ABSTRACT

Mercury is a common contaminant in aquatic habitats and causes many problems for humans. Mercury can exist in a variety of forms in the environment. The elemental form (Hg^0) is very volatile and causes few problems with toxicity. In aquatic environments an ionic form of mercury (Hg[II]) exists which is methylated to methylmercury (CH_3Hg) by bacteria. This methylmercury is very toxic to humans. Fish and other aquatic animals in lakes and rivers may bioaccumulate high levels of methylmercury as they feed. Human consumption of fish with elevated levels of methylmercury can cause serious health concerns. Mercury resistant bacteria have the ability to convert the methylmercury into its less toxic elemental state. The genes that encode for mercury resistance are located on the *mer* operon. The goal of this project is to clone the *mer* operon from a natural aquatic bacterium. Former students have isolated mercury resistant bacteria from their natural aquatic habitats. The DNA from these bacteria will be digested with restriction enzymes and DNA fragments containing the *mer* genes will be identified by Southern blot hybridization. Once identified the *mer* operon will be cloned into *Escherichia coli*. Currently only *mer* operons cloned from clinical isolates are available which may not be relevant to microbial ecology studies performed in aquatic environments. This project will supply the *mer* operon clones from a natural aquatic bacterium in order to provide a better avenue for future research in environmental mercury cycling.

NARRATIVE

Background and Significance

Mercury contamination has caused numerous cases of illness and has been a concern for many years. Mercury is found in many forms in the environment. The elemental form (Hg^0) is very volatile and causes few problems with toxicity. In aquatic environments an ionic form of mercury (Hg[II]) exists which is methylated to methylmercury (CH_3Hg^+) by bacteria (Winfrey and Rudd, 1990; Fig 1). This methylated form of mercury is very toxic to humans, especially the fetus. Methylmercury has a long half life in the human body causing problems if a substantial amount is accumulated. Fish and other aquatic animals in lakes and rivers may bioaccumulate high levels of methylmercury as they feed. Consumption of fish with elevated levels of mercury can cause serious health concerns to humans.

Mercury resistant bacteria have the ability to convert the methylmercury into its less toxic elemental state by a two enzyme system that is encoded for by the *mer* operon (Silver and Mirsa, 1988 Fig 2). The *mer* T and *mer* P gene products are responsible for the transport of mercury into the cell. The *mer* B gene encodes for organomercury lyase which is responsible for the conversion of CH_3Hg^+ to Hg[II] . The *mer* A gene encodes for reductase that converts Hg[II] to Hg^0 which is released into the atmosphere. The *mer* D gene encodes for a regulatory protein, which shuts down the operon in the absence of mercury (Hongri et al, 1996). This proposal focuses on cloning the *mer* operon from a natural aquatic bacterium into *Escherichia coli* to be used in future environmental mercury cycling studies.

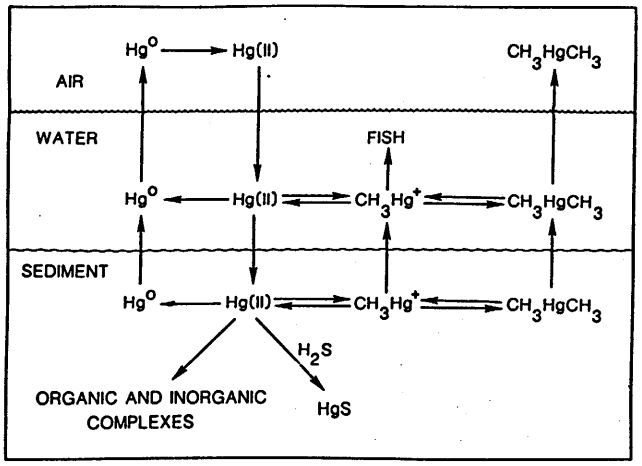

Fig 1: The biogeochemical cycling of mercury in freshwater lakes

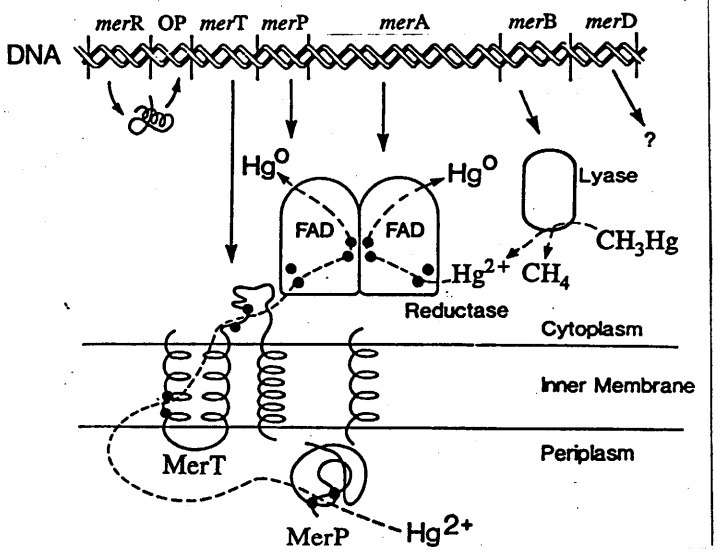

Fig 2: Model of mercury detoxification system

Objectives

The goal of this research is to clone the *mer* operon from a natural aquatic bacterium to be used in further research in detoxifying rivers and lakes.

Methods

1. Isolate the DNA from the mercury resistant bacterial strains. I completed this during fall '99 semester and have DNA from four different mercury resistant strains.
2. The DNA will be digested with several restriction enzymes and separated by agarose gel electrophoresis. The restriction enzymes will cut the DNA into fragments one of which will contain the *mer* operon. The digested DNA will then be transferred to a nylon membrane by the Southern blot technique (Winfrey et al, 1997).
3. A *mer* A gene probe will be purified so it may be used in labeling (Gambill and Summers, 1985). The probe will then be labeled with radioactive ^{32}P using a Nick translation kit (Gibco BRL, 1999).

4. The probe will now be used to identify the fragments of DNA containing the *mer* operon on the Southern blots. The probe will only bind to the complementary sequence of DNA on the nylon membrane if the hybridization conditions are correct. The correct conditions are found by increasing the stingency of the hybridization. Stingency refers to the conditions that affect the probe binding to the target DNA. Once the correct conditions are reached the probe will bind only to the *mer* operon.
5. The fragment of DNA that contains the mer operon will then be identified, purified and the *mer* operon will be cloned into *E. coli* (Winfrey et al, 1997)

Student Involvement

I started on this project the second semester of my sophmore year and will be continuing it until it is complete or until my graduation.

Plans for Dissemination

I plan to present my project at the annual UW- La Crosse Undergraduate Research Symposium. Also upon the completion of my research I may present at the Annual American Society of Microbiology meeting.

Resources

Dr. Winfrey's lab will be the site of my research. This lab and the microbiology program at UW-La Crosse have the necessary equipment for my research.

Other Funding

I may also apply for the undergraduate summer research fellowship for the summer of 2000 in the amount of $2500.

BUDGET

	Unit Cost	Total
1 Nick Translation Kit		$129.00
2 units of α^{32}P dATP radioactive isotope	$51.00	$102.00
RNase A (100mg)		$24.80
2 boxes of type 667 film	$19.99	$39.90
Restriction enzyme (*Sau* 3A I)		$67.00
4 boxes of gloves	$6.00	$24.00
Total		**$386.70**

All prices reflect current UW-L discounts.

Budget Justification

The Nick translation kit and the radioactive α^{32}P dATP are needed for the labeling of the probe. The RNase A is needed to remove the RNA from the DNA of the mercury resistant bacteria. The boxes of type 667 film are needed for taking pictures of the completed gels. The restriction enzyme (*Sau* 3A I) is needed for digesting the DNA into fragments containing the *mer* operon. The boxes of gloves are needed in order to handle the bacterial DNA and enzymes without contamination.

References

1. **Gambill, D.G., A.O. Summers.** 1985. Versatile mercury-resistant cloning and expression vectors. Gene. **39:**293-297.
2. **Gibco BRL.** 1999. Nick Translation kit instructions.
3. **Hongri, Y., L. Chu, T. Misra. 1996.** Intracellular inducer Hg^{2+} concentration is rate determining for the expression of the mercury-resistance operon in cells. 1996. J. Bacteriol. **178:**2712-2713.
4. **Silver, S., T. K. Misra.** 1988. Plasmid-mediated heavey metal resistances. Annu. Rev. Microbiol. **42:**717-743.
5. **Winfrey, M.R., M.A. Rott, and A.T. Wortman.** 1997. Unraveling DNA: molecluar biology for the laboratory. Prentice-Hall Inc., New Jersey.
6. **Winfrey, M.R., and J.W.M. Rudd. 1990.** Environmental factors affecting tge formation of methylmercury in low pH lakes. Environ. Toxicol. Chem. **9:**853-869.