Bioinformatic and phylogenetic analysis of merR gene to determine thereasons for their different sensitivity to mercury

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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 merR respectively. 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 MerR 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.

Conclusion: 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 Hg²⁺ binding site etc. In addition, our phylogenetic analyses suggest that there is an interesting evolutionary relationship in prokaryotes based on merR gene.

Keywords: merR gene, 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 105 to find out which of them are more sensitive than another. The main purpose of this study is selecting the more sensitive merR 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 phylogenetic 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 phylogenetic 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 acids in the given part of 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.
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 of MerR-MS 105 compared with 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 105 may be looser because of incomplete H-T-H domain in this protein. Another subject is the lack of cysteine-126 in MerR-MS 105, may cause less sensitivity to mercury compared with merR-pBS228. This theory results in faster and easier dissociation of MerR-MS 105 from DNA and also less 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.

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