Cloning of a *mer* operon from Naturally Occurring Aquatic Bacteria

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ABSTRACT

Methylmercury is a toxic substance which accumulates in fish and other biota and causes toxicological problems when consumed by humans. Certain mercury resistant bacteria are able to convert the highly toxic methylmercury to a much less toxic form. Several *mer* operons, a group of genes which encode this function, have been cloned and characterized. However, they have been isolated and cloned from clinical isolates, and these may not be adequate models for studying mercury resistance in nature. The purpose of this project is to isolate and clone the *mer* operon from a natural isolate to be used as a tool for studying mercury detoxification in aquatic habitats. DNA from various natural isolates was digested with restriction endonucleases to identify the *mer* operon on a smaller fragment of DNA. These restriction fragments will be probed with a DNA fragment which is complementary to the *mer* operon. After locating the operon on a restriction fragment, it will be cloned into a cloning vector and characterized by restriction mapping. This project will be beneficial because it provides a model for mercury resistance in natural isolates that can be used for studies on mercury detoxification in an environmental setting.

To date, the genomic DNA from strains L016, MB2, and PA07 has been isolated, digested with six different restriction enzymes (Fig. 2), and run on an agarose gel. These gels have been blotted onto a nylon membrane with the Southern blot technique and are ready for hybridization. The fragment that will be used for the hybridization probe has been identified from the *EcoR I/Ava* II digest.

Introduction

Mercury is a toxic heavy metal that has caused numerous cases of human toxicity throughout history. It is found in several forms, both organic and inorganic, in the environment. The elemental state (Hg^{o}) is volatile, causing few problems with toxicity. An ionic form of Hg, $Hg[II_2]$, is found in aquatic environments and can be methylated to the methylmercury (CH_3Hg) by bacteria. Methylmercury is highly toxic and even lethal to humans. Humans are currently affected most by it after consumption of fish that have high levels of accumulated methylmercury. There are certain bacteria (mercury resistant) that have the ability to demethylate this toxic form into the much less toxic Hg[II]. Hg[II] is then enzymatically reduced to Hg^{o} (Nazaret et al, 1994). This resistance mechanism has been studied in an attempt to understand the potential mechanisms of environmental mercury detoxification.

There are two forms of bacterial mercury: broad and narrow spectrum. Broad spectrum resistance is the ability to detoxify both inorganic and organic mercury

compounds. Narrow spectrum resistance refers to the detoxification of only inorganic mercury (Gilbert and Summers, 1988). This study focuses on broad spectrum resistance because this can detoxify methylmercury.

The genes that encode for mercury resistance (*mer* operon) encode several proteins with various functions (Fig 1). The *mer*T and *mer*P gene products transport mercury in the cell. The *mer*B gene product encodes organomercurial lyase which cleaves the carbon-mercury bond in organomercurials producing Hg[II]. The *mer*A gene product (mercuric reductase) then reduces Hg[II] to the volatile Hg^{o} which is released from the cell. The *mer*R gene encodes a regulatory protein that represses the operon in the absence of mercury (Brown et al, 1986).

This purpose of this project is to isolate and clone the *mer* operon from a natural isolate to be used as a tool for studying mercury detoxification in aquatic habitats. This will be a much more appropriate model for these studies than the current *mer* operon that was isolated from clinical isolates.

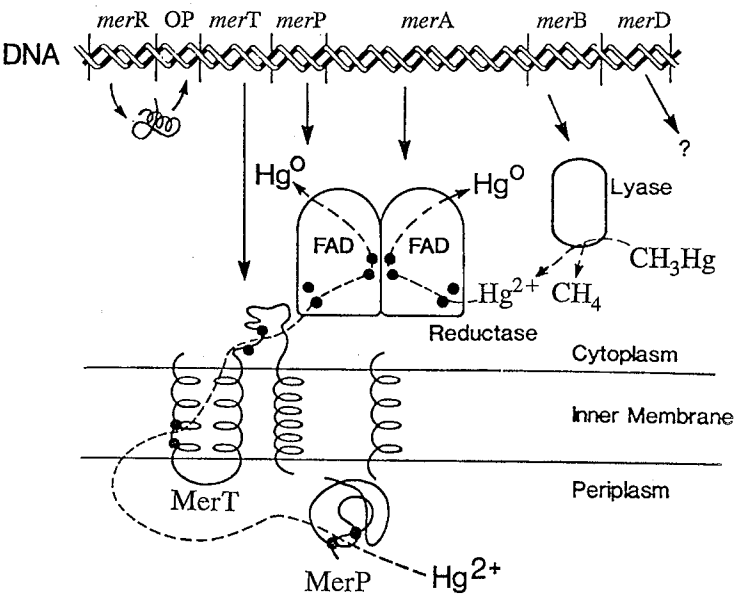


Figure 1. The *mer* operon. This model descirbes the genes in the *mer* operon and the proteins involved in transport and detoxification of methylmercury. (Silver and Misra, 1988.)

MATERIALS AND METHODS

Bacterial Strains and Plasmid. Strains were isolated by Bryan Marks from the Pettibone Lagoon and from stab vials of mercury resistant bacteria isolated by L. Overbye in the summer of 1988 (Table 1.)

The plasmid pDG105 contains the Tn21 *mer* operon inserted into a cloning vector (Gambill and Summers, 1985.)

Table 1. Bacterial strains.

Strain	Source
MB2	Pettibone Lagoon
L016 L.	Overbye (1988)
PA07	L. Overbye1988)

DNA Isolation. L016 was streaked out on R2A Agar with 3 ppm phenyl mercuric acetate (PMA) and incubated at 28°C. An LB broth +3 ppm PMA tube was inoculated with L016 and this culture was transferred to a 100 ml flask of LB+3 ppm PMA to give a 1% inoculum. DNA was isolated following the procedure described by Winfrey et al., 1997. The concentration of the DNA was determined by fluorometry (Cesarone et al., 1979.)

Restriction digestion and Southern blot. Chromosomal DNA from each mercury resistant strain (MB2, L016, and PA07) was digested with six different enzymes (*Hind* III *m, Sst* I, *Pst* I, *BamH* I, *Sal* I, *and Xha* I) in separate digestions at 37°C for eight hours. The resulting fragments were separated by agarose gel electrophoresis on a 0.8% gel, stained with ethidium bromide and photographed on a transilluminator. These resulting fragments were transferred to a nylon membrane by the Southern blot technique (Winfrey et al., 1997.)

Identification of the probe fragment. pDG105 was digested in two digestions at 37°C. Digestion 1 contained *EcoR* I and digestion 2 contained *EcoR* I and *Ava* II. Digested DNA and uncut plasmid was run on a 1.8% agarose gel electrophoresis with a 1 Kb BRL ladder as a size standard. The 1.12 Kb *EcoR* I-*Ava* II fragment was identified and will be used as a probe following labelling.

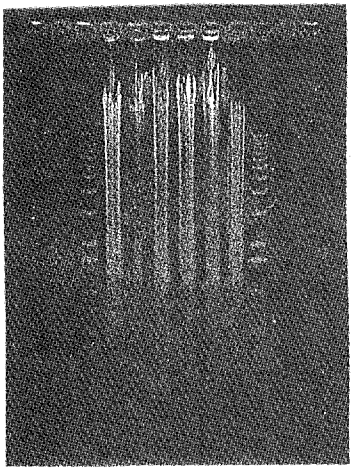
Results

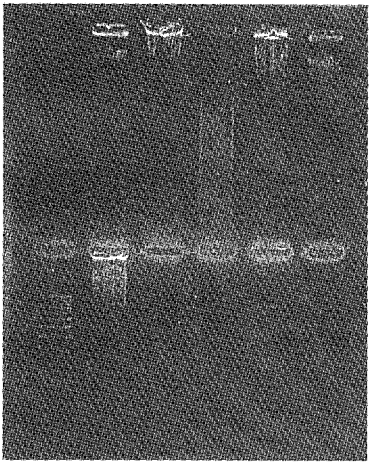
Table 2. Characteristics of mercury resistant bacteria (Data of Bryan Marks).

Strain	Resitance ppm CH_3Hg^+	Gram reaction	Oxidase reaction	Morphology
MB1	2	-	-	single rods
MB2	3	-	-	short rods
MB3	2	-	-	circular rods
MB4	3	-	-	single rods
MB5	4	-/+	+	oval rods
L020	3	-	+	rods
L014	3-4	-/+	+	short rods
L013	4	-	+	short,single rods
YB3	2	-	-	rods
PA07b	5	-	-	short rods
L016	4	-	-	oval rods

[a]MB2, L016, and PA07 were chosen for this study based on their variance in morphology and their high level of methyl mercury resistance.

[b]PA07 is a strain of *Pseudomonas aeruginosa* from the microbiology prep room.





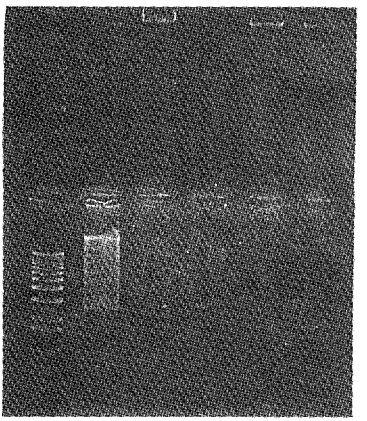


Figure 2. Each genome was digested with the following restriction endonucleases: *Hind* III, *Bam* HI, *Sal* I, Sst I, *Pst* I and *Xba* I. These digestions were performed to obtain the *mer* operon on a clonable fragment. Six different enzymes were used to increase the likelihood that the *mer* operon would be obtained on a clonable (5-12kb) fragment. Following agarose gel electrophoresis, blots of the gels were made onto nylon membranes via the Southern blot technique. A. Genomic digest of LO16. Genomic digest on MB2. This strain carries several plasmids. C. Genomic digest of PA07.

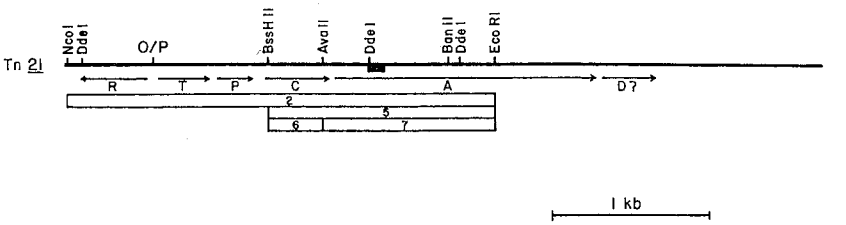


Figure 3. A model of Tn21 *mer* operon. This tranposon is found on the plasmid pDG105. Specific fragments that can be used as hybridization probes to identify *mer* operons are denoted by open boxes. Probe 7 will be used for this project following purification and labeling (Gilbert and Summers, 1998).

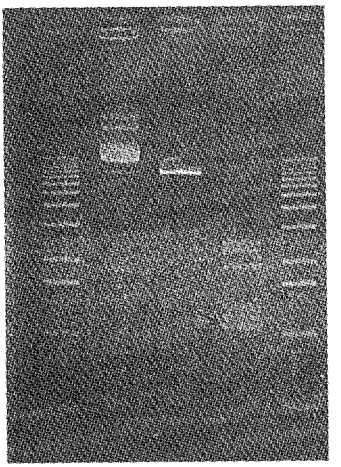


Figure 4. A plasmid (pDG105) that contains the Tn21 *mer* operon, was cut with *Ava* II and *Eco*R I to obtain the *mer*A gene on a 1.12 kb fragment (indicated by arrow). This fragment will be purified from the agarose gel, labeled with ^{32}P-labeled nucleotides, and used as a hybridization probe to identify restriction fragments containing the *mer* operon in the digested genomic DNA from LO16, MB2 and PA07.

CONCLUSIONS/FURTHER WORK

To date, the genomic DNA from strains L016, MB2, and PA07 have been isolated, digested with six different restriction enzymes (Fig. 2), and run on an agarose gel. These gels have been blotted onto a nylon membrane with the Southern blot technique and are ready for hybridization. The fragment that will be used for the hybridization probe has been identified from the *Eco*R I/*Ava* II digest.

The next step in this project is to probe the genomic DNA digests with the *mer*A probe to identify the fragment containing the *mer* operon in the chromosome of each strain. The membranes from the Southern blots will be hybridized with a radio-labeled *mer*A probe. The probe will bind to the digested DNA wherever the sequences have

some homology. The challenge to this procedure lies in the fact that the amount of similarity between the *mer*A probe and the *mer*A genes in the chromosomes is unknown. Therefore, the proper hybridization stringency must be determined. Stringency refers to the conditions that define specificity of the probe binding to its target. For example, under high stringency the probe will bind only to targets with near perfect homology. In contrast, under low stringency the probe will bind to DNA fragments with less homology. At the beginning, low stringency hybridization will be used, which will allow the probe to bind to sequences with low homology. The stringency will be increased in subsequent hybridizations until less binding occurs and ultimately until the probe binds only the desired fragment. Too high of a stringency will not allow any probe to bind. Positive and negative controls will be used to assess the stringency requirements. The positive control is the 1.12Kb *Ava* II/*EcoR* I fragment that will be used as the hybridization probe, therefore, the probe should always bind to this fragment. The negative control is the 1Kb BRL Ladder. The *mer* operon is not found on this DNA and any binding that would result would be caused by too low of a stringency. The stringency could then be adjusted to limit this binding.

After completion of the hybridization, the operon will be cloned into *Escherichia coli* cells. This will provide researchers studying mercury resistance in the environment with a model of the genes that encode for this resistance from a natural source. This will be much more relevant than using the current *mer* operons from clinical isolates.

REFERENCES

Brown, N.L., T.K. Misra, J.N.Winnie, A. Schmidt, M. Seiff, and S. Silver. 1986. The nucleotide sequence of the mercuric resistance operons of plasmid R100 and transposon Tn501: further evidence for *mer* genes which enhance the activity of the mercuric ion detoxification system. Mol. Gen. Genet. 202:143-151.

Cesarone, C.P., C. Bolognesi, and L. Santi. 1979. Improved microfluorometric DNA determination in biological material using 33258 Hoechst. Anal. Biochem. 100:188-197.

Gambill, B.D., and A.O. Summers. 1985. Versatile mercury-resistant cloning and expression vectors. Gene 39:293-297.

Gilbert, M.P.,and A.O. Summers. 1988. The distribution and divergence of DNA sequences related to the Tn21 and Tn501 *mer* operons. Plasmid 20:127.

Nazaret, S., W.H. Jeffrey, E. Saouter, R. Von Haven, and T. Barkay. 1994. *mer*A gene expression in aquatic environments measured by mRNA production and Hg(II) volatilization. Appl. Environ. Microbiol. 60:4059-4065.

Silver, S., and T.K. Misra. 1988. Plasmid mediated heavy metal resistances. Ann.Rev. Microbiol. 42:717-743.

Winfrey, M.R., M.A. Rott, and A. T. Wortman. 1997. Unraveling DNA: molecular biology for the laboratory. Prentice-Hall Inc., New Jersey.