Study of merA Gene in Gram-negative Bacteria from Brazilian Aquatic Systems as a First Step to Select Microorganisms to Bioremediate Mercury Pollution

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Studies about trace metal pollution is a priority in National Health Programs.

**Mercury** is considered the most toxic among trace metals

Neustadt & Pieczenik, 2007

INC 1, 7 - 11 June 2010, Stockholm, Sweden
INC 2, 24 - 28 January 2011, Chiba City, Japan
INC 3, 31 October - 4 November 2011, Nairobi, Kenya,
INC 4, 27 June - 2 July 2012, Punta del Este, Uruguay,
INC 5, 13 - 19 January 2013, Geneva, Switzerland

MERCURY(Hg)

- It is present in several forms:
  - metallic Hg
  - organic Hg
  - inorganic Hg

- It can be founded in three different oxidation states that are easily interconverted in the environment:
  - 0, +1, +2
MERCURY(Hg)

≠ chemical species
≠ physical-chemical property
≠ toxicity

Organomercury
> toxicity

Gherini & Summers, 1988
EXAMPLE: Minamata Disaster
MERCURY (Hg)

Resistance = Hg conversion

mer operon

Narrow (merA)  Broad (merA+merB)

Adapted from Barkay et al., 2003
MERCURY(Hg)

Adapted from Barkay *et al.*, 2003

MerA
mercuric ion reductase

MerR metal-responsive regulatory protein

MerT transport

MerB organomercuriolyase

MerC secondary transport

MerD activation antagonist

MerP periplasmatic protein
Mercury is an important global pollutant.

Mercury biogeochemical cycle has been altered by anthropogenic emissions.

Methylmercury is biomagnified through food chain, reaching high levels at the top.

Bacteria are capable of converting Hg to less toxic form and can be used to reduce Hg emissions to the environment.
OBJECTIVE

Identify *merA* gene in phenotypically Hg resistant bacteria from “*Bacteria Collection Resistant to Environmental Pollutants*” from the Environmental Health and Sanitation Department, National School Of Public Health – Oswaldo Cruz Foundation
Sampling and Isolation,

Determination of Hg’s Phenotypic Resistance Level ($\text{MIC}_{\text{Hg}}$),

Biochemical Identification and Strains Storage

were previously performed by the research group
Water Sampling

Bacterial Isolates

Adapted from Duque, 2012
Bacterial Samples’ Selection

Highest Hg resistant Gram-negative Strains \((\text{MIC}_{\text{Hg}} \geq 4 \text{ mg Hg L}^{-1})\)

DNA Extraction

Sambrook \textit{et al.} (1989)

\textit{merA} Gene’s Detection (\textit{PCR Method})

Primers:
- A1 Forward 5’- ACCATCGGCGGCACCTGCGT-3’
- A5 Reverse 5’- TTGGTCCCCTACCTGACGATGGT-3’

\text{PCR Mix:}\n\begin{align*}
100 \text{ ng A1} &+ 100 \text{ ng A5,} \\
0.05 \text{ mM of each dNTP,} & \\
3 \text{ mM MgCl}_2, & \\
1.5 \text{ U Taq Pol (Promega®, USA),} & \\
\text{final volume 50 }\mu\text{L} &
\end{align*}

\text{Amplifications Condition:}\n\begin{align*}
1 \text{ cycle } 95^\circ\text{C}/5\text{´}, & \\
39 \text{ cycles} & \\
(94^\circ\text{C}/30\text{´}+64^\circ\text{C}/30\text{´}+72^\circ\text{C}/1), & \\
1 \text{ cycle } 72^\circ\text{C}/10\text{´} &
\end{align*}
DNA Sequencing

Chain Termination Method

DNA Sequencing Platform PDTIS/FIOCRUZ-RJ
(http://plataformas.cdtis.fiocruz.br/subunidade/exibe_sub/1)

Primers:

A1 Forward 5’- ACC ATC GGC GGC ACC TGC GT-3’;
A5 Reverse 5’-TTG GTC CCC TAC CTG ACG ATG GT-3’;

merABR F 5’-ACA TTC CCG AAC GCC TTG CAG TAA- 3’
merABR R 5’-TTA CTG CAA GGC GTT CGG GAA TGA-3’

Sanger, 1977
Liebert et al., 1997
De Falco, 2013
1st step: Sequencing using A5 primer

false positive screening

2nd step: Sequencing using A1, merABR F and merABR R primers

Efficient complete sequencing
Analyses of obtained sequences

BioEdit Program → Analyze Sequences Chromatograms
BLAST Program → Identify Sequences as *merA*

Multiple Align of Partials *merA* Genes Detected on the Study

T-COFFEE Multiple Alignment Program

Phylogenetic Tree Design and Analysis

CLUSTAL-W Program
FRAGMENT CONSTRUCTION
FRAGMENT CONSTRUCTION
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FRAGMENT CONSTRUCTION

AAGGGCACTTTAA-CACTTTCGGA\nCCGCGCCCTTTAACTCCTGAAAC\nCCGCGCCCTTTAAAAGAATCGCAAC

... ... ...

AAGGGCACTTTAA-CACTTTCGGA\nCCGCGCCCTTTAACTCCTGAAAC\nCCGCGCCCTTTAAAAGAATCGCAAC

... ... ...

AAGGGCACTTTAA-CACTTTCGGA\nCCGCGCCCTTTAACTCCTGAAAC\nCCGCGCCCTTTAAAAGAATCGCAAC

... ... ...

AAAAACACTTGATAGTATA... + ...AAAAACACTTGATAGTATA... + ...AAAAACACTTGATAGTATA... + ...AAAAACACTTGATAGTATA...
RESULTS & DISCUSSION

Sampling: 191 Bacterial Strains

Selection: 150 Gram-negative Bacterial Strains, $\text{MIC}_{\text{Hg}} \geq 4 \text{ mg L}^{-1}$

Growth: 125 Bacterial Strains

Pure Isolated Strains: 110 Bacterial Strains in Accordance to Required Conditions

$merA$ gene Detection: 69 Bacterial Strains (62.7%)
RESULTS & DISCUSSION

*merA* Gene Sequencing Results:

1*st* step: Sequencing using A5 primer
- 60 sequences

2*nd* step: Sequencing using A1, *merABR* F and *merABR* R primers
- 44 sequences (1115 bp, compared with 1234 bp target)

Sequences Classified by Geographic Origin
- South-East (RJ): 18.2%
- South (RS): 11.4%
- North (RO): 15.9%
- Middle-East (MT): 4.5%

Sequences Classified by Sampling Point
- Domestic & Industrial Effluents: 70.5%
- Fish-Farming: 18.6%
- Mining Effluents: 11.4%
- Dam: 4.5%
RESULTS & DISCUSSION

merA Multiple Alignment Results

All Studied Sequences’ Multiple Alignment

Identity (Sequence vs Target) = 91 to 99%

Global Alignment Identity = 84%

Individual Identity on Global Alignment = 78 to 86%

All Deposited Sequences’ Multiple Alignment

(Limits: UNTIL 06/03/2013; NOT Uncultured bacteria; 50 seq > Identity)

Identity (Sequence vs Target) = 84 to 96%

Global Alignment Identity = 62%

Individual Identity on Global Alignment = 33 to 66%
merA Multiple Alignment Results (Phylogenetic Tree)
RESULTS & DISCUSSION

merA Gene’s Multiple Align Result (Phylogenetic Tree)

Domestic & Industrial Effluents (n=31)
- Mining Effluents (n=6)
  - Fish-Farming (n=5)
    - Dam (n=2)

Klebsiella pneumoniae (n=13)
- Enterobacter cloacae (n=13)
- Pantoea sp. (n=2)
- Klebsiella oxytoca (n=1)
- Escherichia coli (n=1)
- Enterobacter sp. (n=1)

Sampling Point

Biochemical Identity

Geographic Origin

- Rio de Janeiro (n=24)
- Mato Grosso (n=8)
- Rio Grande do Sul (n=7)
- Rondônia (n=5)
Presence of merA Gene Polymorphism

Individual Identity on Global Alignment

- RO: 100%
- RJ: 87.5%
- RS: 100%
- MT: 60.0%

88.6% to 86%
The *merA* gene was found in the majority of the isolates;

The use of two new primers - *merABR R* and *merABR F* - has increased the sequencing efficiency;

The bioinformatics analysis of multiple alignments showed high identity between the sequences;

The identity increased if the data was grouped based on the collection points, supporting the hypothesis of horizontal communication among bacteria that belonged to the same aquatic environment;

A sequence deletion was detected in 86.4% of the studied strains.
FUTURES GOALS

- Identify all the studied strains;
- Detect \textit{merB} gene on the strains;
- Analyze the 12pb deletion with proteomic study, including 3D modeling, coupled with activity assays of the MerA enzyme;
- Analyze the ability of the most interesting strains to grow in bioreactor condition.
ACKNOWLEDGMENTS

- Oswaldo Cruz Foundation – Fiocruz, Ministry of Health, Brazil
- National Research Council – CNPq
- Research Support Foundation of Rio de Janeiro State – FAPERJ
- Genomic Platform - DNA Sequencing of Oswaldo Cruz Foundation – PDTIS/FIOCRUZ
...THANK YOU FOR YOUR ATTENTION
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<th>Region</th>
<th>Aquatic System Sampled</th>
<th>N° samples</th>
<th>Consensus (%)</th>
<th>Max (%)</th>
<th>Min (%)</th>
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