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BACTERIAL *MER* GENES ARE POTENTIAL GENETIC MATERIAL FOR BIOREMEDIATION ON MERCURY POLUTION

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ABSTRACT

Remediation over mercury-contaminated areas become major concern and needs to be solved. Recently, various parts of ecosystem were contaminated because of increase of mercury emission into environment. Methyl-mercury is the most toxic form, which commonly found in contaminated area. This pollutant was rapidly increased due to uncontrolled emission of mercury from power plan, gold mining process and from other sources. The one possible and reasonable method in mercury bioremediation is by using genetically modified plant expressing bacterial-mercury resistance gene. Group of *Mer* gene was characterized encode mercury transporter and mercury detoxification protein in bacteria. On this study, Blast search on database based on known Mer protein sequence, it shows that these proteins were conserved among bacteria. These groups of genes are potentially to be cloned and transformed into plant for phytoremediation purposes. In order to enhance mercury uptake and reduce its toxicity, multiple genes transformation are considered to be effective approach for transgenic plant construction. This transgenic plant should survive in medium with high concentration of mercury and it should have activities in mercury uptake, detoxification, and Volatilization.

Keywords: ***Mercury, Mer Gene, Blast, Remediation.***

ABSTRAK

Pembersihan kontaminasi logam berat merkuri di berbagai habitat merupakan suatu tantangan yang harus segera diatasi. Akhir-akhir ini kontaminasi merkuri terjaadi semakin meningkat karena emisi yang semakin tidak tekendali dari bernagai kegiatan termasuk pembangkit listrik, penambangan emas, dan dari sumber lainnya. Bentuk logam ini yang paling beracun adalah Metil-merkuri, yang umum ditemukan di tempat yang terkontaminasi. Oleh karena itu, perlu dikembangkan metode dalam mengurangi tingkat kontaminasi logam berat tersebut. Salah satu cara yang banyak dipikirkan ilmuwan adalah dengan tekhnologi phytoremediasi menggunakan tanaman transgeic yang mampu menyerap merkuri dan mengurangi tingkat toxisitasnya. Gen yang bisa digunakan dalam tujuan ini adalah gen Mer yang berasal dari bakteri. Proein Mer memiliki konservasi yang tinggi pada bakteri, dan sangat potensial digunakan sebagai materi genetika dalam penyusunan tanaman transgenik. Dalam rangka meningkatkan

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efficiency dan efektifitas penyerpan logam merkuri, perlu dipertimbangkan untuk melakukan rekayasa dengan menggunakan gene lebih dari satu. Diharapkan, dengan metode ini dapat dikembangkan suatu tanaman transgenik yang mampu menyerap merkuri, mendetoksikasi dan mengubahnya ke bentuk lain.

Kata Kunci : ***Merkuri, Mer Gene, Blast, Remediasi.***

INTRODUCTION

Various pollutants increase rapidly due to human activity in industries, mining, and other pollutants sources in addition to natural sources. High accumulation of pollutants, especially the toxic ones, will threaten human's and other organism's health and ecosystems.

One serious problem in health and ecosystems is significant increase of mercury in environment, which directly and seriously effect to human health (Mozaffarian, 2009, Gardner *et al.*, 2010). Mercury is one ubiquitous global environmental pollutant and the most toxic heavy metal particle, which emitted from power plans, and gold mining process. Recent estimates calculate that the annual global emission is between 4,800–8,300 tons per year (Ruiz and Daniell, 2009). In developed countries, detoxification and environmental clean up were usually done by highly cost technologies (reach to tens of thousands of dollars), which usually based on physical and chemical process. Therefore, finding alternate remediation approaches is a challenging and urgent need. Perhaps, it will be useful and applicable tool, not only for developed and rich countries but also for poor and developing countries.

Bioremediation is reasonable, attractive and challenging technology for environmental decontamination of polluted area. This technology was recently developed intensively due to its benefit, including environmental friendly, cheaper in cost compare to the conventional method, and it can be used in large scale of clean up. In principle, this technology is tried to use exist biological system in order to enhance the absorption and detoxification of pollutants in certain contaminated area. To date, by applying advance cellular and molecular technology, and manipulate the available genetic material, we can construct "bioremediation engine" in various plant and bacteria which very useful in environmental - decontaminations.

Group of *Mer* gene was widely used in this purpose (Hussein *et al*., 2007; Ruiz and Daniell, 2009). Several homologue genes were also reported and thought to be potential genetic materials for mercury detoxification and remediation. Here in this study we try to identify the potential source of material genetics that usually used and review the posibility in developing the geneticly modified plant, which can be used in phytoremediation.

METHODOLOGY

BLAST (Basic Local Alignment Search Tool) on homologue genes ware performed on the NCBI (National Centre for Biotechnology Information) database, using known amino acid sequences as queries. The amino acid sequences were then aligned using the ClustalX program. The phylogenetic tree was inferred using the neighbor-joining method. The data were later visualized as phylogenetic trees using the treeview program.

RESULT

Bacterial genes *MerA* and *MerB* are already identified, as genes that confer bacteria become resistance to mercury compounds. *MerA* gene codes for mercuric ion reductase, which catalyzes the reduction of Hg^{2+} into elemental mercury (Hg^0). *MerB* is a 638 bp gene that encodes a 24 kDa enzyme that undergoes the detoxication of organomercurials by cleavage of the carbon-mercury bond (Hussein *et al.*, 2007). Other homologue of *Mer* gene called *MerC*, MerG, *MerP* and *MerT* were also reported having activity in transporting Hg^{2+} into the cell (Ruiz and Daniell, 2009). *MerC* gene was also successfully to be transformed into Arabidopsis and enhance mercury uptake into the plant, suggesting that plat can be genetically modified with bacterial metal transporter gene (Sasaki *et al.*, 2006).

By performing Protein Blast search on database, and based on known protein sequence of Mer protein we can identify the homologue proteins/genes from other organisms. In this study we used sequence of MerA protein from *Serratia marcescens* as query. Identified homologue proteins were shown in Table 1. The homology of these proteins can also be seen from phylogenetic trees, which drown from the alignment data as shown in Fig. 1. This result indicating that, MerA proteins highly conserved among bacteria. In addition, those bacteria were also can be isolated easily from their habitat. Salmonella, Pseudomnas, Shigella, Bacillus, and Acinetobacter are common bacteria, which usually as a contaminant in foods. In practical view, we can easily to get genetic

material source as a template for cloning and transformation.

We also perform similar Blast search for gene/protein of MerB, and MerG. Table of Blast data were not shown here, but similar result can be drown from the data. *MerB* and *MerG* genes were also conserved among bacterial genes/proteins, as shown in Fig. 2 and Fig. 3 respectively.

DISCUSSION

Mer proteins are the conserved among bacteria, including alpa-protobacteria, gama- protobacteria, proteobacteria and enterobacter. Several genes of this group are already well characterized and already shown to be cloned and to be transferred in to plant. Their activity in transporting mercury element into the cell, and converting the mercury compound into less toxic form were promising process for bioremediation. Blast search on genomic database shows that *Mer* genes, including *MerA*, and *MerG*, can be cloned from various bacteria. This data also suggesting that other *Mer* gene homologue will also easy to be found from various bacteria. Mesophyllic bacteria like Salmonella and shigella are commonly found as food contaminans, and can be isolated by relatively cheep method. This fact is a positive point in developing transformation system in order to generate transgenic plant. We can get the material without any significant difficulties and we do not have to pay just for *Mer* plasmid.

Previous study already showed that transgenic plant expressing MerA (mercuric ion reductase) and MerB (organomercurial lyase) proteins, enhance mercury uptake and detoxification by plant (Heaton et al., 2003; Hussein *et al.*, 2008). Those Transgenic plants (tobacco, rice, Arabidopsis) could accumulate higher mercury within their cell, reduce the toxicity and transform into Hg^0, which is volatilized from plants. Unfortunately, mercuric ion reductase did not protect against the high toxic and environmentally relevant organic-Hg. High accumulation within the cell is potentially disturbing normal cellular function. Consequently, at least both the *merA* and *merB* genes are needed to protect cells from organic-Hg. Other mercury transporter such as *MerG* gene is also potential to be used in generating of mercury-resistance-transgenic plant. *MerG* from Pseudomones genome have been described to be involved in phenylmercury resistance (Kiyono and Pan-Hou, 1999).

Other points should be put on consideration in generating transgenic plant are: 1) which species of plant is suitable for target of mercury remediation, 2) which

genes should be set-up in one construct, 3) where those set of genes, should be inserted (into nuclear genome or into chloroplast genome), and 4) to which sub-cellular location, the mercury compound will be deposited. Several plant species including Arabidopsis, tobacco, poplar, rice, peanut, salt marsh grass and Chlorella have been successfully transformed with *MerA* and *MerB* genes. These transgenic plant shows high tolerant to either organic or anorganic mercury contaminant (Ruiz and Daniell, 2009). But in practice purpose, we should consider in where of area that transgenic plant would be used. For instance, for remediation in seashore, chlorella

Table 1. *MerA* genes/proteins homologue were identified based on Blast Search again MerA protein sequence of *Serratia marcescens.*

Accession	Description
YP_001102037.1	mercuric reductase [Salmonella enterica subsp. enterica serovar Newp
ZP_07794006.1	mercuric reductase [Pseudomonas aeruginosa 39016] >gb|EFQ39102.1|
ADV39899.1	mercuric reductase [Salmonella enterica subsp. enterica]
ADV39896.1	mercuric reductase [Salmonella enterica subsp. enterica]
CAC80891.1	mercuric ion reductase [Acinetobacter sp. ED23-35]
CAD10785.1	putative mercuric reductase [Pseudomonas putida]
CAJ77060.1	Mercuric ion reductase [Acinetobacter baumannii]
EGB77432.1	mercuric reductase [Escherichia coli MS 57-2]
CAC86905.1	mercuric ion reductase [Pseudomonas putida]
NP_569364.1	putative mercuric reductase [Salmonella enterica subsp. enterica serov
CAA70231.1	mercuric reductase [Enterobacter cloacae]
YP_556423.1	putative mercuric reductase [Burkholderia xenovorans LB400] >ref|ZP_
P94702.1	RecName: Full=Mercuric reductase; AltName: Full=Hg(II) reductase >en
YP_001353439.1	putative mercuric reductase [Janthinobacterium sp. Marseille] >gb|ABF
YP_003162664.1	MerA [Pseudomonas putida] >gb|ACU65296.1| MerA [Pseudomonas pu
NP_840913.1	mercuric reductase [Nitrosomonas europaea ATCC 19718] >emb|CAD8
CAD31047.1	mercuric ion reductase [Acinetobacter sp. ED45-25]
ZP_05829612.1	mercuric reductase [Acinetobacter baumannii ATCC 19606] >gb|EEX02
ZP_05826565.1	mercuric reductase [Acinetobacter sp. RUH2624] >sp|Q52109.1|MERA_
ZP_06067642.1	mercuric reductase [Acinetobacter junii SH205] >gb|EEY91713.1| merc
CAD31101.1	mercuric ion reductase [Acinetobacter junii]
YP_004293226.1	mercuric reductase [Nitrosomonas sp. AL212] >gb|ADZ28047.1| mercu
NP_361073.1	MerA protein [Plasmid pSB102] >emb|CAC79204.1| MerA protein [Plas
NP_085424.1	Tn501, mercuric reductase [Shigella flexneri 5a] >ref|YP_145636.1| puta
YP_001427358.1	putative mercuric reductase [Pseudomonas aeruginosa] >emb|CAO917
YP_004704641.1	Tn501 mercuric reductase [Pseudomonas putida S16] >gb|AEJ15761.1|
ACN60170.1	MerA [Bacillus sp. KHg2]
AAC38220.1	MerA [Pseudomonas stutzeri]

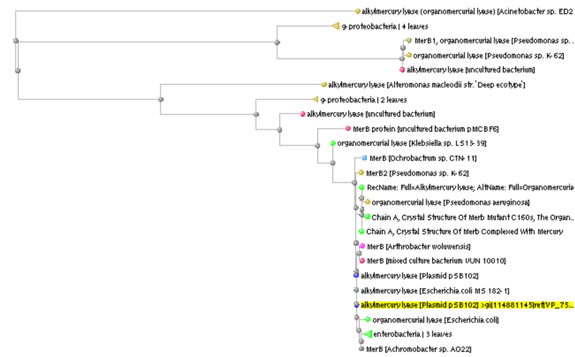

Figure 1. Unrooted phylogenetic trees of MerA proteins. Blast search was performed based on sequence of *Escherichia coli* MerB protein. The trees show conservation of MerB protein in bacteria, including Beta-protobacteria (brown), Gama-protobacteria (dark blue), proteobacteria (green), enterobacter (magenta).

could be better rather than using Arabidopsis or tobacco. Phytoremediation in mercury-contaminated garden will be suitable if we use non-edible plant such as vegetable, in order to avoid mismanagement in harvesting.

In order to set up the best possible transgenic cell/plant for bioremediation, the following model could be proposed: 1) multiple genes could be introduced into one cell/plant, 2) the genes should consisting of transporter to enhance mercury uptake from outside of the cell into inside of the cell (for example *MerG* and *MerC*), 3) there should also containing gene which encode enzyme for reduction of toxic form /detoxification such as *MerA* and *MerB*, 4) there also gene which responsible in accumulation of less

toxic form of mercury into vacuole (need certain transporter of molecule from cytoplasm into vacuole), 5) to reduce the toxic effect of high accumulated mercury within the cell, that could also coupled to a chelator protein like polyphosphate kinase (ppk) or metallothionein (mt), 6) these multiple transgenic could be done based on current possible method, the genes will be introduced either into chloroplast or into nuclear genome.

In summary, cleaning up of contaminated area containing mercury pollutant could be done by phytoremediation using transgenic plant expressing bacterial genes. Transgenic plant, which resistance to mercury, can be generated by introducing multiple genes into suitable plant. So far all evidences were based on experimental scale and need to be tested in the field.

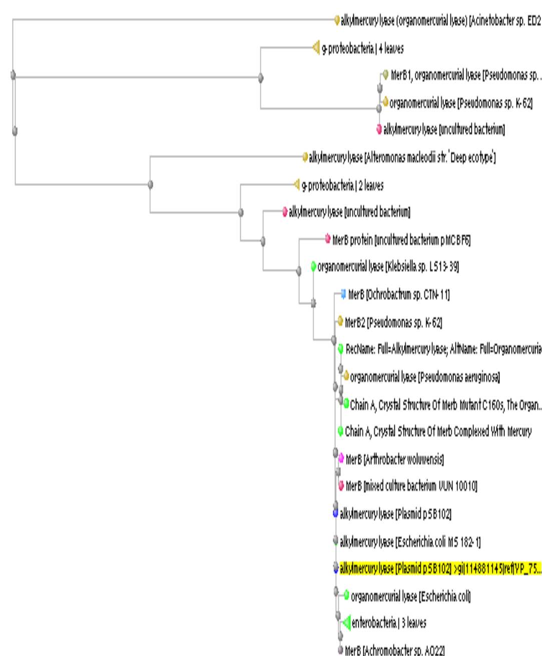

Figure 2. Unrooted phylogenetic trees of MerB proteins. Blast search was performed based on sequence of *Eschecichia coli* MerB protein. The trees show the conservation of MerB protein in bacteria, including Beta-protobacteria (magenta), Gama-protobacteria (dark blue), proteobacteria (brown), enterobacter (light blue).

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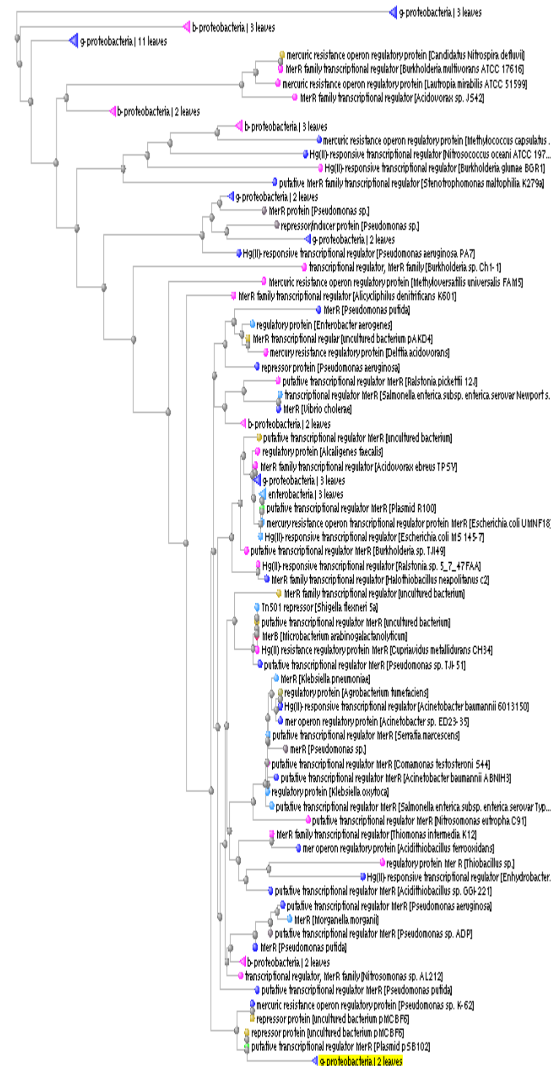

Figure 3. Unrooted phylogenetic trees of MerG proteins. Blast search was performed based on sequence of *Pseudomonas aeruginosa* MerG protein. The trees show the conservation of MerB protein in bacteria, including Beta-protobacteria (magenta), Gama-protobacteria (dark blue), proteobacteria (brown), enterobacter (light blue).