum Collections | 2015-07

University of Colorado Denver

Unimpacted - behind ears and behind hind legs



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**Narrative Final Report (Attachment C)  
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**Title Page**

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**Institution/Organization:** Regents of the Univ. of Colorado, University of Colorado Denver

**Principal investigator:** Dr. Timberley Roane

**Project team:** Denver Museum of Nature and Science (not originally proposed)

**Principal investigator contact information:**

Department of Integrative Biology  
Campus Box 171  
P.O. Box 173364  
University of Colorado Denver  
Denver, CO 80217-3364

Phone: (303) 556-6592  
Fax: (303) 556-8440  
Email: [Timberley.Roane@ucdenver.edu](mailto:Timberley.Roane@ucdenver.edu)

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### Executive Summary

This study has identified the presence of diverse bacterial communities inhabiting the surface of collections throughout the museum setting regardless of storage, history, subject, and preservation treatment. Equally present on collection items from educational to zoological to anthropological, identified bacteria represent commonly-occurring species, many of which are human associated as would be expected given the handling and storage conditions of many collections. Interestingly, the types of bacteria seem to be heavily influenced by both the presence of arsenic (for arsenic-preserved collections) and the material composition of the item (e.g., feather versus hide). The continued identification and characterization of museum-associated bacteria will lead to an increased understanding of the degradation of artifacts, as well as possible new approaches to the handling and storage of sensitive collections. Finally, this work has provided needed background information on the bacteria already existing on the surface of arsenic-treated materials which may influence the efficiency of a newly proposed, novel bacterial-based remediation technology for the removal of arsenic and mercury from museum collections.

### Introduction

The microbiology of museums is unknown and remains an unexplored habitat for microorganisms. The bacteria, in particular, have long been associated with degradation of artifacts and collections under museum conditions; however, few comprehensive studies, if any, have been conducted to assess the presence of diverse bacterial communities on museum materials. This study proposed the use of an advanced molecular method, high throughput sequencing, to perform a comprehensive analysis of the bacterial communities present on several museum collections.

While the elucidation of the microbiology of museums is novel and will contribute to our understanding of the presence, spread, and potential role in degradation, understanding the bacteria present on collections will also help optimize a newly developed method of mercury and arsenic detoxification of metal-preserved artifacts using metal-resistant bacterial species. In order for this technology to be most efficient, the presence of other potentially competing *in situ* bacteria needs to be addressed.

Based on preliminary analyses of several arsenic-preserved educational collections at the Denver Museum of Nature and Science (DMNS), an initial screening showed the presence of complex bacterial communities on the collection surfaces. The complexity of these communities was surprising and indicated the need for further investigation. This study aimed to explore the composition and diversity of the bacteria present on DMNS collection items.

### Materials and Methods

*In the original proposal, work was to be conducted on mercury-preserved collections from the Arizona State Museum. However, once the grant award was made in the fall of 2013, the PI could no longer travel to AZ due to work-related obligations (e.g., teaching and service). As such, the PI reached out to the Denver Museum of Nature and Science for possible access to their arsenic-treated collections. Access was quickly granted, and so the proposed work began as planned but with the change in collection source (and as a result the influencing metal. DMNS does not have many identified mercury-preserved collections.).*

Working with several department managers at the Denver Museum of Nature and Science, including education, zoology, and anthropology, access to arsenic-treated and non-treated collections were granted. Dampened with sterile 0.1% phosphate buffered saline, sterile pieces of Whatman #2 filter paper were used to wipe a designated collection surface area (approximately 4 sq. in). The paper swatch was then placed into a sterile test tube for transport at -20°C to the PI's laboratory on the University of Colorado Denver campus for analysis. Upon bacterial/DNA collection, items were also tested for arsenic (and quantified where possible using the LaMotte Arsenic Test Kit, Chestertown, MD).

Upon return to the laboratory, bacterial DNA was extracted and purified from the swatches using the MoBio PowerMax Soil DNA Isolation Kit (Carlsbad, CA). DNA extractions were then used to create 16S rDNA libraries which were then used for bacterial DNA sequencing using Illumina high throughput sequencing (performed at the Genomics and Microarray Core Facility on the CU Denver Anschutz Medical Campus). DNA sequences were identified and analyzed using the Ribosomal Database Project, QIIME microbial ecology software, and R statistical software.

### Results and Discussion

This was a complex study addressing the bacterial communities associated with a variety of museum collections. Collections representing different material types, e.g., composed primarily of feathers, hide, fur or textile, representing both arsenic-treated and non-treated materials, and representing items from education, zoology, and anthropology were used in this study. High throughput MiSeq 16S rDNA sequencing was used to genetically identify the bacterial representatives found on the surfaces of these collections. What resulted is a first of its kind comprehensive bacterial analysis of microorganisms in the museum setting. Minimally, this study will contribute to our understanding of the types of bacteria that may be present on museum collections. Future work building on the preliminary findings presented here will increase our understanding of how bacteria may be contributing to degradation processes in the museum and how storage and handling of collections are conducted.

The research here is providing foundational information for several on-going questions.

- Are bacteria broadly distributed on collections throughout the museum setting?  
*Figure 1 shows the bacterial community composition found on 17 collection items from the Denver Museum of Nature and Science. It was evident that bacteria were ubiquitous on collection items.*
- Are the bacteria present on an individual collection item evenly distributed?  
*This data is still being processed. On a subset of collections sampled, two physically separated sample areas were determined, e.g., all bird collections were sampled on the breast and the center of the back between the wings. So far a high degree of consistency is being observed; however, additional sampling is needed to confirm or dispute this early observation.*
- Do the bacteria present on an item differ depending on whether the item has been treated with arsenic or not?  
*It appears that while the presence of arsenic on a collection does influence the types of bacteria present (see Figure 2), it does not prevent the occurrence of bacteria. Further sampling is needed to determine if the types of bacteria selected for by the presence of arsenic is meaningful or not, and remains an open area of research. It was noted that the arsenic concentrations associated with individual collections varied from below detection limits (<1 ppb) to >2000 ppb per area sampled (~4 sq. in).*
- Are the bacteria present on a collection item specific to the item's material type, e.g., feathers on a headdress or a textile in a garment?  
*Approximately 32% of the bacterial differences seen among sampled collections was due to material type (see Figure 3). In other words, the bacteria present on a given item seem to be due to the materials used in the item. Different communities were observed on feather, fur, textile, hide, and other types of collections; however, within a given material type, e.g., feathers, a high degree of similarity was observed ( $p$ -value <0.5).*
- Does a collection's storage and handling influence the bacteria present on its surface?  
*This remains to be determined; however, intriguing patterns are beginning to appear in the data. For example, from the educational and zoological collections sampled, several human-associated bacteria were found, e.g., the bacterial group called Enterobacteriaceae. Enterobacteriaceae are associated with human skin and fecal material, and is now considered ubiquitous in human-impacted environments. While not necessarily highly pathogenic, some members of this group are associated*

with human illnesses such as gastroenteritis. This is perhaps not unexpected given the increased handling by museum visitors these collections experience.

Future work needs to continue with sampling for bacteria on collections throughout the museum in order to determine if any broad-scope conclusions can be made regarding the microbiology of collections. Continuing elucidation of the bacterial data collected in this study will immediately help optimize the PIs proposed bacterial-based arsenic remediation protocol (proposed in a 2006/07 NCPPT grant).

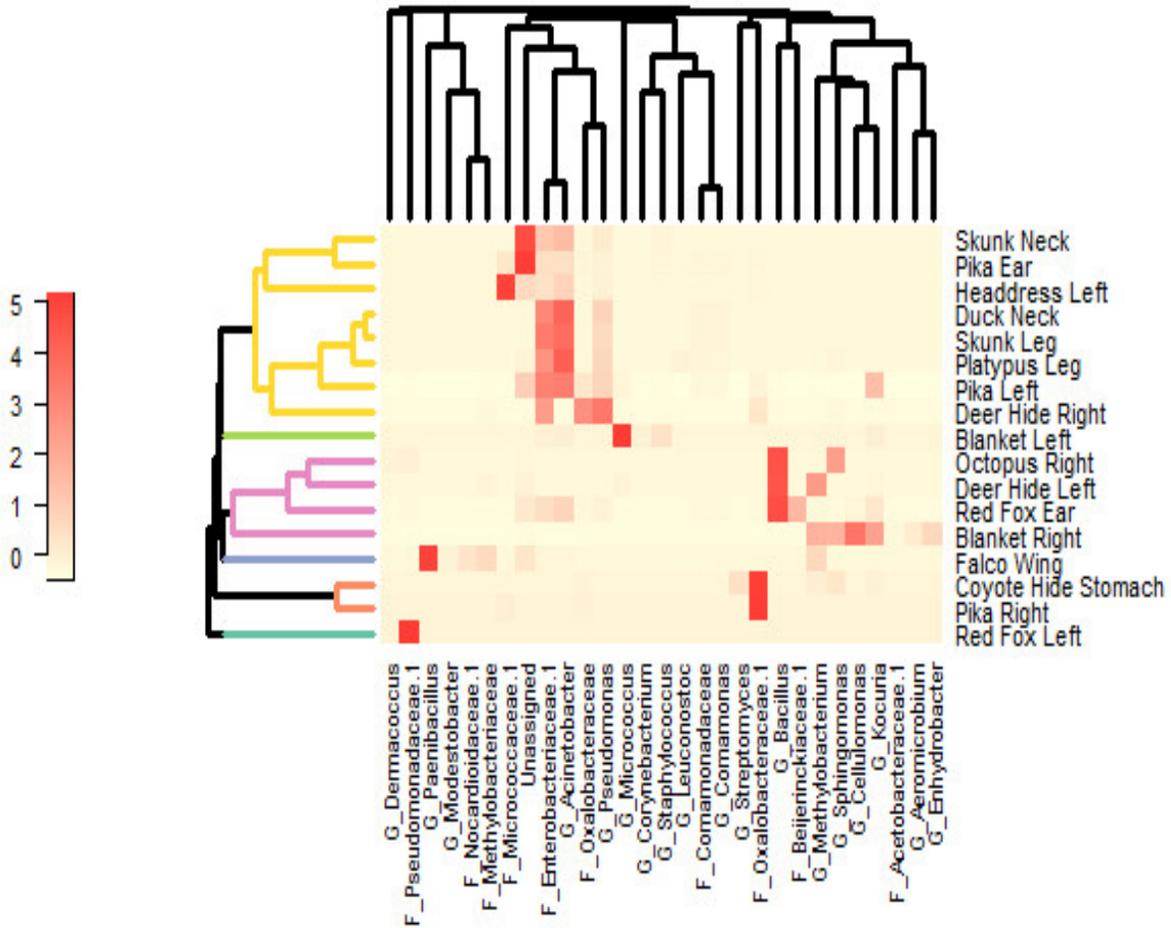


Figure 1. Bacterial diversity of DMNS collections at the family/genus level. The items sampled are listed on the right. Deeper red color indicated a high presence of the corresponding group of bacteria (on the x-axis). Yellow color indicated a decreased presence of the bacterial group.

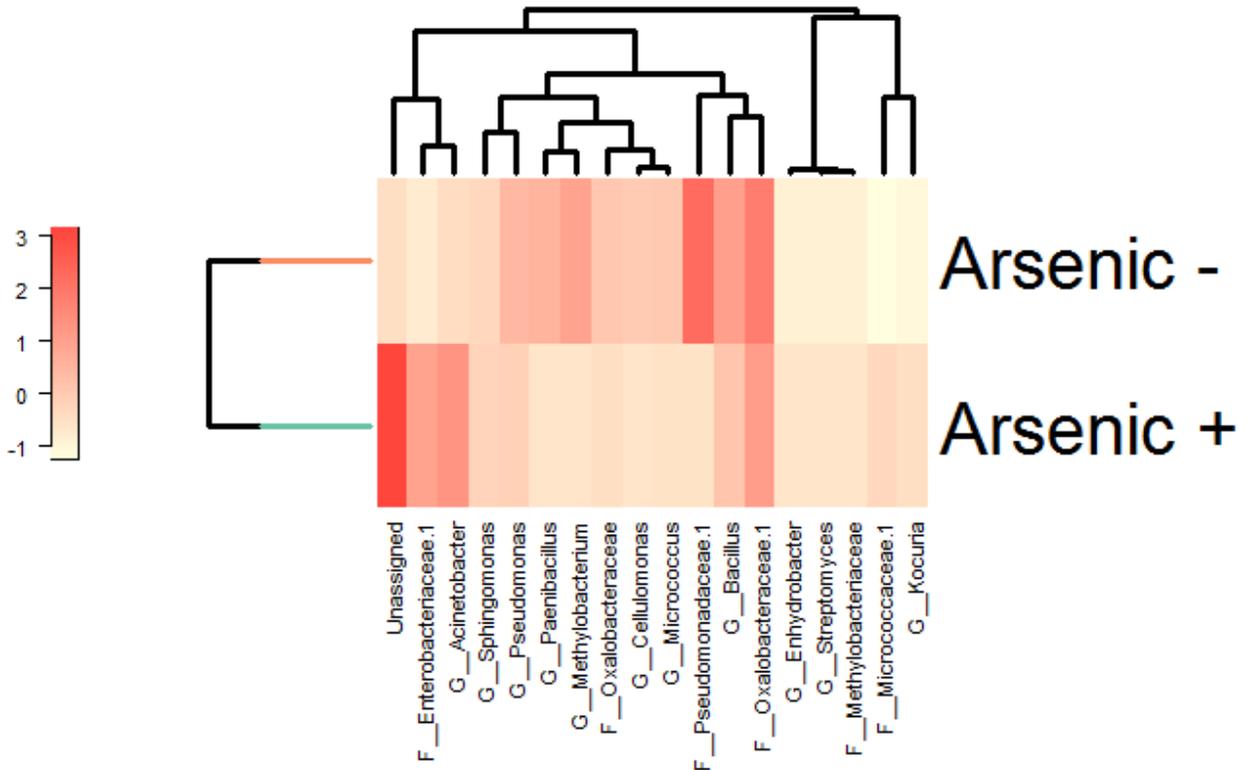


Figure 2. Bacterial diversity of DMNS collections at the family/genus level based on whether the item was previously treated with an arsenic preservative. All items with and without arsenic were grouped together for analysis and are simply indicated as “Arsenic -” (no treatment) and “Arsenic +” (treatment). Deeper red color indicated a high presence of the corresponding group of bacteria (on the x-axis). Yellow color indicated a decreased presence of the bacterial group.

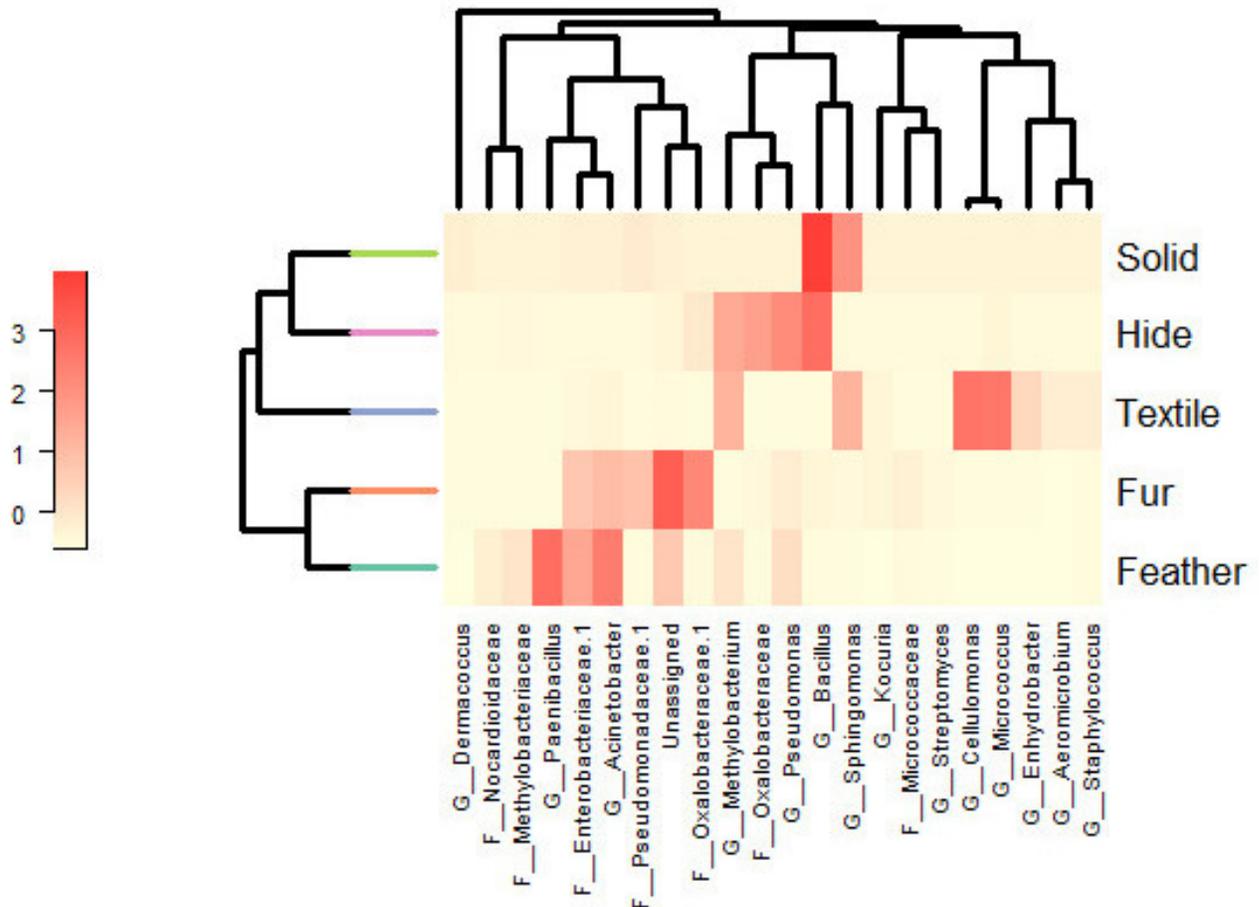


Figure 3. Bacterial diversity of DMNS collections at the family/genus level based on whether the dominant material composition classification, e.g., feather, fur, etc. Deeper red color indicated a high presence of the corresponding group of bacteria (on the x-axis). Yellow color indicated a decreased presence of the bacterial group.

### Conclusions

There appears to be an active bacterial biome in the museum setting. Sources of bacteria are numerous including *in situ* to the collections themselves, handling personnel, museum visitors, and airborne particulates. What information can be predicted from the presence of bacteria on collections regardless of storage, material types, presence/absence of arsenic, and age (as well as other factors not noted) remains to be seen. However, this untapped resource of bacterial data is bound to contain information that is likely to have great influence on how collections are viewed and handled.

Additionally, the presence of an inherent bacterial flora on museum collections, especially those previously treated with arsenic-based pesticides, will have to be a consideration in the use of bacteria in the remediation of arsenic. Since bacteria readily compete with each other, the presence of indigenous bacteria could potentially decrease the efficiency of bacterial arsenic removal. Further testing will need to be conducted to determine the impact the presence of an *in situ* flora will have on bacterial-based arsenic mitigation efforts.

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**References**

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