

A CONSERVATIVE REGION OF THE MERCURIC REDUCTASE GENE (*MER*A) AS A MOLECULAR MARKER OF BACTERIAL MERCURY RESISTANCE

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SHORT COMMUNICATION

ABSTRACT

The most common bacterial mercury resistance mechanism is based on the reduction of Hg(II) to Hg⁰, which is dependent of the mercuric reductase enzyme (MerA) activity. The use of a 431 bp fragment of a conservative region of the mercuric reductase (*merA*) gene was applied as a molecular marker of this mechanism, allowing the identification of mercury resistant bacterial strains.

Key-words: *merA* gene; mercury resistant; bacteria; molecular marker

The contamination of aquatic environments by mercury (Hg) is of great concern because of its toxicity and ubiquity. Mercury levels have risen due to human activities, such as coal and petroleum burning, and industrial uses (e.g. lamps, catalysts, instruments, etc.), with a consequent release of mercury into the air, aquatic and terrestrial ecosystems. These activities can increase local mercury levels several thousand-fold above background (1,9,17). In aquatic environments, mercury can be naturally converted to its most toxic species, methylmercury a potent neurotoxin, which is bioaccumulated and biomagnified through the trophic chain (2,9).

Some bacteria developed a mercury resistance mechanism, based on a group of genes located in an operon (*mer* operon). It allows bacteria to detoxify Hg(II) into volatile metallic mercury through its enzymatic reduction (14,16,18,23,24). Bacterial mercury resistance has been found in a wide range of Gram-negative and Gram-positive bacteria. The operon varies in the

number of genes present and is usually located on plasmids (3,5,8,19,25) and chromosomes (4,13,18,28); they are often components of transposons (5,11,15,20) and integrons (4,13,18) in a striking diversity of arrangements, often involving duplications and distributions of the enzymes, transporters or regulators among several replicons in one cell. Moreover, two major *mer* genes, the regulator *merR* and the *merA*, which codes the NADPH-dependent flavoenzyme mercuric reductase [E.C. 1.16.1.1] that is responsible for the reduction of Hg(II) to the volatile and low-toxicity elemental form, are each composed of discrete modules observed in paralogs with distinct but related roles in prokaryotic physiology. Most mercury resistance loci have some means of controlling the expression of the proteins involved in mercury resistance, the Hg(II) uptake and reduction system. This function is carried out by *merR*, a metal-specific activator/repressor of the *mer* operon that also encodes the *merT*, *merP*, and *merA* structural genes. These three components

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(transport, catalysis, and regulation) are the central functions of mercury resistance found in both Gram-negative and Gram-positive eubacteria (3).

Recently, the microbial mercury resistance, dependent of MerA mechanism, has been used for the remediation of mercury contaminated wastewater (3,7,27). Therefore, the identification of Hg resistant strains is essential to the technological development of mercury bioremediation strategies.

The aim of our study was to determine a conservative region of the *merA* gene that could be used as a molecular marker of the *mer* operon, allowing the identification of mercury resistant bacterial strains.

Mercury resistance tests had been made using 100 bacterial strains, isolated from aquatic systems of the Amazon region, Brazil. Cells were grown in Luria-Bertani (LB) broth at 30°C for 24 hours. The growth in the slurries was assessed through optical density readings at 600 nm, and necessary dilutions were made to inoculate multi-wells plates according to the method described by Cursino *et al.* (6) with modifications. The cells were inoculated in agar nutritional medium containing mercury, with the help of a replicator. The mercury concentrations used varied from 0.5 µM Hg to 25 µM Hg, with 2.5 µM Hg intervals. The growth was evaluated after 24, 48 and 72 hours (12).

The alignment of the sequences, deposited in the GeneBank database until the 09/11/2005, that codes the mercuric reductase enzyme (MerA) in bacteria was analyzed using a matrix of similarity of the sequences. The BLASTn program was used with the initial searcher sequence of the partial sequence of the mercuric reductase enzyme, with length of 1141 base pairs (bp), for the *Citrobacter freundii*, access number AJ418049 (8). The alignment of the sequences, in which two regions with high similarity had been identified by visual approach in color key alignment score bigger or equal to 200, is shown in Fig. 1. Among the sequences deposited for the *merA* gene, 86% showed these consensus regions, suggesting that these regions are highly conserved within the *merA* gene. Therefore, they can be used for the development of a very specific molecular marker of the *merA* gene. The selected sequences were employed to design primers using the program available in Stanford Genomic Resources (21). The initial parameters were: Tm equal to 50 and 60°C, amount of CG 40%, and size of the primers between 18 and 25 nucleotides (nt). Primer F1merA hybridized with the Crick ribbon (3' - 5'), in position 251 to the 268 nt of the gene *merA*, while primer F2merA hybridized with the Watson ribbon (5' - 3'), in position 676 up to 693 nt. The sequence of primers: Forward F1merA - 5' TCGTGATGTTTCGACCGCT 3' and Reverse F2merA - 5' TACTCCCGCCGTTTCCAAT 3', 431 base pairs of *merA* fragment with an optimized PCR protocol.

Among the bacteria tested, nine strains showed the highest mercury resistance, they were able to grow in a medium containing 17.5 µM Hg (12). These strains were screened for the presence

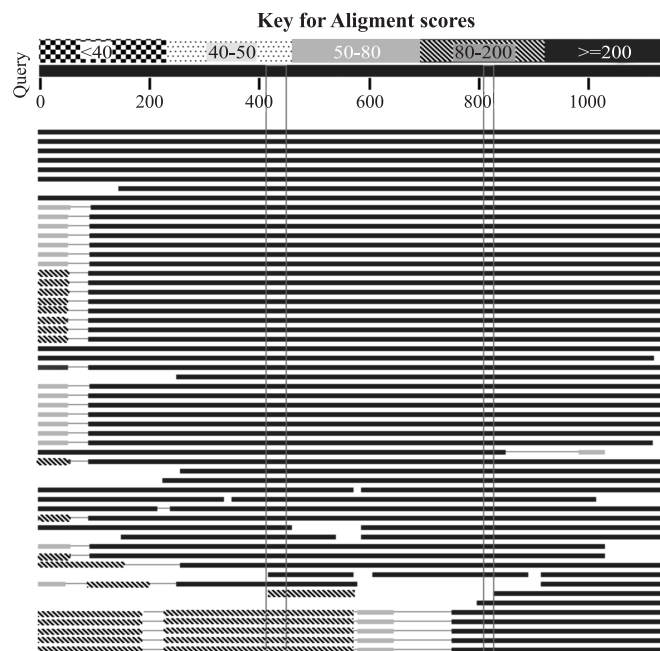


Figure 1. Demonstrative figure generated from the alignment of the sequences for bacterial *merA* gene, described by the pattern key alignment score.

of the *merA* gene in the genomic and plasmidial DNA material. To test the specificity of the method, five bacterial strains sensitive to mercury were randomly selected and used as negative controls (*Morganella morganii* – CPqLMD 12, *Serratia rubidea* – CPqLMD 69, *Proteus mirabilis* – CPqLMD 70, *Serratia marcescens* – CPqLMD 63, *Salmonella sp* – CPqLMD 98). The standard strain *Acinetobacter baumannii* (provided by Dr. Nascimento from UFMG), in which the *merA* gene was shown previously, was used as positive control. The DNA extraction was made as described previously by Sambrook *et al.* (22) and submitted to PCR reaction. The best conditions of amplification reactions were performed in a total volume of 20 µL by using gradient thermal cycler (Mastercycler Gradient, Eppendorf, Hamburg, Germany). The PCR reaction mixture contained 1 U/µL Recombiant *Taq* polymerase (Invitrogen®), 1X Enzyme buffer, 200 µM of each dNTP (Invitrogen®), 1.25 mM MgCl₂, 50 ng of DNA template and 0.5 µM of each primers. The optimized amplification conditions included a pre-denaturation step at 94°C for 1 min; 30 cycles of 94°C for 1 min, 55°C for 1 min, and an extension step at 72°C for 1 min, and end extension at 72°C for 7 minutes. The PCR products were analyzed using gel electrophoresis (1.5% agarose gel, using 90 volts for 1 hour). The gel was dyed with ethidium bromide (0.5 mg/mL) and visualized in transilluminator, using a UV lamp at 365 nm.

Among the nine bacterial strains investigated for the presence of *merA* gene, two strains showed it in genomic DNA:

Enterobacter cloacae (CPqLMD 56) and *Escherichia coli* (CPqLMD 105); while it was detected in plasmid DNA of eight strains: *Klebsiella pneumoniae* (CPqLMD 47), *Enterobacter cloacae* (CPqLMD 56), *Salmonella sp* (CPqLMD 10), *Providencia rettgeri* (CPqLMD 57), *Escherichia coli* (CPqLMD 105), *Morganella morganii* (CPqLMD 32), *Providencia rettgeri* (CPqLMD 93), *Morganella morganii* (CPqLMD 40). Only one bacterial strain analyzed, *Serratia marcescens* (CPqLMD 79), did not show the target fragment either in the genomic or plasmid DNA (Fig. 2), suggesting an alternative Hg resistance mechanism as described elsewhere in the literature (3,10,11). The negative controls showed no amplification, while it was detected a fragment of 431 base pairs in the positive control (data not shown).

The presence of the *merA* gene, detected by PCR analysis in eight strains, was later confirmed by the measurement of the enzymatic activity of the MerA as described in Takeuchi *et al.* (11,24). While in the only strain, where *merA* gene was not detected by PCR, it was also not possible to measure mercuric reductase activity.

Therefore, the results demonstrated the effectiveness of our approach, where a molecular marker for the *merA* gene was developed, and used to identify mercury resistant bacterial strains. This is an essential step in the development of bioreactors, which has a potential use in the bioremediation of mercury contamination.

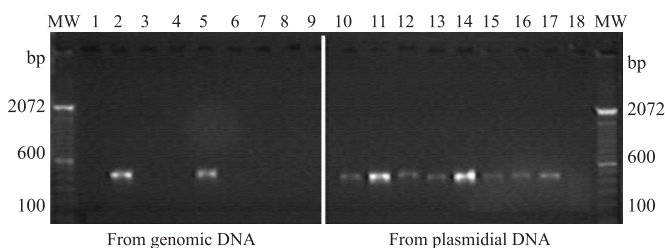


Figure 2. Amplification of 431 bp *merA* fragment from mercury resistant strains using an optimized PCR protocol with primers F1 and F2 on an ethidium bromide-stained agarose gel. On the left hand (lines 1-9) samples from genomic DNA and on the right hand (lines 10-18) from plasmidial. Lines 2 and 11 - *Enterobacter cloacae*; Lines 5 and 14 - *Escherichia coli*; Lines 1 and 10 - *Klebsiella pneumoniae*; Lines 3 and 12 - *Salmonella sp*; Lines 4 and 13 - *Providencia rettgeri*; Lines 6 and 15 - *Morganella morganii*; Lines 7 and 16 - *Providencia rettgeri*; Lines 8 and 17 - *Morganella morganii*; Lines 9 and 18 - *Serratia marcescens*.

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RESUMO

Região Conservada do Gene da Mercúrio Redutase (*merA*) como Marcador Molecular da Resistência Bacteriana ao Mercúrio

O mecanismo de resistência bacteriana ao mercúrio mais comum é baseada na redução do Hg(II) a Hg⁰, através da atividade da enzima mercúrio reductase (MerA). O uso do fragmento de 431 pb amplificado de uma região conservada do gene *merA*, que codifica a enzima MerA, foi utilizado como marcador molecular deste mecanismo, permitindo a identificação de bactérias resistentes ao mercúrio.

Palavras-chaves: gene *merA*; resistência ao mercúrio; bactéria; marcador molecular

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