CLONING AND EXPRESSION OF *PSEUDOMONAS AERUGINOSA* PA01 GENES IN *E.COLI* DH5A FOR THE DETECTION OF ARSENIC IN CONTAMINATED WATER SAMPLE

A report

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UNDER THE GUIDANCE

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1. **TITLE OF THE PROJECT & PROJECT PROPOSAL REFERENCE NO.:** CLONING AND EXPRESSION OF *PSEUDOMONAS AERUGINOSA* PA01 GENES IN *E.COLI* DH5A FOR THE DETECTION OF ARSENIC IN CONTAMINATED WATER SAMPLE

**PROJECT PROPOSAL REFERENCE NO.:** 40S_BE_1001

2. **NAME OF THE COLLEGE & DEPARTMENT:** PES Institute of Technology, Bangalore, Department of Biotechnology.

3. **NAME OF THE STUDENTS & GUIDE (EMAIL ID & CELL NO.):** Ms. Jaishree S (USN 1PI13BT015) & Ms Sanjana B (USN1PI13BT043); Dr. Sasmita Sabat (sasmitasabat@pes.edu, 9845103846)

4. **KEY WORDS:** Arsenic, Whole cell biosensor, *Pseudomonas aeruginosa* PA01, GFP.

5. **INTRODUCTION:** The large population in various parts of the world is using portable water contaminated with arsenic concentration much higher to its permissible level. Thus arsenic is becoming the most abundant toxic element in the environment and also considered as threat to living organisms. Detection & identification of such contaminant knowing its bioavailability in the environment for consuming becomes very important. Arsenic is mainly present in two forms: organic and inorganic, the inorganic being highly toxic. The legal limit for arsenic in water by WHO is 10μg/L.

Over the recent past, the increased concentration of Arsenic in drinking water has caused an ecological threat for people around the globe. Utilization of ground water specifically in India and Bangladesh has found to cause new health issues due to arsenic contaminations. The Asian countries are more affected in the recent past. In the year 2000 it was observed that there were only five major places of Arsenic contamination in Asian countries (India, Bangladesh, China, Nepal and West Bengal). But during 2000-2005, Arsenic related health issues started showing up in different parts of Asia, some of which are China, Nepal, Pakistan, Afghanistan etc. Arsenic as it is being termed as one of the heavy metal is not abundantly found in the Earth’s crust compared to other elements. Arsenic can be readily solubilized in ground waters which are influenced by pH, temperature, redox conditions and solution compositions. The occurrence of arsenic in natural water sources above 10μg/L makes it highly unfit for consumption. Man-made sources like mining of ores, waste processing, poultry and pesticides have majorly caused arsenic contamination in soil and ground water.

The chemical methods formulated for the quantification has been found to be uneconomical and even hazardous. To overcome these difficulties it would be rather possible to develop a biosensor which can help in the detection of the analyte rapidly and also remains cost-effective. Whole cell biosensor consists of genetically engineered genes in bacteria which are capable for sensing presence of the analyte, coupled with a reporter gene like GFP. These biosensors are rapid,
portable and enable the detection process with high sensitivity and accuracy for the analyte present in various sources.

In the present project work, the gene sequences selected from NCBI database were arsR, arsC and arsB genes which were isolated from P. aeruginosa PA01. The clones developed during this work can measure the arsenic between 10ppb-100ppb effectively. These results are remarkable as the permissible level for arsenic in domestic water recommended by WHO is 10ppb. The intensity of fluorescence indicates the presence or absence of arsenic in the given water sample. The level of gene expression was confirmed using IPTG as an inducer and performing Native PAGE to confirm the protein concentration. The quantification of arsenic was found to be 8.579μg/L for 10ppb and 2.451μg/L for 100ppb concentrations by performing AAS.

**6. OBJECTIVES:**

- a. Isolation of genomic DNA from *Pseudomonas aeruginosa* PAO1 using and LB broth.
- b. Designing of primer for the selected genes (Ars B, Ars C, Ars R).
- c. Amplification of genes using PCR.
- d. Expression of genes into pUC18 & pBluescript for two fragments (Fragment I: GFP+ArsR+ArsC & Fragment II: Ars B+His Tag respectively).
- f. Expression of the genes into whole cell bacteria (*E.coli* DH5α).
- g. Isolation of plasmid DNA and screening of the transformed clones from the *E.coli* DH5α.
- h. Quantification of arsenic in water sample using Atomic Absorption Spectrophotometry.

**7. METHODOLOGY:**

- a. Selection of the organism *Pseudomonas aeruginosa* PAO1 MTCC 3541 and procured from IMTECH, Chandigarh.
- b. Revival of the Culture in the nutrient broth and subcultured in nutrient agar.
- c. Inoculated in the Luria Bertini broth for quantification of DNA.
- d. Isolation of genomic DNA and observation by gel electrophoresis.
- e. Primer designing using Primer 3 tool and amplified the genes ArsR, ArsC & ArsB identified from NCBI database using PCR. Gel electrophoresis for the genes site confirmation and the identification of the molecular size of the DNA fragment.

**PCR conditions:**

<table>
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<tr>
<th>94°C</th>
<th>94°C</th>
<th>55°C</th>
<th>72°C</th>
<th>72°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>30 sec</td>
<td>30 sec</td>
<td>1 min</td>
<td>10 min</td>
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</table>

35 cycles
Design of the Experiment

f. Cloning of Fragment I in pUC18 and Fragment II in pBluescript cloning vector with the restriction sites Ndel/EcoRI and EcoRI/Xhol respectively.
g. DNA ligation with the cloning vectors. Isolation of plasmid DNA and run of gel from the h. Transformation in competent cell E.coli strain DH5a.
i. Screening and selection of clones from the transformed clones.
j. Analysis if arsenic in the contaminated sample using UV radiation and AAS.

Design of the Experiment – Fragment I

Design of the experiment – Fragment II

a. Quantification of arsenic intake by clones using AAS:

Inoculation of the clones from fragment 1 and 2 in LB broth:

Once the cells were transformed on the LB agar plates, one colony each from fragment 1 and fragment 2 clones were inoculated in 10ml of LB broth kept for growth at 37ºC overnight.

Induction using IPTG

The overnight grown cells were added with different concentrations of Sodium Arsenite (0.01ppm, 0.1ppm, 1ppm, 10ppm, 100ppm, 1000ppm) and IPTG of 1mM(10µl in each vial) kept for incubation at 37ºC for 4 hours.

Detection and Quantification of arsenic using expression host

Transformed clones were inoculated in 10ml of LB broth and incubated at 37ºC for 18 hours.
After 18 hours of incubation sodium arsenite of different concentrations in ppb (blank, 20, 40, 60, 80, 100) were added to the culture. Incubated at 37ºC for four hours. Then AAS was performed to determine the concentration of arsenic uptake by the clones and test water sample.

8. RESULTS & CONCLUSION:
a. Isolation of genomic DNA after revical in nutrient broth medium. Gel images of gDNA and quantification using UV spectrophotometry.

Genomic DNA band observed under UV transilluminator

Absorbance of Genomic DNA at 260nm was found to be 0.278

1% agarose gel showing bands of the amplified genes
1- PCR amplicon of Ars R gene (350bp)
2- PCR amplicon of Ars C gene (450bp)
3- PCR amplicon of Ars B gene (1.3 Kb)
L1-100bp DNA Ladder
L2-500bp DNA Ladder
Transformed Clones of Fragment I and fragment I

Clones of Fragment I and Fragment II without IPTG
Emission of Fluorescence by clones after addition of sodium arsenite of various ranges from: 0.01ppm-1000ppm

Fluorescence under UV light by Clones of fragment I and fragment II with different concentration of IPTG (0.0mM-0.5mM) induction added sodium arsenits 10ppb and 100ppb after 2hours.

**Table:** Concentration of arsenic intake by clones

*(Note: 1ppb = 0.001mg/L)*

<table>
<thead>
<tr>
<th>Concentration of arsenic added to the clones(ppb)</th>
<th>Concentration of arsenic intake by clones(µg/L)</th>
</tr>
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<tbody>
<tr>
<td>Blank</td>
<td>0.00</td>
</tr>
<tr>
<td>20</td>
<td>6.925</td>
</tr>
<tr>
<td>40</td>
<td>3.814</td>
</tr>
<tr>
<td>60</td>
<td>3.041</td>
</tr>
<tr>
<td>80</td>
<td>2.524</td>
</tr>
<tr>
<td>100</td>
<td>2.451</td>
</tr>
<tr>
<td>contaminated water sample</td>
<td>1.336</td>
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Protein bands obtained by Native PAGE

Lane description:
- L1: control
- L2: fragment 1
- L3: fragment 2
- L4: 0.1mM IPTG + fragment 1 + fragment 2
- L5: 0.2mM IPTG + fragment 1 + fragment 2
- L6: 0.3mM IPTG + fragment 1 + fragment 2
- L7: 0.4mM IPTG + fragment 1 + fragment 2
CONCLUSION:
The main aim of this project was to construct a bacterial based whole cell biosensor with a novel
gene of interest, which can be used for the detection of arsenic in the contaminated water. So to
close the novel gene into an expression vector is essential. The combination of genes selected from
\textit{P.aeruginosa} PA01 in order to fulfill the objectives was found to be excellent in detecting the arsenic
sample in contaminated water to lower concentration of 10ppb. The vectors used in this study were
primarily cloning vectors with high copy number which help in amplification of the insert genes
also can induce expression using IPTG as an inducer. The \textit{E.coli} DH5α was used as the cloning
strain, as it shows higher transformation efficiency factor and also ensures stability of the inserted
genes. The transformed clones were further used for the detection and analysis of sodium arsenite at
different concentrations. The expression of proteins was induced by addition of IPTG.

Native PAGE was performed to check the expression of both the fragments as well as analyze the
best IPTG concentration used in bringing about the expression of the proteins.

Atomic Absorption Spectrophotometry was carried to check for the concentration of arsenic taken
up by the engineered organism for different concentration of sodium arsenite added along with
arsenic contaminated water as the test sample.

9. SCOPE OF THE WORK:

a. To measure the arsenic concentration in water sample using genetically modified organism as a
biosensor. This biosensor is a non-polluting, non-hazardous, long-term tool, cost effective and rapid
analysis for the presence of the contaminant.