BRAZILIAN JOURNAL OF MICROBIOLOGY

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Environmental Microbiology

Analysis for the presence of determinants involved in the transport of mercury across bacterial membrane from polluted water bodies of India

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ARTICLE INFO

Article history:
Received 7 January 2015
Accepted 14 June 2015

Associate Editor: Miguel Juan Beltran-Garcia

Keywords:
Bioremediation
Mercury
Resistance determinants
Mercury transport

ABSTRACT

Mercury, which is ubiquitous and recalcitrant to biodegradation processes, threatens human health by escaping to the environment via various natural and anthropogenic activities. Non-biodegradability of mercury pollutants has necessitated the development and implementation of economic alternatives with promising potential to remove metals from the environment. Enhancement of microbial based remediation strategies through genetic engineering approaches provides one such alternative with a promising future. In this study, bacterial isolates inhabiting polluted sites were screened for tolerance to varying concentrations of mercuric chloride. Following identification, several *Pseudomonas* and *Klebsiella* species were found to exhibit the highest tolerance to both organic and inorganic mercury. Screened bacterial isolates were examined for their genetic make-up in terms of the presence of genes (*mer*P and *mer*T) involved in the transport of mercury across the membrane either alone or in combination to deal with the toxic mercury. Gene sequence analysis revealed that the *mer*P gene showed 86–99% homology, while the *mer*T gene showed >98% homology with previously reported sequences. By exploring the genes involved in imparting metal resistance to bacteria, this study will serve to highlight the credentials that are particularly advantageous for their practical application to remediation of mercury from the environment.

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http://dx.doi.org/10.1016/j.bjm.2015.11.023

Introduction

Pollution with toxic metals has accelerated dramatically since the beginning of the industrial age. Mercury is the sixth most abundant toxic element among 6 million known toxic substances. Being recalcitrant to biodegradation, it persists in the environment though bioaccumulation, thereby presenting a great threat to human health. Soon after its release into the environment in metal or ionic form, mercury is able to become methylated to highly toxic organomercurial compounds.[1,2] Mercury contamination presents a major health problem owing to its ability to cross the placental and blood-brain barrier.[3,4] Intentional or unintentional exposure to mercury results in acquisition of resistance in bacteria, enabling them to thrive in environments with concentrations far above normal levels. Mercury resistance determinants that occur globally in bacteria from natural environments facilitate their transformation to overcome their deleterious effects on human health.[5–7] The most studied mechanism involves enzymatic transformation based on clustering of different determinants in an operon (*mer* operon). The *mer* operons, which show some genetic variation in structure, are composed of genes encoding functional proteins for regulation (*mer*R, *mer*D), transport genes (*mer*T, *mer*P) and genes involved in reduction (*merB*, *merA*).[8,9] Additionally, genes such as *mer*C, *mer*E, *mer*H and *mer*F (all membrane proteins) are believed to assist in transport functions,[10–12] and *mer*G confers resistance to phenyl mercury.[13,14]

Environmental decontamination of polluted sites remains one of the main challenges for sustainable development. In our previous study, we showed that, among the screened bacterial isolates, only three (*Pseudomonas aeruginosa* (ARY1), *Klebsiella* sp. (ND3) and *Klebsiella pneumonia* sp. (ND6)) contained the broad spectrum mercury resistance operon.[15] These results indicated that resistance in most of our isolates is mediated by other genes of *mer* operons. Although this bacterial resistance system represents a model for biological detoxification of organic mercury, these findings indicate that studies of determinants involved in the transport of mercury across the bacterial membrane is essential before they can be employed to achieve mercury remediation from polluted sites. In continuation of our previous study, the present investigation was carried out to examine the genetic make-up of mercury resistant bacteria in terms of the presence of different genes of the *mer* operon either singly or in combination to deal with toxic mercury. Despite the fact that mercury-reducing bacteria represent an important tool for remediation of contaminated sites, it is still necessary to investigate the genes involved in the transport of mercury (Hg^{2+}) into the cell for reduction to the volatile elemental form to enable design of strategies to combat its removal from the environment. As microbe based detoxification of mercury is on forefront of remediation strategies, studies based on characterization of mercury resistant determinants involved in the transport would provide a good foundation for understanding the complete structure of typical mercury resistance modules among screened bacteria isolates to facilitate their manipulation for bioremediation of contaminated sites.

Materials and methods

Screening of bacteria and growth inhibition assay

Following cold vapor atomic absorption spectroscopy (CVAAS) of collected water samples for the determination of mercury load, screened bacterial isolates were checked for their tolerance to varied concentration of mercuric chloride (10 μM, 100 μM, 1000 μM), by inoculating them in luria broth, followed by incubation at 37 °C for 16–18 h on a rotator platform incubator shaker (SCIGENICS) operating at 250 rpm. *Pseudomonas*

Table 1 – Growth of bacterial isolates in presence of varying concentrations of mercuric chloride.

Conc	*Pseudomonas aeruginosa* (ATCC 9027)	ARY1 (FJ613642)	ARY4 (FJ613643)	ARY2 (FJ613644)	ARY7 (HM149547)	ARY3 (FJ613645)	ARTK3 (HM149545)	ARH4 (HM149546)	ARFA (HM149549)	ARFB (HM149550)
0.1 μM	++	++	++	++	++	++	++	++	++	++
1 μM	++	++	++	++	++	++	++	++	++	++
10 μM	++	++	++	++	++	++	++	++	++	++
100 μM	+	++	++	+	++	+	++	+	++	++
1000 μM	–	++	++	–	++	–	++	–	–	–
10,000 μM	–	–	–	–	–	–	–	–	–	–

Conc	ARKK (HM149548)	ARSA3 (HM149552)	ARSA4 (HM149551)	ARR4 (HM149544)	ND1 (JF927778)	ND2 (JF927779)	ND3 (JF927780)	ND5 (JF927781)	ND6 (JF927782)	ND7 (JF927783)
0.1 μM	++	++	++	++	++	++	++	++	++	++
1 μM	++	++	++	++	++	++	++	++	++	++
10 μM	++	++	++	++	++	++	++	++	++	++
100 μM	++	+	+	++	++	++	++	++	++	–
1000 μM	–	–	–	++	–	–	++	++	++	–
10,000 μM	–	–	–	–	–	–	–	–	–	–

++, good growth; +, less (late) growth; –, no growth.