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Mini Review

Operon *mer*: Bacterial resistance to mercury and potential for bioremediation of contaminated environments

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ABSTRACT. Mercury is present in the environment as a result of natural processes and from anthropogenic sources. The amount of mercury mobilized and released into the biosphere has increased since the beginning of the industrial age. Generally, mercury accumulates upwards through aquatic food chains, so that organisms at higher trophic levels have higher mercury concentrations. Some bacteria are able to resist heavy metal contamination through chemical transformation by reduction, oxidation, methylation and demethylation. One of the best understood biological systems for detoxifying organometallic or inorganic compounds involves the *mer* operon. The mer determinants, *RTPCDAB*, in these bacteria are often located in plasmids or transposons and can also be found in chromosomes. There are two classes of mercury resistance: narrow-spectrum specifies resistance to inorganic mercury, while broad-spectrum includes resistance to organomercurials, encoded by the gene *merB*. The regulatory gene *merR* is transcribed from a promoter that is divergently oriented from the promoter for the other *mer* genes. MerR regulates the expression of the structural genes of the operon in both a positive and a negative fashion. Resistance is due to Hg^{2+} being taken up into the cell and delivered to the NADPH-dependent flavoenzyme mercuric reductase, which catalyzes the two-electron reduction of Hg^{2+} to volatile, low-toxicity Hg^{0}. The

potential for bioremediation applications of the microbial *mer* operon has been long recognized; consequently, *Escherichia coli* and other wild and genetically engineered organisms for the bioremediation of Hg^{2+}-contaminated environments have been assayed by several laboratories.

Key words: Operon *mer*, Bacterial mercury resistance, Bioremediation

INTRODUCTION

Mercury, the 6th most toxic in a universe of 6 million substances, exists naturally in small amounts in the environment, being the 16th most rare element on Earth. However, its levels have risen due to environmental contamination from human activities, such as burning coal and petroleum products, use of mercurial fungicides in paper making and agriculture and mercury catalysts in industry, with a consequent release of mercury into the air and water and on the land. These activities can increase local mercury levels several thousand-fold above background (Tuovinen, 1984). In Brazil, huge amounts of mercury are used at prospecting sites for amalgam formation in gold extraction. An average of 1.32 kg of mercury is used for each kilogram of gold produced (Lacerda and Solomons, 1991). As a consequence, metallic mercury is introduced into the environment, representing one of the major sources of aggression against man and the environment. Its use in seed and bulb dressings directed against bacteria and fungi on fruit trees has introduced much of the mercury that contaminates agricultural land. Therefore, environmental pollution is an increasing problem both for developing and developed countries.

Minamata disease, discovered in 1956 around Minamata Bay, Japan, is the first instance on record of severe methylmercury poisoning, having affected thousands of people, 887 of whom were killed (Daher, 1999). It resulted from the consumption, mainly by fishermen and their families, of large amounts of fish and shellfish contaminated with methylmercury, resulting from the transformation of the $HgCl_2$ discharged from a chemical plant (Chisso Co. Ltd.). Methylmercury is a neurological poison primarily affecting the central nervous system, liver and kidney. When ingested, almost all of the methylmercury is absorbed. Its half-life is about 44 days. Most methylmercury is converted and excreted into the feces and urine (Abelsohn et al., 2002). The other chemical forms of mercury, vapor and inorganic mercury, accumulate in the brain (Hg^0) and kidney (Hg^{2+}). The kidney is the main target organ for inorganic mercury. The typical symptoms of Minamata disease include neurological disorders, such as sensory disorders, cerebellar ataxia, constriction of the visual field, auditory disturbances, tremoring of the visual field, and disequilibrium (Langford and Ferner, 1999). Furthermore, many of the affected individuals in Minamata were congenitally affected by methylmercury. Their mothers had only mild or no manifestation of poisoning (Harada, 1978). This fact demonstrates the much higher vulnerability of fetuses than adults and shows that methylmercury easily passes through the placenta and affects the fetus (Nishigaki and Harada, 1975).

MERCURY CYCLE IN THE ENVIRONMENT

The environmental mercury cycle is mediated by both geological and biological processes. Mercury vapor (metallic mercury) emitted from both natural and anthropogenic sources is globally distributed in the atmosphere. The major form of mercury in the atmosphere is vapor mercury (Hg^0), which is volatile and is oxidized to mercuric ion (Hg^{2+}) as a result of its

interaction with ozone in the presence of water (Munthe and McElroy, 1992; DeMagalhaes and Tubino, 1995; Pleijel and Munthe, 1995). Most of the mercury entering aquatic environments is Hg^{2+}. Inorganic mercury, present in water and sediments, is subject to bacterial conversion to methylmercury compounds that are bioaccumulated in the aquatic food chain. Organomercury compounds are translocated rapidly through the food chain, with tragic consequences. Predatory organisms at the top of the food chain generally have higher mercury concentrations, found as organic forms of methylmercury.

The major chemical forms of mercury to which humans are exposed are mercury vapor, Hg^0, and methylmercury compounds, which are highly toxic to all living organisms. The toxicity of inorganic and organic mercury compounds is due to their strong affinity for sulfur-containing organic compounds, such as enzymes and other proteins. For this reason these compounds are extremely toxic to biological systems. However, bacteria, fungi and plants have evolved mechanisms of resistance to several of these different chemical forms. The bacteria play a major role in the global cycling of mercury in the natural environment. Bacterial resistance to mercury and their role in mercury cycling have been extensively studied (Osborn et al., 1997). This mini-review focuses predominantly on mercury resistance *mer* operons.

BIOCHEMICAL BASIS OF BACTERIAL MERCURY RESISTANCE

As a response to toxic mercury compounds globally distributed by geological and anthropogenic activities, microorganisms have developed a surprising array of resistance systems to overcome the poisonous environment. An extensively studied resistance system, based on clustered genes in an operon (*mer* operon), allows bacteria to detoxify Hg^{2+} into volatile metallic mercury by enzymatic reduction (Komura and Izaki, 1971; Summers, 1986; Misra, 1992; Silver, 1996; Osborn et al., 1997). Mercury-resistance determinants have been found in a wide range of Gram-negative and Gram-positive bacteria isolated from different environments. They vary in the number and identity of genes involved and are encoded by *mer* operons, usually located on plasmids (Summers and Silver, 1972; Brown et al., 1986; Griffin et al., 1987; Radstrom et al., 1994) and chromosomes (Wang et al., 1987; Inoue et al., 1991); they are often components of transposons (Misra et al., 1984; Kholodii et al., 1993) and integrons (Liebert et al., 1999).

Two main *mer* determinant types have been described: narrow-spectrum *mer* determinants confer resistance to inorganic mercury salts only, whereas broad-spectrum *mer* determinants confer resistance to organomercurials such as methylmercury and phenylmercury, as well as to inorganic mercury salts (Misra, 1992; Silver and Phung, 1996; Bogdanova et al., 1998).

The biochemical basis of resistance to inorganic mercury compounds such as $HgCl_2$ appears to be quite similar in several different species. It involves the reduction of Hg^{2+} to volatile Hg^0 by an inducible enzyme, mercuric reductase. This enzyme has been characterized in plasmid-carrying strains of *Pseudomonas*, *Escherichia coli* and *Staphylococcus aureus* (Summers and Silver, 1978; Bhriain and Foster, 1996; Silver and Phung, 1996; Osborn et al., 1997). This reductase is a flavoprotein, which catalyzes the NADPH-dependent reduction of Hg^{2+} to Hg^0. Since mercury has such a high vapor pressure, it volatilizes and the bacterial environment is left mercury free. This mercuric reductase is found intracellularly and is inducible by subinhibitory concentrations of mercuric ions and a variety of organomercurial substances (Furukawa and Tonomura, 1972; Summers, 1972; Schottel, 1978).

Based on a comparison with other bacterial periplasmic binding, protein-dependent transport systems, it has been proposed that Hg^{2+} diffuses across the outer membrane (Brown, 1985). Mercuric ions are transported outside the cell by a series of transporter proteins. This mechanism involves the binding of Hg^{2+} by a pair of cysteine residues on the MerP protein located in the periplasm. Hg^{2+} is then transferred to a pair of cysteine residues on MerT, a cytoplasmic membrane protein, and finally to a cysteine pair at the active site of MerA (mercuric reductase) (Hamlett et al., 1992). Next, Hg^{2+} is reduced to Hg^0 in an NADPH-dependent reaction. The non-toxic Hg^0 is then released into the cytoplasm and volatilizes from the cell.

The biochemical mechanism for broad-spectrum resistance to organomercurials involves, in addition to mercuric reductase, another inducible, soluble enzyme: organomercurial lyase. This enzyme cleaves the organometallic linkage to yield Hg^{2+}, and then the reductase uses NADPH to reduce the elemental mercury form, which volatilizes from the cell (Schottel, 1978).

STRUCTURE OF THE *MER* OPERON

The *mer* operons vary in structure and are constituted by genes that encode the functional proteins for regulation (*merR*), transport (*merT*, *merP* and/or *merC*, *merF*) and reduction (*merA*) (Figure 1). In some cases, known as broad-spectrum mercury resistance, additional *merB* genes are required to confer resistance to many organomercurials, such as methylmercury and phenylmercury, by hydrolyzing the C-Hg bond before Hg^{2+} reduction. In general, the additional *merB* genes are found downstream of the *merA* gene in the *mer* operon (Osborn et al., 1997).

Most *mer* operons contain a regulatory gene, *mer R*, which is transcribed separately and divergently from the structural *mer* genes. However, in Gram-positive bacteria the *merR* genes of pI258 from *Staphylococcus aureus* and RC 607 from *Bacillus* sp. are transcribed in the same direction as the structural genes (Laddaga et al., 1987; Wang et al., 1989). MerR, the metalloregulatory protein, binds the promoter-operator region, where it both positively and negatively regulates the expression of the divergently transcribed structural genes, and also negatively regulates its own expression. MerR protein activates transcription of the operon in the presence of inducing concentrations of Hg^{2+}. It represses transcription of the structural genes from the *mer* operon (*merTPCFAD*) in the absence of Hg^{2+}, and activates transcription in the presence of Hg^{2+}. The most distal promoter gene, *merD*, which is co-transcribed with the structural genes, down-regulates the *mer* operon. MerD, a secondary regulatory protein, also binds the same operator-promoter region as MerR, although very weakly (Nucifora et al., 1990; Mukhopadhyay et al., 1991).

A number of structural genes are found downstream of the operator/promoter site; the proteins they code for are involved in mercuric ion transport. All the *mer* operons have *merT* and *merP*, however, some operons, such as transposon Tn21, have merC (the first example found with the *merC* gene). The additional *merC* gene is located between *merP* and *merA*. However, it seems not to be essential for Hg^{2+} resistance since it is absent from Tn501, which confers identical Hg^{2+} resistance levels (Bhriain and Foster, 1986; Summers, 1986). Both *merT* and *merP* are required for full expression of Hg^{2+} resistance, but loss of *merP* is less deleterious than loss of *merT*. In contrast, mutating *merC* had no effect on Hg^{2+} resistance, though it decreased the level of expression. Recently, one more *mer* gene implicated in mercuric transport, *merF*, was found in plasmid pMER327/419 of *Pseudomonas fluorescens* between *merP* and *merA* (Wilson et al., 2000).

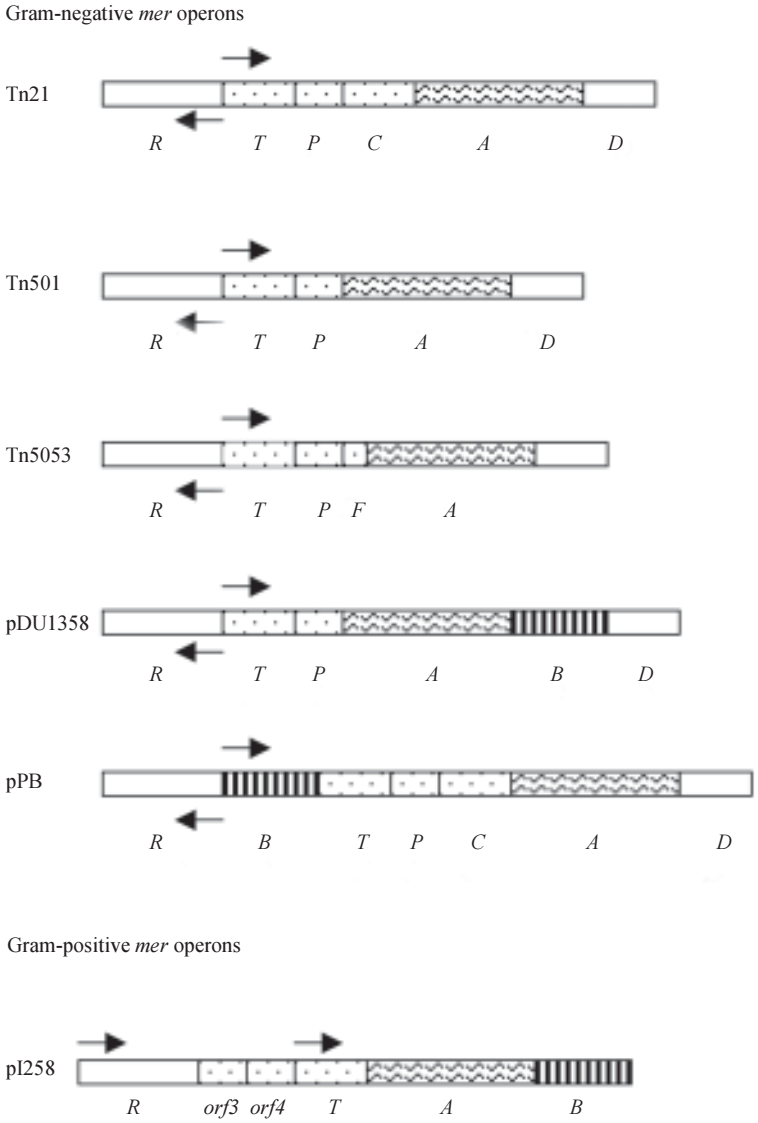

Figure 1. Schematic representation of Gram-negative and Gram-positive *mer* operons, derived from DNA sequence data: transposon Tn21 of the plasmid NR1 from *Shigella flexneri*; transposon Tn501 of the plasmid pVS1 from *Pseudomonas aeruginosa*; plasmid pDU1358 from *Serratia marcescens*; transposon Tn5053 of the plasmid pMR from *Xanthomonas* sp.; plasmid pPB from *Pseudomonas stutzer*, and plasmid pI258 from *Staphylococcus aureus*.

The *merA* gene, determining mercuric reductase, and *merB*, if present, encoding the enzyme organomercurial lyase, are immediately followed by genes encoding transport function. However, as observed in *Pseudomonas stutzeri*, the *merB* gene is found between *merR* and *merT*, together with an extra operator-promoter region (Weiss et al., 1977; Walsh et al., 1988; Reniero et al., 1995). The other genes encoding organomercury resistance have been identified and designated *merG* and *merE*, located between *merA* and *merB* on the broad-spectrum *mer* operon (Huang et al., 1999; Kiyono and Pan-Hou, 1999). Furthermore, *merB* seldom occurs in Gram-negative bacteria (Laddaga et al., 1987; Wang et al., 1989; Sedlmeier and Altenbuchner, 1992; Bogdanova et al., 1998).

Various mercury detoxification mechanisms, without mercury-reducing activity, have been reported, such as reduced uptake of mercuric ions due to reduction in cellular permeability to Hg^{2+} ions (Pan-Hou et al., 1981), demethylation of methylmercury by *Clostridium cochlearium* T-2P, which involves the decomposition and inactivation of inorganic mercury with hydrogen sulfide (H_2S) (Pan-Hou and Imura, 1981), mercury methylation by certain bacteria that use methylation as a resistance/detoxification mechanism (Trevors, 1986) and sequestration of methylmercury (Silver and Misra, 1984).

MERCURY AND ANTIBIOTIC RESISTANCE

Mercury pollution can contribute to increased antibiotic resistance (McArthur and Tuckfield, 2000). The combined expression of antibiotic resistance and mercury may be caused by selection, as a consequence of the mercury present in an environment (Sant'ana et al., 1989). Mercury resistance operons, which are often found in conjugative plasmids and transposons, provide a suitable model system for the study of horizontal gene transfer in natural populations of bacteria. Bacterial plasmid resistance systems (*mer* gene) for mercurials and organomercurials are the best understood of such systems at the biochemical and molecular genetic levels (Kalyaeva et al., 1988; Silver, 1994).

BIOTECHNOLOGICAL APPLICATIONS OF *MER* GENES TO MERCURY DECONTAMINATION AND RECOVERY

Industrial use of mercury led to pollution of the environment. Consequently, mercury removal is a challenge for environmental management. Common processes to remove mercury from contaminated sources, based on adsorption with ion-exchange resins or biosorbents, have been found to be sensitive to environment conditions (Ritter and Bibbler, 1992; Chang and Hong, 1994). Biological processes have been employed in bioremediation, including metal recovery, and are potentially low cost. The use of bacteria for removing metal from contaminated environments is a promising technology. However, passive adsorption and immobilization treatments produce a large volume of mercury-loaded biomass, the disposal of which is problematic. Microorganisms in contaminated environments have developed resistance to mercury and are playing a major role in natural decontamination (Cursino et al., 1999).

The bacterial plasmid/transposon resistance systems for mercurials and organomercurials (mer systems) are the best understood at the biochemical and molecular genetic levels (Silver, 1994), and are of great interest since they represent a natural strategy for the detoxification of mercury-contaminated environments. The potential of the microbial *mer* operon, which functions by active enzymatic reduction of mercury ions to water-insoluble metallic mercury, has been recognized for a long time, because of its high levels of efficacy and specificity. Inside the cell, Hg^{2+} is reduced to metallic mercury (Hg^0), which passively diffuses out of the cell and its environment (with no energy expenditure) (Saouter et al., 1994; Silver, 1996; Silver and Phung, 1996; von Canstein et al., 1999; Chen et al., 1999; Nies, 1999). Therefore, the bacterial biomass acts continuously as a catalyst, without the accumulation of large volumes of biomass. However, currently there are no records of the use of the *mer* operon for the treatment of industrial waste or of other environments contaminated with mercury (von Canstein et al., 1999).

Some experiments have been conducted in the form of a microcosmos (a glass apparatus with different chambers) used to perform environmental simulations (river, lake, etc.). In a

central chamber the contaminated medium is treated with mercury-reducing bacteria. The concentration and form of mercury can be monitored in the different chambers. Mercury reduction from Hg^{2+} to Hg^{0} can reach a 95% rate when the Hg^{2+} in the first chamber (entry) is compared to that in the last one (exit), demonstrating the high biotechnological potential of mercury reduction by the *mer* operon (Saouter et al., 1994).

Other studies, some of them conducted in our laboratory, have described mercury-reducing bacterial strains, with emphasis on *Escherichia coli*, obtained and genetically improved by means of *mer* operon cloning and by other recombinant DNA techniques (Hou et al., 1988; Nascimento et al., 1992a,b; Chen and Wilson, 1997; Cursino et al., 2000). MerA has been found to be active in yeast (Rensing et al., 1992) and plants (Rugh et al., 1996, 1998).

Techniques to detect mercurial compounds in the environment using mechanical analysis procedures, such as atomic spectrophotometry (Omang, 1971) or cold-vapor atomic fluorescence detection (Bloom and Fitzgerald, 1988), have been developed. However, the preparation of samples is very laborious. An alternative will be the use of bacterial sensors. Bioassays can complement analytical chemical methods for the detection of biologically available mercury in environmental samples. Bacterial biosensors have been engineered to contain a report plasmid that carries gene fusions between the regulatory region of the *mer* operon (*merR*) and bacterial luminescence genes (*lux-CDABE*) that quantitatively respond to Hg^{2+} (Selifonova et al., 1993; Ramanathan et al., 1997; Rasmussena et al., 2000).

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