



Microbial Detoxification of Mercury Contaminated Museum Collections | 2006-03

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**Narrative Final Report (Attachment C)
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Title Page

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Executive Summary

Many cultural museum artifacts were treated with mercury-based pesticides to protect the item from insect and microbial damage. While acting as a long-term preservative, mercury poses serious health risks with exposure. While many mercury-treated materials can be contained in the museum setting, they cannot be controlled once returned to tribal ownership, in accordance with the 1990 Native American Graves Protection and Repatriation Act (NAGPRA). NAGPRA requires federally-funded museums to return cultural items to native peoples upon request. Physical repatriation of mercury-containing materials has been generally halted while remediation technologies are developed to address the serious health risks these artifacts pose. This research funded by the NCPTT program is a novel, microbiologically-based mercury mitigation method for the remediation of contaminated cultural collections.

Some microorganisms are known to be metal-resistant and can reduce toxic metal concentrations by turning the metal into a gaseous form that can be safely collected and properly disposed of. Mercury-volatilizing bacteria can be used to reduce the concentration of mercury associated with a particular environment, or in this case material. Given that many of the traditional methods for mercury-mitigation from museum artifacts are potentially destructive, the overall goal of this research was to determine if mercury-volatilizing bacteria can be used as a less destructive, culturally sensitive approach to metal mitigation on sensitive museum collections, especially those earmarked for repatriation.

Sampling of mercury-treated cultural and herbarium materials at the Arizona State Museum resulted in sixteen different bacterial isolates. Each isolate represented a commonly occurring bacterial species. Each species exhibited varying degrees of mercury resistance, ranging from parts per billion (ppb) to several tens of parts per million (ppm) mercury. Four highly resistant isolates were examined for their ability to turn dissolved and solid mercuric chloride into a gaseous form. One isolate, in particular, *Arthrobacter* sp. 2604 was able to convert up to 2 ppm of mercuric chloride into gaseous mercury (with a 10 ppm starting concentration), removing up to 20% of the mercury in both solution and solid matrices, such as amended agar and paper. The highest amount of removal was observed when *Arthrobacter* sp. 2604 was applied with a minimum cell concentration of 10^6 cells/mL in a glucose-based nutrient solution to maintain cell growth during mercury volatilization.

This research has resulted in the initial development of a novel methodology addressing the removal and detoxification of mercury from contaminated items in museum collections throughout the U.S.

Introduction

Museums throughout the U.S. and Canada have mercury- and arsenic-contaminated materials in their collections. Original collectors frequently applied arsenic- and mercury-based salts to items for preservation purposes, protecting them primarily from insect and rodent damage. Treatment with such metal-based salts also reduced microbial activity, thereby further reducing material degradation and deterioration. These materials, some more than a hundred years old, are now housed in museums and are still contaminated. Their contamination is extensive enough to be toxic and pose health risks to those who handle the items. While the practice of using arsenic and mercuric salts ceased in the 1970s, the passing of the Native American Graves Protection and Repatriation Act (NAGPRA) in 1990 has resulted in active pursuit to understanding the extent of the problem, the true risks associated, and remediation

efforts to mitigate those risks. NAGPRA requires the return, upon request, of cultural items to their native owners. Tribes across the United States actively seek the return of their artifacts, such as headdresses, ceremonial masks, pipes and clothing. Less chemically intensive methods of preservation are now in use, including freezing and treatments with insect pheromones.

Current proposed remediation practices include material washing, scrapping, heating, and cross-linking the metal to the material via UV light exposure. While successful under some circumstances, these approaches are considered too harsh or too aggressive for some materials. Additionally, some native communities are uncomfortable with these practices being used on their artifacts. With our research, we propose using naturally-occurring mercury-removing bacteria as an alternative to current practices.

Some microorganisms, such as bacteria, are known to be metal-resistant and can reduce toxic metal concentrations in a process called volatilization where the metal is converted into a gaseous form that can be collected and properly disposed of. Readily found in the environment and generally non-pathogenic, metal-volatilizing bacteria can be used to mitigate and remediate metal-contaminated soil and water systems. The metal volatilizing bacteria in our research here are being examined for a culturally-sensitive approach to the remediation of materials that cannot be remediated using current methods, such as washing and scrapping.

Bacteria capable of converting various forms of mercury, including mercuric chloride (HgCl_2), into volatile, gaseous forms can be found in a variety of environments, including, as this research shows, from the museum setting. Naturally exposed to low background levels of mercury or high anthropogenic sources of mercury, these mercury-resistant bacteria provide a unique opportunity for the removal of mercury from museums. The gaseous forms of mercury, which include methyl mercury and primarily elemental mercury, can be easily contained and properly handled for disposal. The activity of mercury-volatilizing bacteria can reduce the concentration of mercury associated with a material as the gaseous form is collected. This was the basic premise behind the research here. The objective of this research was to identify mercury-volatilizing bacteria collected from mercury-contaminated museum collections capable of reducing mercury concentrations associated with treated materials. The overall goal of this research is to determine if mercury-volatilizing bacteria can be used as a less destructive, culturally sensitive approach to metal mitigation on sensitive museum collections, especially those earmarked for repatriation.

To achieve our objective, we collected bacteria already existent on the surface of mercury-treated museum collections. Upon collection, individual bacteria were identified and screened for their mercury resistance. The ability of the bacteria to grow in the presence of varying mercury concentrations reflected their maximum resistance level (MRL). Once an isolate was found resistant, the ability of the bacterium to convert mercury into a gaseous form was examined using a mixture of cultural and analytical techniques. When grown in the presence of a known concentration of mercury, a mercury-volatilizing isolate will reduce the amount of mercury in the surrounding medium as the mercury is converted into a gas. Final experimentation involved applying remediating organisms to the surface of mercury-amended paper to monitor the removal of mercury.

Our research has been very successful. We have found two bacterial organisms, *Arthrobacter* sp. 2604 and *Cupriavidus metallidurans* CH34, capable of removing 20% and 40%, respectively, of a 10 mg/Kg mercury application to paper within 10 days. Future research will focus on optimizing this process for these and other potential remediating organisms,

monitoring for any material degradation during the remediation process, and constructing a list of material types for this type of remediation.

Materials and Methods

The objective of this research was to identify mercury-volatilizing bacteria capable of reducing mercury concentrations associated with mercury-contaminated museum materials. The following methods/approaches were used to achieve this objective.

Sample collection: Access to several mercury-contaminated materials was granted by the Arizona State Museum in collaboration with Nancy Odegaard, Director of Curation, for non-destructive swab sampling. Contaminated items within collections had been previously screened by museum personnel using X-ray fluorescence (XRF) for surface-associated mercury. Such items included Native American headdresses, pouches, and herbarium Harvard cabinets.

For bacterial collection, cotton swabs were gently used to wipe the surface of various locations associated with each item. Microorganisms collected on the swabs were grown in standard microbiological growth media (tryptic soy agar and R2A), at 28°C. Upon appearance of individual colony types, based on color, size and shape, individual bacterial isolates were further isolated and purified. Purified isolates were stored at 4°C until analysis.

Bacterial isolate identification: Individual bacterial isolates obtained from the swabbing of museum materials were identified using the molecular method of 16S rDNA sequencing. The DNA from each purified isolate was extracted and subjected to the polymerase chain reaction (PCR) using universal primers specific for the 16S gene in bacteria. The 16S gene is unique in each bacterial species and so provides an accurate method for identification. Following PCR, each segment of DNA was sequenced and compared to a national database for bacterial identification (NCBI GeneBank).

Bacterial mercury resistance: Bacterial isolates that were identified as non-pathogenic based on the 16S rDNA sequencing were examined for their maximum level of mercury resistance. To assess mercury resistance, each isolate was grown in media (either nutrient agar or Luria Bertani agar) amended with mercuric chloride concentrations ranging from 10 ppb to 13.5 ppm. Those capable of growing in the presence of 13.5 ppm mercuric chloride (HgCl₂) or 10 ppm mercury (Hg) were further tested in higher concentrations of mercury up to 135.3 ppm HgCl₂ (100 ppm Hg). Growth was monitored as visible turbidity, indicative of bacterial activity.

Mercury growth curve: One highly mercury resistant isolate was chosen for growth analysis in the presence of mercury. The growth, as an increase in cell numbers, of isolate *Arthrobacter* sp. 2604 in 13.5 ppm HgCl₂ was monitored with time using culturable plate counts. This involved diluting and plating cells onto Luria Bertani agar every 24 hrs. Cell enumeration was performed by counting the number of resulting colonies on each plate at each dilution.

Mercury removal from broth: The potential of four mercury-resistant bacterial isolates to convert mercuric chloride into a gaseous form was examined. In this experiment, each isolate capable of growing in 13.5 ppm HgCl₂ was inoculated into Luria Bertani broth amended with 13.5 ppm HgCl₂ and incubated at 120 rpm at 28°C. Upon visible turbidity, acid digestion of the culture was performed and mercury quantified using a mercury analyzer. The difference

between the amount of mercury left in the broth as compared to uninoculated mercury-amended controls reflected the amount of mercury removal.

Acid digestions: A modified method for mercury analysis (modified from EPA Method 1631: Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation (EPA-821-R-01-013)) was developed for this research. Prior to mercury quantification using a Leeman Labs PS200II cold vapor atomic absorption spectrophotometer, the cultures were microwave (600 Watts for 2.5 min.) acid digested with concentrated nitric acid.

Mercury removal from solid matrices (agar and paper): The potential of isolate *Arthrobacter* sp. 2604 to remove mercury from amended agar (gelatin) and paper was assessed. Similar to the broth study above, agar and paper (Whatman 22) were treated with 13.5 ppm HgCl_2 . Mercury application was similar to that originally used by collectors and museum personnel with the mercury initially applied as a solution that was allowed to dry. Upon drying, the paper was then dipped in a nutrient solution (Luria Bertani broth) containing approx. 10^6 to 10^8 cells/mL. The paper was then aseptically placed in an empty sterile Petri plate for incubation at 28°C for 10 days. For the agar experiments, the mercury was included in the agar prior to solidification.

Acid digestions: The same method used above in the broth experiments was used to prepare the agar/paper samples for analysis. Following incubation, both the agar and the paper were microwave acid digested prior to mercury quantification on the Leeman Labs mercury analyzer. Subtractive analysis comparing inoculated versus uninoculated treatments reflected mercury removal.

Results and Discussion

Nine mercury-contaminated items from the Arizona State Museum were swab sampled (Figure 1). From the nine materials, a total of 16 isolates were collected (Table 1). The mercury levels associated with the materials ranged from 23 to $2,147 \mu\text{g}/\text{cm}^2$, as determined by XRF.



(from left to right) Native American spear thrower, red textile, and headdress.

Native American pouch



Figure 1. Example mercury-contaminated materials.

Other than the Harvard cabinet, each of the items was estimated to be up to a hundred years old. The items were composed of various material types including wood, human and horse hair, leather, feathers, and cloth. Bacterial isolates found on the surfaces identified as commonly

occurring environmental species, such as *Pseudomonas*, *Bacillus*, and *Arthrobacter* spp. Two isolates, one from a Native American headdress and the other from a red Native textile, did not match any 16S sequences in the NCBI database and so are currently unidentified. Further testing needs to be done to confirm the uniqueness of these isolates; however, these isolates may be new to the microbiological community. Interestingly, *Agrococcus*, *Arthrobacter*, *Bacillus* and *Pseudomonas* have been found associated with artwork degradation. In an examination of red stains on the marble of the Certosa of Pavia, Italy, researchers found lead-resistant *Pseudomonas vesicularis* and *Bacillus cereus* (Zanardini et al., 1997). *Agrococcus citreus* was isolated from a medieval wall painting in the Castle Herberstein, Austria, chapel (Wieser et al., 1999). Both *Pseudomonas* and *Kaistobacter* are linked to bioremediation of environmental contamination, and so perhaps it is not surprising to find these organisms associated with mercury-treated materials. However, according to their maximum mercury resistance levels, they did not necessarily represent the most resistant of the isolates.

Table 1. Materials and their corresponding mercury levels sampled at the Arizona State Museum, and the mercury resistant bacteria found on the materials.

Source	Mercury on material ($\mu\text{g}/\text{cm}^2$)	Isolate identification (16S rDNA sequencing)	Maximum mercury resistance (MRL)
Leather bag	93	<i>Arthrobacter</i> sp. 2604	50 ppm
Turtle fetish	undetermined	<i>Bacillus megaterium</i> 1487 <i>Pseudomonas</i> sp. 1487 <i>Kocuria rosea</i> 1487 <i>Bacillus niacini</i> 1487	5 ppm 2 ppm <10 ppb <10 ppb
Spear thrower	2,147	<i>Bacillus</i> sp. 16975	10 ppm
Headdress 1	280	Unknown	<10 ppb
Headdress 2	1,076	<i>Kocuria</i> sp. 2336	<10 ppb
Moccasin	23	<i>Chelatococcus asaccharyorans</i> 1493 <i>Arthrobacter</i> sp. 1493	<10 ppb 100 ppb
Leather pouch	undetermined	<i>Agrococcus jenensis</i> 26033	<10 ppb
Red textile	370	Unknown	100 ppb
Harvard cabinet	300	<i>Pseudomonas synxantha</i> 20350 <i>Kaistobacter koreensis</i> 20350 <i>Arthrobacter</i> sp. 20350 <i>Bacillus</i> sp. 20350	1 ppm <10 ppb 100 ppb <10 ppb

Arthrobacter sp. 2604, isolated from a Native leather bag, showed the highest degree of mercury resistance, up to 50 ppm Hg. Other highly resistant isolates worth noting include *Bacillus* sp. (up to 10 ppm Hg) from the spear thrower, and *Bacillus megaterium* and *Pseudomonas* sp. (5 and 2 ppm Hg, respectively) from the turtle fetish. Finally, the *Pseudomonas synxantha* isolate from the Harvard cabinet was resistant up to 1 ppm Hg. The other isolates were resistant up to ppb levels of mercury. Interestingly, the degree of resistance did not correlate with the amount of mercury on the material. For example, *Arthrobacter* sp. 2604 (50 ppm MRL) came from the leather bag with 93 $\mu\text{g}/\text{cm}^2$ mercury, while *Bacillus* sp. (10 ppm MRL) came from the spear thrower with 2,147 $\mu\text{g}/\text{cm}^2$. The varying degrees of resistance may reflect organism tolerance for the mercury toxicity, but may also be related to the expected varying concentration of mercury distributed throughout the surface of the material.

The high mercury resistance of *Arthrobacter* sp. 2604 made it an ideal candidate for mercury removal. A common mechanism of mercury resistance is the conversion of mercury into volatile forms, such as methylmercury (CH_3Hg) or elemental mercury (Hg^0). The vapor pressure of elemental mercury allows for abiotic volatilization. In both cases, CH_3Hg and Hg^0 , the gaseous mercury diffuses away from the cell, thereby reducing the effective toxic concentration of mercury surrounding the cell. This protects the cell from mercury toxicity through decreased exposure, but can also decrease the overall amount of mercury associated with a material as the concentration of mercury on the material decreases as the gaseous mercury diffuses away.

Noting the ability of *Arthrobacter* sp. 2604 to remove mercury, the growth of the bacterium in the presence of 10 mg/L Hg was followed (Figure 2). Note the initial decrease in cell numbers (indicating a decrease in cell viability) within 24 hrs of exposure to the mercury. The decrease indicates mercury toxicity. However, resistance is observed as cell numbers recover and increase to a maximum of 10^9 CFU/mL. The maximum levels reached were almost equal to those achieved when the isolate was grown in the absence of mercury.

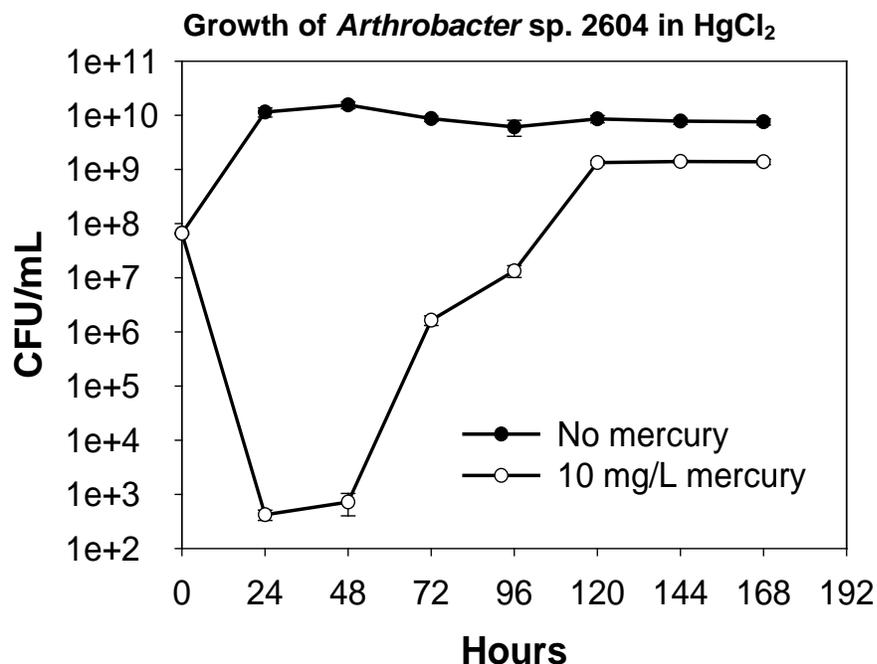


Figure 2. Growth of the mercury-resistant isolate *Arthrobacter* sp. 2604 in the presence of mercury.

For *Arthrobacter* sp. 2604 to be effective at mercury removal, mercury volatilization has to occur. Mercury volatilization can be monitored using two approaches: through examination of the mercury composition of collected gas or through examination of the remaining mercury following treatment. In this study, a decrease in the remaining mercury associated with a particular mercury-treated medium reflected mercury removal when compared to mercury controls. In Figure 2, *Arthrobacter* sp. 2604 was able to remove up to 20% of the mercury from 13.5 mg/L HgCl₂ amended broth, agar (a solid), and paper.

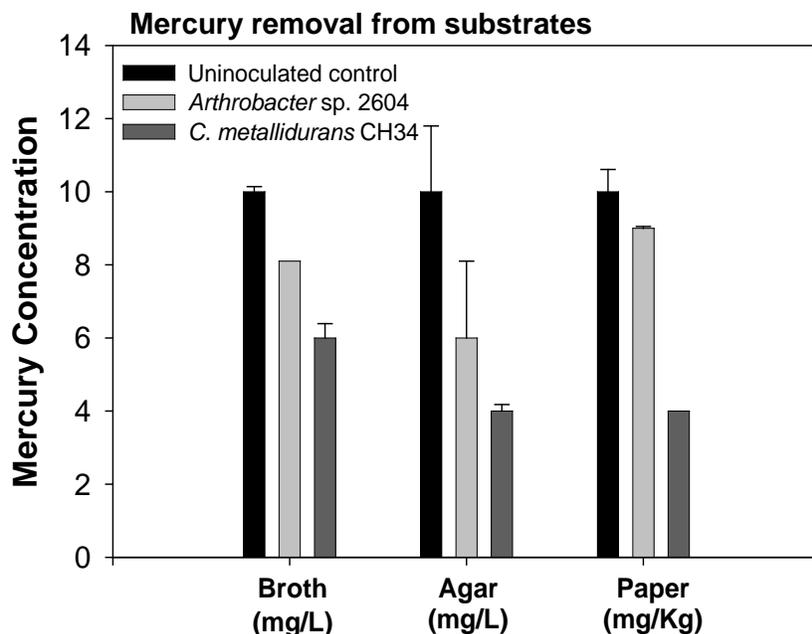


Figure 3. Mercury removal from amended broth, agar and paper by mercury-resistant bacterial isolates *Arthrobacter* sp. 2604 and *Cupriavidus metallidurans* CH34.

Finally, many experiments included the known mercury-volatilizing bacterium *Cupriavidus metallidurans* CH34, for comparison purposes. Interestingly, *C. metallidurans* also showed the ability to remove mercury from broth, agar, and paper, removing almost double the amount of mercury than *Arthrobacter* sp. 2604. For example, on 13.5 mg/Kg HgCl₂-treated paper, up to 40% of the mercury was removed within 10 days (Figure 3). Given the success of *C. metallidurans* CH34 to remove mercury, it shows great potential for use as a bioremediation agent, and its use in future studies will continue.

The use of microorganisms in the remediation or restoration of artwork is gaining attention. Cappitelli et al. (2006) propose the use of sulfate-reducing bacteria in the removal of black crusts on stonework. Restoration of frescos using direct application of *Pseudomonas stutzeri* A29 to dissolve interfering adhesive animal glue was examined by Ranalli et al. (2005). Other scientists are examining the microbial composition of Paleolithic paintings (Schabereiter-Gurtner et al., 2004). Finally, Ramirez et al. (2005) calls for more research on the use of biotechnology for the preservation and restoration of cultural heritage. The research here addresses a novel use for microorganisms in the removal of toxic mercury from cultural collections in museums. As cultural items are earmarked for repatriation, the detoxification of mercury is imperative to the health of individuals and to maintaining our cultural integrity.

Conclusions

This study shows potential for the use of mercury-resistant bacteria in the removal of mercury from treated museum collections. Many of the isolates have been found associated with artwork, such as cave paintings and stonework. However, none have been found associated with cultural assets, in particular mercury-treated cultural materials. *Arthrobacter* sp. 2604 and *Cupriavidus metallidurans* CH34 show potential for use in removing mercury from contaminated museum materials. Additional studies addressing optimal growth conditions and

enhanced mercury removal will increase the potential for bioremoval of mercury from complex, sensitive museum materials. This study presents a novel approach for the mitigation of mercury toxicity associated with museum collections.

Acknowledgements

(a) We would first and foremost like to thank the National Center for Preservation Technology and Training for supporting this work. The support from NCPTT has led to the development of a new methodology in the restoration of culturally-sensitive museum collections.

(b) We would like to acknowledge the participation of the Arizona State Museum in providing access to their collections. As a forerunner in the issue of pesticide mitigation in museums, Nancy Odegaard, Head Conservator, and the Arizona State Museum were instrumental in the success of this research. We look forward to continued collaborations with the Museum.

(c) We would like to thank Mr. Jeff Boon of the Shared Analytical Services Laboratory at the University of Colorado at Denver and Health Sciences Center for his analytical expertise and support for the project.

(d) We would like to thank Dr. Mary Striegel, Environmental and Materials Research Program Director, for her support and enthusiasm for our work.

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Abstracts and Summaries for presentations generated by NCPTT funding

Presentation at the International Symposium on Microbial Ecology (ISME), August 2006:

The Bacterial Diversity of Mercury-Treated Ethnographic Collections in a U.S. Museum
T.M. Roane¹, L.J. Snelling¹ and M.H. Albuthi¹

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Abstract:

In the United States, the use of mercury- and arsenic-based antimicrobials to preserve educational and ethnographic collections ceased nearly four decades ago. Mercury and arsenic still present on treated materials, however, pose health risks to museum personnel and to indigenous peoples upon repatriation and return to cultural use. While remediation technologies are investigated, little is known of the microbial populations associated with museum collections and in particular with metal-treated collections. Representing a unique metal-contaminated environment, the aim of this study was to identify and characterize the mercury-resistance of microorganisms associated with mercury-treated cultural items from the Arizona State Museum. Cultural and molecular methods, including 16S rDNA sequencing, were used to purify and identify isolates. Maximum mercury-resistance levels were determined by growth of individual isolates in mercury-amended broth. Mercury concentrations were confirmed using cold vapor atomic absorption spectroscopy. Sixteen bacterial isolates were collected from the ethnographic materials, with mercury levels from 23 to 2,147 $\mu\text{g}/\text{cm}^2$, as determined by X-ray fluorescence analysis. While belonging to common genera, including *Arthrobacter*, *Bacillus*, and *Pseudomonas*, isolated showed varying resistance when grown in the presence of mercury concentrations ranging from 100 $\mu\text{g}/\text{L}$ to 50 mg/L mercury. Upon growth, some isolates showed evidence of mercury volatilization. Determination of the bacterial diversity associated with metal-treated museum materials will contribute to our understanding of material deterioration and preservation.

Presentation given at the National Park Service sponsored meeting on Contaminated Cultural Collections for the Sault St. Marie Tribe of the Chippewa Indians, June 2006

Use of Microorganisms in the Removal of Mercury from Museum Materials
T.M. Roane¹ and L.J. Snelling¹

¹Department of Biology, University of Colorado at Denver and Health Sciences Center, CO

General presentation summary:

Microorganisms, such as bacteria, are very small living systems that are too small to be seen with the eye alone. Despite their small size, bacteria are crucial to our well being. While a select few can cause illness, majority of bacteria help us digest our food, protect us against disease, and clean up our environment. Found everywhere, we are interested in studying how bacteria can help us restore toxic systems.

Mercury is a metal that cannot be destroyed but can be removed from contaminated systems. In environmental clean-up, bacteria have been used in the removal of mercury from contaminated soils and waters. In the research being presented today, we want to study how bacteria can be used to remove mercury from mercury-treated collections in museums, including from cultural and educational materials.

In our research, we have found several bacteria growing on the surface of mercury-treated museum collections, including ceremonial masks and headdresses. All of the bacteria found are commonly found in soils and waters, and all can grow in the presence of mercury. We want to see if the bacteria living on the surface of the collections can be used to remove mercury from the material.

Our work is still very early. However, we have found that some bacteria can remove up to 40% of the mercury from artificially treated materials, such as paper. Before we use the bacteria directly on museum materials, we need to continue our analyses in order to develop a safe method for cultural and museum use.

Specific presentation summary:

Overall goal: The long-term goal of this research is to design a method where mercury-resistant bacteria can be used to reduce mercury concentrations associated with treated museum materials.

Background: The **objective of the current study** is to examine the ability of mercury-resistant bacteria isolated from museum collections to reduce the concentration of mercury associated with solid substrates, such as paper.

- ❖ Our laboratory traditionally studies bacteria living in highly toxic metal-contaminated environments, such as acid mine drainage and industrial waste. We are interested in how these microorganisms deal with toxic metals, and how we can use these organisms to clean-up contaminated soils and waters. Based on our experience, we are interested in using bacteria to mitigate mercury associated with contaminated museum collections.
- ❖ What are bacteria? Bacteria are a type of microorganism found everywhere on surfaces and in our food, water and air. While a few bacteria can cause disease, most bacteria are harmless and are actually beneficial to us. For example, bacteria in soil help us grow our food. Bacteria on our skin protect us from infections. Bacteria in our intestines help us digest our food. Some bacteria can also detoxify and degrade harmful chemicals.
- ❖ While toxic to all biological systems, some bacteria have developed ways of resisting mercury toxicity.
- ❖ Mercury-resistant bacteria can remove mercury from substrates by converting mercuric salts into gaseous mercury. Gaseous mercury can be collected for proper disposal.

Experimental approach:

- ❖ Commonly found in mercury-contaminated environments, several mercury-resistant bacterial isolates have been collected from mercury-treated museum collections. These isolates are being screened for the ability to reduce mercury concentrations.
- ❖ Bacteria are applied directly to mercury-contaminated materials and allowed to incubate while gaseous mercury coming from the material is collected.

Results:

- ❖ Twenty different bacterial isolates were found on the surface of mercury-treated cultural collections. All 20 of these isolates can grow in the presence of mercury. Three of the isolates can grow in up to 60 mg/L (ppm) of mercury.
- ❖ Some bacterial isolates are capable of removing up to 40% of the mercury associated with liquid and solid substrates showing the potential for mercury removal from collections.

Conclusions:

- ❖ Early results are promising and show potential for the use of bacteria in the removal of mercury from materials. We will continue to examine mercury removal from different materials, including hair and cloth.

*This work was funded by a grant from the National Center for Preservation Technology and Training, a division of the National Park Service.

Presentation given to the National Park Service sponsored Intercontinental Region Tribal Consultation Meeting, Santa Fe, NM, July 2005

A Microbial Method of Mercury Removal from Museum Materials

L.J. Snelling¹ and T.M. Roane¹

¹Department of Biology, University of Colorado at Denver and Health Sciences Center, CO

Summary:

Overall goal:

- The long-term goal of our research is to design a method where mercury-resistant bacteria can be used to reduce mercury concentrations associated with treated museum materials.

Background:

- Our laboratory traditionally studies bacteria living in highly toxic metal-contaminated environments, such as acid mine drainage and industrial waste. We are interested in how these microorganisms deal with toxic metals, and how we can use these organisms to clean-up contaminated soils and waters. Based on our experience, we are interested in using bacteria to mitigate mercury associated with contaminated museum collections.
- What are bacteria? Bacteria are a type of microorganism found everywhere on surfaces and in our food, water and air. While a few bacteria can cause disease, most bacteria are harmless and are actually beneficial to us. For example, bacteria in soil help us grow our food. Bacteria on our skin protect us from infections. Bacteria in our intestines help us digest our food. Some bacteria can also detoxify and degrade harmful chemicals.
- While toxic to all biological systems, some bacteria have developed ways of resisting mercury toxicity.
- Mercury-resistant bacteria can remove mercury from substrates by converting mercuric salts into gaseous mercury. Gaseous mercury can be collected for proper disposal.

The **objective of the current study** is to examine the ability of mercury-resistant bacteria isolated from museum collections to reduce the concentration of mercury associated with solid substrates, such as paper.

Experimental approach:

- Commonly found in mercury-contaminated environments, several mercury-resistant bacterial isolates have been collected from mercury-treated museum collections. These isolates are being screened for the ability to reduce mercury concentrations.
- Bacteria are applied directly to mercury-contaminated materials and allowed to incubate while gaseous mercury coming from the material is collected.

Results:

- Twenty different bacterial isolates were found on the surface of mercury-treated cultural collections. All 20 of these isolates can grow in the presence of 10 mg/L (ppm) of mercuric chloride. Three of the isolates can grow in up to 100 mg/L (ppm) of mercuric chloride.
- Some bacterial isolates are capable of removing up to 80% of the mercury associated with paper treated with 10 mg/Kg (ppm) mercury chloride.

Conclusions:

- Early results are promising and show potential for the use of bacteria in the removal of mercury from materials. We will continue to examine mercury removal from different materials, including hair and cloth.

*This work was funded by a grant from the National Center for Preservation Technology and Training, a division of the National Park Service.

Presentation given at the American Society for Microbiology annual meeting, May 2006:

Mercury Mitigation from Museum Collections Using Mercury-Resistant Bacteria

L.J. Snelling¹ and T.M. Roane¹

¹Department of Biology, University of Colorado at Denver and Health Sciences Center, CO

Abstract:

Historically, cultural museum collections were often treated with mercury as a preservative against insect and microbial damage. Still contaminated, these materials pose current health risks upon exposure. An effective method of mercury mitigation from treated cultural materials is currently not available. However, in protecting their cellular functions, mercury-resistant bacteria have the ability to convert mercury to a gaseous form. The objective of this research was to use resistant bacteria to remove mercury from treated materials in an effort to mitigate the toxicity associated with some museum collections. Twenty bacterial isolates were recovered from ethnographic collections, with mercury concentrations ranging from 51 $\mu\text{g}/\text{cm}^2$ to 2150 $\mu\text{g}/\text{cm}^2$, as determined by X-ray fluorescence. Using 16S rRNA gene sequencing, 50% of the isolates identified as *Bacillus* spp. Of the 20 isolates, three showed growth in 10 mg/L mercury-amended nutrient broth within 7 days at 24°C at 150 rpm. Each of the three isolates, identified as *Bacillus megaterium* 1487, *Pseudomonas fluorescens* 16975 and *Bacillus* sp. 2604, showed unique substrate utilization patterns upon BIOLOG analysis. When

examined for their maximum mercury-resistance level, one isolate in particular, *B. megaterium* 1487, showed a 10-fold increase in cell numbers in the presence of 20 mg/L mercury within 24 hrs. When used as an inoculum to remove mercury from laboratory-treated paper, *B. megaterium* 1487 removed 8 mg/Kg mercury from the paper within 7 days at 24°C, as determined by microwave acid digestion and cold vapor atomic absorption spectroscopy. Removal efficiency was enhanced when supplied with a dilute nutrient solution and increased humidity. The observed mercury reduction from inoculated paper demonstrates the potential use of bacteria in the removal of mercury from contaminated museum collections.

Presentation given at the American Society for Microbiology annual meeting, June 2005:

Microbial Removal of Mercury from Treated Museum Materials

L.J. Snelling¹ and T.M. Roane¹

¹Department of Biology, University of Colorado at Denver and Health Sciences Center, CO

Abstract:

Museums across the United States have collections which collectors once treated with mercuric salts to inhibit insect and microbial damage. Since metals are non-degradable and are long-lived these metal-based pesticides continue to pose toxicity concerns. Under the Native American Graves Protection and Repatriation Act (NAGPRA), enacted in 1990, cultural museum materials may be earmarked for return to native tribes. As such, treated museum materials pose health risks to museum personnel and native peoples. A widely accepted method of mercury removal from museum specific materials has yet to be identified. Microorganisms, however, are known for their diverse abilities to detoxify metals, such as mercury reduction. The objective of this research was to isolate bacteria from mercury-treated museum materials that can reduce mercury to a gaseous form that can be collected and properly disposed of, thereby reducing the effective toxic concentration of mercury associated with the material. Based on differential colony morphology, 20 bacterial isolates were collected from the surfaces of mercury and arsenic treated ethnographic and natural science specimens. Materials were identified as containing up to 2000 µg/cm² mercury using X-ray fluorescence. Specimens were sampled non-invasively using gentle swabbing with sterile cotton applicators. The applicators were then streaked onto nutrient agar and R2A for culturing and isolation. In broth and solid complex media amended with mercury, confirmed by the use of a mercury analyzer, 4 bacterial isolates were able to volatilize 10 mg/L of Hg to less than 4 mg/L Hg within 7 days. All isolates have been identified using 16S rRNA gene sequencing.

Presentation given at the Eastern Analytical Symposium annual meeting, November 2004:

Microbial Detoxification of Mercury Contaminated Museum Collections

L.J. Snelling¹ and T.M. Roane¹

¹Department of Biology, University of Colorado at Denver and Health Sciences Center, CO

Abstract:

Use of integrated pest management strategies, such as freezing and controlled atmosphere, is a current option to safeguard museum specimens from microbial and arthropod damage. However, historically arsenic- and mercury-based pesticides were sometimes used to preserve materials. As metals, arsenic and mercury are long lived and not readily degradable. For this reason, some arsenic- and mercury-treated specimens currently pose health risks to individuals coming into contact with them. Microorganisms are known for their abilities to volatilize mercury, converting it into a gaseous form that can be collected and properly disposed of. To reduce the risk associated with some museum specimens, we propose a novel strategy of microbial-based remediation of the mercury. Based on colony morphology, fifty-seven bacterial isolates were collected from the surfaces of arsenic- and mercury-treated ethnographic and natural science specimens. Specimens were sampled non-invasively using gentle swabbing with sterile cotton applicators. The applicators were then streaked onto nutrient agar and R2A for culturing and isolation. In mercury solutions and confirmed via analysis with a mercury analyzer, approximately 40 bacterial isolates were able to volatilize 7.4 $\mu\text{g/ml}$ Hg to non-detectable levels within 7 days, and 20 isolates could reduce 74 $\mu\text{g/ml}$ Hg to 20-40 $\mu\text{g/ml}$ Hg within 7 days. These mercury-volatilizing bacteria are being examined for use in the removal of mercury from artificially-treated materials in the laboratory.

Appendix A

Press releases



University of Colorado at Denver and Health Sciences Center

FOR IMMEDIATE RELEASE

Oct. 13, 2004

Office of Media Relations
Michele Ames
(303) 556-2523

UCDHSC researcher works to remove toxic metals from native artifacts

A researcher on the downtown Denver campus of the University of Colorado at Denver and Health Sciences Center is working to clean American Indian artifacts of toxic metals after years of contamination caused by antiquated preservation methods.

With the help of a grant from the National Center for Preservation Technology and Training, an office of the National Parks Service, Timberley Roane, assistant professor of biology, is researching a means to resolve an environmental quandary involving pesticides and artifacts such as kachina dolls, pipes, pottery, blankets, mounted animals and ceremonial masks.

“Historically, artifacts might have been treated with a variety of different pesticides to preserve the objects from insects and microbial damage,” explained Roane. “Two of the most prevalent pesticides that we’re most concerned with now are mercury and arsenic, as the toxicity of these metals to biological systems is under review.”

Roane, who is of the Lumbee tribe, and a Navajo friend who works with the Environmental Protection Agency, collaborated to come up with the use of bacteria as a possible means to extract mercury from these artifacts without damaging them. Due to the presence of mercury, for example, and the risk of dermal or inhalation exposure, some of these artifacts cannot be put back into cultural use.

She has been awarded a \$37,000 one-year renewable grant from the National Center for Preservation Technology and Training to work with the Arizona State Museum on the project. The National Center was established by an act of congress in 1992 to promote the use of new technologies to preserve cultural resources. This grant was one of nine awarded from 72 submissions received in this year’s grant competition.

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Other proposed methods of removing the toxic metals from the collections include treatments, such as chemicals, ultraviolet light and heat, which can sometimes damage materials.

“You have to treat them gently and with respect, especially since some of these materials are considered living by their native peoples,” Roane said. “New methods, such as those proposed by the grant procedures, may offer new hope.”

In essence, Roane hopes that her work with bacteria already living on the artifacts will allow her to change mercury into a gaseous form which can then be disposed of properly. This work echoes other successful work Roane has done in the past to deal with environmental cleanup through the use of naturally occurring bacteria.

Roane was granted access to Native American collections at the Arizona State Museum. Dozens of samples will be taken and documented. After the bacteria are grown in the lab they are screened for their ability to turn mercury into a gaseous form. Those forms are then tested further.

While much is not known about contamination levels in native artifacts, Roane’s research represents a promising step toward dealing with the contamination from the past while preserving these significant cultural artifacts for the future.

“We’re really taking baby steps,” Roane said. “This is the beginning of a process.”

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The Denver Post

Saving artifacts from toxic isolation

By Katy Human

Denver Post Staff Writer

Sunday, December 12, 2004 -

Decades ago, museum staffs around the country dipped, dusted or sprayed animal pelts and American Indian artifacts with pesticides now known to be toxic. The chemicals - laced with arsenic, mercury and other poisons - kept bugs at bay but penetrated deeply into headdresses, textiles and deer pelts.

Many artifacts remain so contaminated they can't be returned to tribes or used in traditional ceremonies. Museums can do little but protect staffers with gloves and masks, and keep the most noxious items locked away.

Denver biologist Timberley Roane has a new idea about how to clean up contaminated objects.

She's searching for natural bacteria that can strip away toxics, using techniques gleaned from her work at former industrial sites.

"This has a very personal significance for me," said Roane, a University of Colorado at Denver biologist. She is also member of the Lumbee tribe in North Carolina, and although her people are not aching for objects held in limbo because of contamination, Roane said, she has colleagues across the country who are.

A Navajo friend called Roane a few years ago with the idea of siccing bacteria on museum contaminants.

Museum toxics are so widespread that the Hopi Nation has stopped repatriating museum items deemed rightfully theirs.

"We found one item just so laden with arsenic that ... under EPA standards, it was hazardous waste," said Leigh Kuwanwisiwma, director of the Hopi cultural preservation office in Kykotsmovi, Ariz. "That's frightening."

Now armed with a \$37,000 renewable grant from the National Park Service, Roane is beginning lab work. She's starting with mercury but hopes to later extend the work to other toxics.

The first step: Identify bacteria already living on mercury-contaminated items that are nibbling slowly away at the poison. Then: Give the microbes what they need to thrive, whether that's warmth, humidity or a nutrient. Finally: Make sure the bulked-up microbes won't further damage sacred materials.

"That's the harder part," Roane said.

But it's worth trying, said a colleague, Nancy Odegaard, a conservator at the Arizona State Museum.

Until the 1970s, many museums and private collectors drenched American Indian objects with pesticides, not knowing the chemicals' toxicity.

"In most places, this was such a mundane thing, it was not even recorded," Odegaard said.

For American Indians, it was a devastating discovery, Kuwanwisiwma said.

When a federal repatriation law passed in 1990, many Hopis were overjoyed that they could bring sacred objects home, he said. A trip to Harvard University's Peabody Museum doused his own hopes.

"We were issued gowns, face masks, rubber gloves and everything," he said. "We were advised not to touch or fondle the objects too much."

Now, he's telling his people they can't bring sacred objects home yet. "You have this sense of bewilderment, you have anger," Kuwanwisiwma said.

Back in the laboratory, Roane and her assistant, Lisa Snelling, are already growing tiny pink, orange and white colonies of bacteria - snagged on cotton swabs from museum collections - on petri dishes.

Before next year is out, Roane said, she expects to isolate microbes that transform mercury metal into gas. Then she'll test them on pseudo artifacts, deliberately seeded with mercury.

She has used similar techniques before and is optimistic. For her Ph.D., Roane recruited microorganisms to clean soils contaminated with heavy metals and pesticides.

For museum items, the technique is better than others, she said. Washing, scraping or exposing to ultraviolet light, which can chemically alter toxics, are inappropriate for sacred objects.

"Some of these objects are considered living spirits," Roane said. "You need to handle them the same way you'd handle anything alive."

Staff writer Katy Human can be reached at 303-820-1910 or khuman@denverpost.com.

Post / Kathryn Scott Osler

Biologist Timberley Roane and her assistant, Lisa Snelling, are growing tiny pink, orange and white colonies of bacteria — snagged on cotton swabs from museums — on petri dishes.

UCDHSC Researcher Shows Progress in Helping to Safely Restore Native Artifacts

Aug. 4, 2006 – Efforts to conserve artifacts of ancient life have been undertaken by many well-meaning conservationists throughout human history. But some of the methods and materials used by early conservationists have since been determined to cause additional damage and make exposure to such items hazardous to humans.

University of Colorado at Denver and Health Sciences Center (UCDHSC) Associate Professor of Biology Timberley Roane has been working on ways to safely remove harmful chemicals from artifacts under a grant from the National Center for Preservation Technology and Training, an office of the National Park Service.

“Early methods of preserving many native artifacts, such as headdresses, pipes, blankets and ceremonial masks, relied heavily on the use of pesticides,” says Roane. “Two common ingredients in those pesticides were mercury and arsenic. Concentrations of those chemicals now make it risky for humans to come into contact with the artifacts.”

Roane, who is of Lumbee descent, discussed the idea with a Navajo colleague at the Environmental Protection Agency, and came up with the use of bacteria as a possible means to extract mercury from artifacts without damaging them. Roane has found 20 types of bacteria that are able to grow in high concentrations of toxic mercury with one bacterium capable of removing approximately 20% of the mercury from a surface within two weeks. Due to the presence of mercury, the risk of skin exposure or inhalation makes it dangerous for artifacts to be used in their historic and cultural contexts. “These bacteria may be the key to helping return artifacts to the people who created them, and to return them without endangering individuals coming in contact with the items,” says Roane.

Other proposed methods for removing toxic materials include using chemicals, or ultraviolet light and heat. But such techniques could damage the items. Roane’s approach is to use bacteria to change the mercury into a gaseous form which then can be disposed of safely. In other work, Roane uses a similar approach to manage environmental cleanup with naturally occurring bacteria.

When Roane began her work with native artifacts, not much was known about contamination levels. But through the renewable grant, she was able to begin her work with the Native American collections at the Arizona State Museum. “It is very important to handle the items with great care because they are considered to be living by the tribes from which they come,” says Roane. “So we believe this research offers hope to ensure their continued legacies.”

The University of Colorado at Denver and Health Sciences Center is Colorado’s premier urban university offering more than 100 degrees and programs in 12 schools and colleges and serving more than 27,000 students in Metro Denver and online.

For University of Colorado system Latitude Publication (2006)-in press

Timberley Roane
Biology

Significant national artifacts contaminated by mercury and pesticides may be one step closer to reclamation and repatriation. In collaboration with the Arizona State Museum, Associate Professor of Biology Timberley Roane has begun testing bacteria as a possible means to extract mercury from artifacts without damaging them.

“Early methods of preserving many native artifacts—such as headdresses, pipes, blankets and ceremonial masks—relied heavily on the use of pesticides,” Roane says. “Two common ingredients in those pesticides were mercury and arsenic. Concentrations of those chemicals now make it risky for humans to come into contact with the artifacts.”

Roane has been working on a means to safely remove harmful chemicals from artifacts under a grant from the National Center for Preservation Technology and Training, an office of the National Park Service, and with the help of a grant facilitated by the Center for Faculty Development.

“We are starting off testing non-culturally significant materials, such as pieces of felt from contaminated herbarium cabinets,” she explains. “This is exciting as it represents the next phase of our work toward actual removal of mercury from museum collections.”

Roane, who is of Lumbee descent, discussed the idea with a Navajo colleague at the Environmental Protection Agency, and came up with the use of bacteria as a possible means to extract mercury from artifacts without damaging them. She has found 20 types of bacteria that are able to grow in high concentrations of toxic mercury with one bacterium capable of removing approximately 20 percent of the mercury from a surface within two weeks.

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When Roane began her work with native artifacts, not much was known about contamination levels. But through the grants, she was able to begin her work with the Native American collections at the Arizona State Museum. “It is very important to handle the items with great care because they are considered to be living by the tribes from which they come,” Roane says. “We believe this research offers hope to ensure their continued legacies.”

