Bioinformaticand phylogenetic analysisof merR gene to determine thereasons for their different sensitivity to mercury

RoointanAmir[1],Shabab Noushin[1],Rahmani Ali Reza[2], KarimiJamshid[1],AlikhaniMohammad Yousef[3]and SaidijamMassoud[*1]

1. Molecular Medicine and Genetics Department,School of Medicine and Research Center for Molecular Medicine, Hamadan University of Medical sciences, Hamadan, Iran

2. Department of Environmental Health Engineering, Faculty of Health and Research Center for Health Sciences,Hamadan University of Medical Sciences, Hamadan, Iran

3. Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

Corresponding author
SaidijamMassoud ,Associate Professor, Molecular Medicine and Genetics Department, Research Center for Molecular Medicine, School of Medicine, Hamadan University of Medical sciences, Hamadan, Iran,
sjam110@yahoo.com

Abstract: Mercury is a known toxic metalloid that can inhibit many biochemical processes. The *mer* operon which encode mercuric resistance have been found in multicopy plasmids in both gram negative and gram positive bacteria. This operon contains several important genes include *merA*, *merB*, *merT*and*merRrespectively*. Several researches based on molecular techniques showed obvious homology of this operon in plasmids among number of bacterial species. These results suggest that the *mer* operons have an evolutionary precursor from an ancient strain. Moreover, the experimental studies indicated that *mer* operons in bacterial plasmids maybe have different sensitivity to mercury compounds. One of the probable factors for this phenomenon may be *merR* gene (the first cistron of *mer* operon) and its product, MerR protein, which is a trans-acting repressor that regulates expression of *mer* operon. The overall goal of our study was to carry out a set of comparative analyses of *merR* gene and *Mer*R protein in plasmid pBS228 from *Pseudomonas aeruginosa* and merR gene and protein from *Escherichia coli* MS 145 to understand which of them are more sensitive than another. **Methods** : PDB and NCBI databases and Chimera, Mega4, CLC main workbench softwares and CPHmodels 3.2 Server and Prosite servers were applied to perform this study. By using these softwares and servers, multiple analyses including determination of residue composition, secondary structure and motifs, 3D structure, conserved regions, etc. were applied. **Results** : Our results suggest that the sensitivity of mer operon in plasmid pBS228 to mercury compound is more than another one from *E.coli* MS 150. **Conclution** : This deduction may be due to the related MerR protein characteristics such as amino acids composition in alpha helix regions, secondary and tertiary structure, level of interaction with DNA, the N-terminal Hg^{2+} binding site etc. In addition, our phylogenetic analyses suggest that there is an interesting evolutionary relationship in prokaryotes based on *merR* gene.

Keywords: *merRgene, mercury, plasmid pBS228, protein secondary and tertiary structure*

1. Introduction

Mercury is a known toxic metalloid and is among the most toxicant environmental pollutant at the global level (1). Some of the bacteria include gram positives and gram negatives are able to resist into mercury with encoding the proteins in operons called mer operon (2).two main mer operon types have been described : narrow- spectrum mer operons confer resistance to inorganic mercury salt only whereas broad-spectrum mer operons confer resistance to organomercurial compounds (2). Mer operon contains several gene responsive to transport (merT, merC, merP), reduction (merA) and specially regulation (merR) (2). Several researches based on molecular techniques showed obvious homology of this operon in plasmids among number of bacterial species (3) These results suggest that the *mer* operons have an evolutionary precursor from an ancient strain. Moreover, the experimental studies indicated that *mer* operons in bacterial plasmids maybe have different sensitivity to mercury compounds (3). One of the probable factors for this phenomenon may be *merR* gene (the first cistron of *mer* operon) and its product, MerR protein, which is a trans-acting repressor that regulates expression of *mer* operon. In this study we try to compare merR gene and protein from Pseudomonas aeruginosa plasmid pBS228 and Escherichia coli MS 145 to find out which of them are more sensitive than another. The main purpose of this study is selectingthe more sensitivemerR among these two strains.

2. Material and Methods

The merR gene and protein sequences were gotten from NCBI databases. For multiple alignment we used CLC main workbench software (4). In order to phylogenic analyses, construction of evolutionary trees and computation were done by using Mega4 software (5-7). Comparison of conserved domains and 3D-structures were done via Prosite and CPHmodels 3.2 servers and Chimera software (8).

3. Results

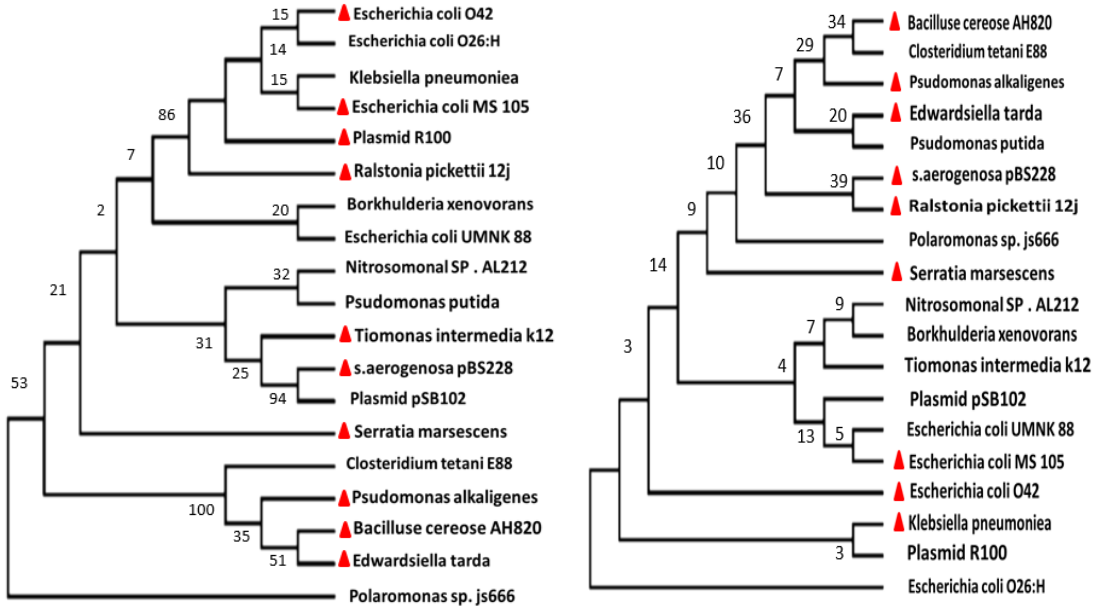
The phylogenic trees were constructed based on nucleotide sequences and amino acid sequences of *merR*gene. In figure 1A and 1B Evolutionary relationships of 19 taxa has been shown.The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 365 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4.

In these figures(1), some taxa which were more relative to *merR* gene and MerR protein from pBS228 or which were have been favorable in *mer* operon studies are marked. Amino acid sequences of the marked taxa are aligned and the important conserved regions are shown in box in figure 2. This region is one of the helix-turn-helix motifs in MerR protein, which is responsible for interaction with DNA sequences and binding to DNA (9).

In the next part we focus on *merR* gene and MerR protein from pBS228 and *E.coli MS 105.* In figure 3 we align nucleotide and amino acid sequences of *merR* gene. You can see the high level of similarity between these two *merR*, but in comparative view,it's easy to see that MerR protein in MS 105 Lacks amino acidsinthe givenpartof the protein and because of this absence,there are no HTH in this part compared with MerR protein from pBS228. Moreover in MerR protein from pBS228 there are three cysteine residues that responsible for binding to Hg^{2+} but in MerR protein from MS 105 because the lack of relevant part, there are only two cysteine residues. This phenomenon is also shown in figure 4.

Figure 4 shows the three dimensional structure of MerR proteins. Here you can see that MerR protein from pBS228 have complete H-T-H domain compared with that is in MS 105. H-T-H motifs are shown in yellow color. Cysteine residues are shown in cyan. These 3D structures were predicted by CPHmodels 3.2 server and edited by using Chimera software

.



A. B.

Fig.1.Phylogenetic tree based on amino acid sequences of MerR protein (A). Phylogenetic tree based on nucleotide sequences of merR gene (B).

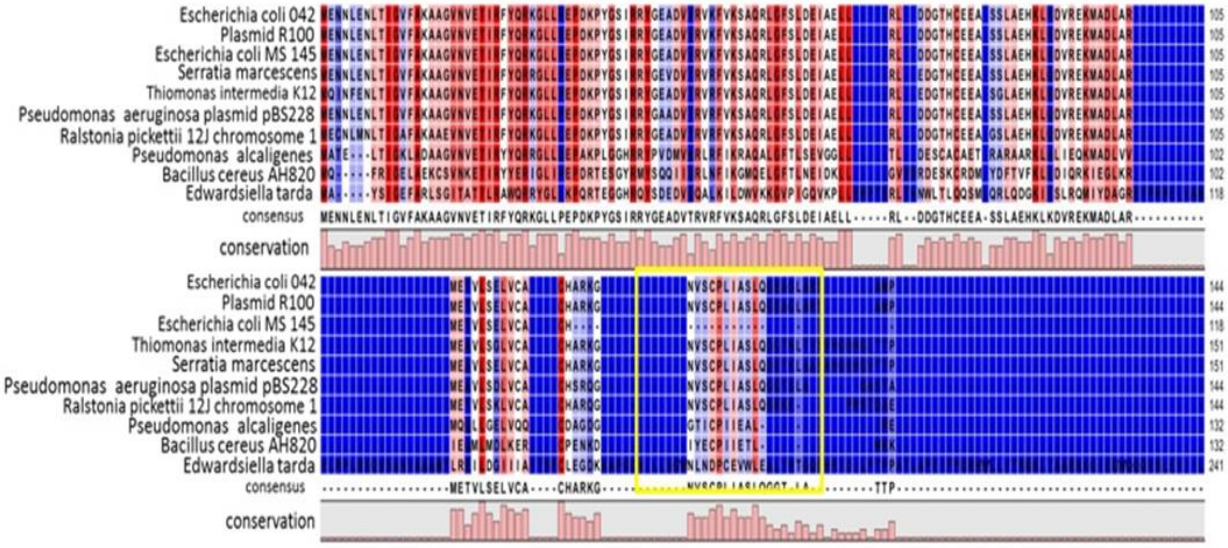


Fig.2. Alignment of amino acid sequences of MerR protein for marked taxa in fig.1A. Alignment was done via CLC main workbench software. Regions which more red color is more conserved

Fig.3. Alignments. The three cysteine are marked with black arrows. You can see here that there are no third cysteine in MerR protein sequence from MS 145

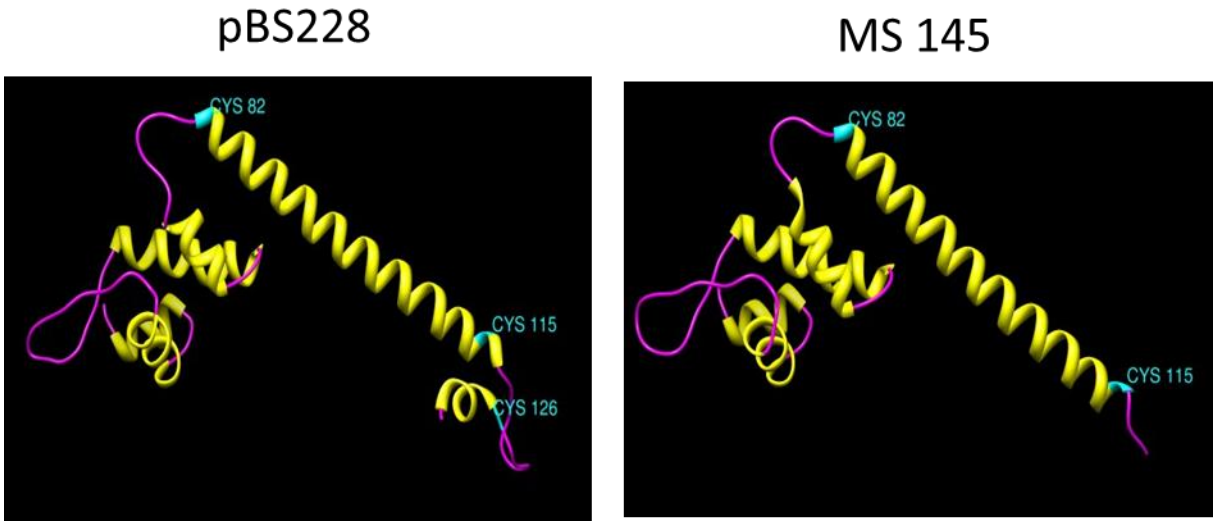


Fig.4. Three dimensional structures of MerR-pBS228 and MerR-MS 145. Structure prediction and post edition were done via 3D-jigsaw and chimera.

4. Discussions

MerR is a member of proteins with winged helix topology and interact with DNA via H-T-H motif .we suggest that difference in amino acid composition related to H-T-H motif may play an important role in different level of sensitivity expression to mercury compounds between merR gene from pBS228 and *E.coli MS 105* through trace on conformation of protein and its reaction with DNA.Due to the absence of several amino acids in H-T-H region ofMerR-MS 105comparedwith merR-pBS228, and also the lack of one cysteine residue at 126 position that respond to mercury binding, there are two suggestion: interaction with DNA in MerR-MS 105may be looser because of Incomplete H-T-H domain in this protein. AnotherSubject isthe lack of cysteine-126 in MerR-MS 105Maycauselesssensitivity to mercury compared with merR-pBS228. This theory results in faster and easier dissociation of MerR-MS 105 from DNA and alsoless sensitivity in the presence of mercury and mercury compounds.The bacterial metalloregulator MerR is the index case of an eponymous family of regulatory proteins, which controls the transcription of a set of genes (the mer operon) conferring mercury resistance in many bacteria. Understanding the functional mechanism of the MerR family will require characterization of the structure, mechanics, and dynamics of the protein and DNA as a function of inducer binding (9).Overall goal of our study was to carry out a set of comparative analyses of merR gene and

MerR protein in plasmid pBS228 and *Escherichia coli* MS 145 to select the best of them for later studies. Our results suggest that such high sensitivity to mercury compounds in mer-containing plasmid pBS228 from *Pseudomonas aeruginosa* may be due to the related MerR protein characteristics such as amino acids composition, Completeness in H-T-H motif, level of interaction with DNA, existence of three cysteine for mercury binding etc.

5. Acknowledgment

This project has been done as a MSc thesis andsupported by deputy of research, HamadanUniversity of Medical Sciences.

.

References

1. Anne Rantala, Luminescent bacteria-based sensing method for methylmercury specific determination, Anal BioanalChem (2011) 400:1041–1049

2. Nascimento AM, Chartone-Souza E. Operon mer: bacterial resistance to mercury and potential for bioremediation of contaminated environments. Genetics and Molecular Research. 2003; 2(1):92-101.,

3. Schlüter A, Szczepanowski R, Pühler A, Top EM. Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. FEMS microbiology reviews. 2007; 31(4):449-77.

4 . www.clcbio.com/products/clc-main-workbench

5 . Kumar S, Tamura K, Nei M. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in bioinformatics. 2004; 5(2):150-63

6. Ronquist F, Huelsenbeck JP. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics. 2003; 19(12):1572-4.

7. Tamura K, Nei M & Kumar S , Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA), (2004), 101:11030-11035.

8. Pettersen, E.F"UCSF Chimera - A Visualization System for Exploratory Research and Analysis." J. Comput. Chem, (2004), 25(13):1605-1612

9. Hao-Bo Guo, Structure and Conformational Dynamics of the Metalloregulator MerR upon Binding of Hg (II), J. Mol. Biol. (2010) 398, 555–557