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Gene expression analysis of the arsC Gene involved in arsenic detoxification in Pseudomonas stutzeri and Pseudomonas putida

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Abstract:

Arsenic bioremediation using microorganisms is an effective environmental solution for reducing heavy metal pollution, due to its high efficiency and low cost compared to traditional methods. In this study, we focused on analyzing the expression of the *arsC* gene, known for its vital role in reducing the highly toxic trivalent arsenic [As(III)] to its less toxic pentavalent form [As(V)], in two bacterial strains: *Pseudomonas stutzeri* and *Pseudomonas putida.* These strains were treated with escalating concentrations of sodium meta-arsenate ($NaAsO_2$) at 25, 50, 100, 150, and 200 ppm. Gene expression levels were measured using RT-qPCR using the ΔCT and ΔΔCT methods. The results showed that *arsC* gene expression gradually increased with increasing arsenic concentration in the culture medium, indicating that the strains responded to toxic stress in a regulated manner. In *P. putida,* fold change values ranged from 9.63 at the lowest concentration to 57.47 at the highest concentration, while *P. stutzeri* showed a higher response, ranging from 15.71 to 85.91, respectively. Statistical analyses (ANOVA) revealed significant differences between the different concentrations ($p < 0.0001$), reinforcing the hypothesis that *arsC* gene activation is related to the severity of arsenic exposure.

These results highlight the effective role of both *P. putida* and *P. stutzeri* in resisting arsenic through precise gene regulatory mechanisms. This makes them promising strains for use in bioremediation techniques for arsenic-contaminated environments and opens new avenues for employing molecular analysis to evaluate the effectiveness of microorganisms in environmental remediation.

Keyword: Arsenic , arsC Gene , *Pseudomonas stutzeri , Pseudomonas putida.*

Introduction:

Agricultural soil is a vital natural resource that directly impacts the sustainability of food production, human health, and the ecosystem as a whole. With increasing environmental pressures and intensive human activities, particularly the excessive use of chemical fertilizers and pesticides, rates of soil contamination with heavy metals have increased, leading to a deterioration in soil quality and a threat to its biological functions. Among these elements, arsenic occupies a special position due to its high toxicity and its ability to accumulate in soil and plants, as well as its transformation into various forms that differ in their ability to be absorbed and transferred within the food chain (Pikuła et al., 2021; Xiang et al., 2021; Saleh and Al-Hassani, 2025).

Arsenic is transferred to agricultural soil either through natural sources, such as the decomposition of rocks containing it, or through human sources, such as the use of contaminated irrigation water, pesticides, and phosphate fertilizers, leading to its accumulation over time in the surface layers of the soil (Patel et al., 2023; Khan et al., 2021). The danger of arsenic lies in its solubility and translocation to plants, making it a food contaminant with serious health consequences, especially if it enters agricultural products consumed by humans (Tang & Zhao, 2021; Khalaf and Rahim, 2025).

In response to these challenges, bioremediation technologies have emerged as a sustainable environmental solution that relies on the ability of certain microorganisms to transform or fix toxic elements. Among the most prominent of these organisms are *Pseudomonas* bacteria, particularly *Pseudomonas stutzeri* and *Pseudomonas putida,* which have demonstrated remarkable arsenic resistance thanks to their genetic system, which includes genes such as *arsC*, responsible for reducing toxicity or expelling arsenic from the cell (Zhao et al., 2023; Sevak & Pushkar, 2023).

The development of molecular techniques, particularly quantitative gene expression analysis techniques such as qPCR, has deepened our understanding of arsenic resistance mechanisms at the cellular level. The expression of *ars* genes is an effective indicator for assessing the efficiency of microorganisms in contaminated environments (Chen et al., 2022; Ali et al., 2021). This methodology is an important tool for selecting promising bacterial strains for use in soil bioremediation applications.

Based on the above, there is a need for systematic studies aimed at exploring the potential of indigenous bacteria to cope with arsenic pollution and understanding the factors affecting their bioefficiency. This will open new horizons for practical and safe solutions to the environmental challenges facing modern agriculture.

The physical and chemical properties of agricultural soil significantly influence the mobility and accumulation of arsenic within the farming environment. Factors such as soil pH, organic matter content, and the composition of various minerals play a crucial role in determining the bioavailability of arsenic to plants and microorganisms. Therefore, understanding these environmental factors is essential for developing effective bioremediation strategies, as modifying soil conditions can enhance the activity of arsenic-resistant bacteria and improve their detoxification capabilities.

Moreover, analyzing the gene expression of arsenic resistance-related genes, such as arsC, serves as an important tool for assessing bacterial adaptation and response to contamination under different environmental conditions. This approach allows for insights into the dynamics of genetic regulation and the degree of activation of resistance mechanisms when exposed to varying levels of arsenic stress. By integrating molecular studies with environmental assessments, advanced bioremediation techniques can be developed that contribute to maintaining soil health and supporting sustainable agricultural systems.

Materials and Methods:

This study was designed to evaluate the efficiency of *Pseudomonas stutzeri* and *Pseudomonas putida* in expressing the arsC gene, which plays a critical role in reducing arsenic toxicity through detoxification mechanisms.

Bacterial Preparation and Arsenic Treatment:

solates of *Pseudomonas putida* and *Pseudomonas stutzeri* were initially revived and cultivated in liquid Luria Bertani (LB) broth, a nutrient-rich medium commonly used to support the growth of a wide range of bacteria. To enhance the bacterial metabolic activity and biomass production, the LB medium was supplemented with 1% (w/v) glucose as an additional carbon source. This supplementation helps ensure optimal growth conditions and supports bacterial cells in entering a robust, active growth phase.

The bacterial cultures were incubated at 30°C with constant shaking at 150 revolutions per minute (rpm) to maintain adequate aeration and homogeneity. Growth was monitored periodically by measuring the optical density at 600 nm (OD_{600}) using a spectrophotometer. The cultures were allowed to grow until they reached mid-logarithmic phase (exponential phase), a stage characterized by rapid cell division and heightened metabolic activity, which is optimal for studying gene expression responses.

Once the desired growth phase was achieved, bacterial cultures were subjected to treatment with sodium meta-arsenite ($NaAsO_2$), a trivalent arsenic compound known for its high toxicity. The arsenic was applied in graded concentrations of 25, 50, 100, 150, and 200 parts per million (ppm), which represent sub-lethal to relatively high toxic levels. This gradient of arsenic concentrations was carefully chosen to simulate varying environmental arsenic stresses and to investigate the bacterial adaptive responses and tolerance mechanisms at the molecular level.

Following exposure, the bacterial cultures were incubated under the same conditions for a defined period to allow for arsenic uptake and induction of stress-related gene expression. These conditions aimed to mimic environmental contamination scenarios and to facilitate the assessment of differential expression of genes involved in arsenic detoxification, such as arsC, as well as other stress response pathways.

Nucleic Acid Extraction and Quantification:

Genomic DNA (gDNA) was isolated from bacterial cells using the Presto™ Mini gDNA Bacteria Kit, a commercially available kit designed for rapid and efficient purification of high-quality DNA from bacterial cultures. The extraction procedure was conducted in strict accordance

with the manufacturer's instructions to ensure maximum yield and purity. Briefly, bacterial cells harvested from cultures were subjected to cell lysis using a specialized buffer containing detergents and enzymes that disrupt the bacterial cell wall and membranes. This was followed by removal of proteins and other contaminants through a series of binding, washing, and elution steps performed in spin columns, resulting in purified genomic DNA suitable for PCR amplification and other molecular biology applications.

In parallel, total RNA was extracted from the bacterial cells using the easy-BLUE™ Total RNA Extraction Kit. This kit facilitates the isolation of intact RNA by effectively lysing cells and protecting RNA from degradation by RNases. The procedure involved cell lysis with RNA stabilization reagents, phase separation using acidic phenol-chloroform to remove DNA and proteins, and RNA precipitation with isopropanol. The RNA pellet was then washed and dissolved in RNase-free water. Throughout the extraction process, stringent RNase-free techniques were employed to prevent RNA degradation.

To further ensure the purity of RNA samples and eliminate any contaminating genomic DNA that may interfere with downstream applications such as reverse transcription and quantitative PCR, the extracted RNA was treated with DNase I enzyme. This enzymatic treatment selectively degrades residual DNA without affecting the RNA integrity.

The quality and concentration of both DNA and RNA extracts were assessed using a NanoDrop spectrophotometer, which measures the absorbance of nucleic acids at specific wavelengths. The absorbance ratio at 260 nm and 280 nm (A260/A280) was used as an indicator of purity, with values around 1.8–2.0 indicating high-quality, protein-free nucleic acids. Additionally, the RNA integrity was confirmed by assessing the 28S/18S rRNA ratio through gel electrophoresis or using an automated bioanalyzer when applicable. These quality control measures ensured that the nucleic acid samples were suitable for sensitive downstream molecular analyses, including gene expression studies and genomic investigations.

cDNA Synthesis and RT-qPCR:

Following the extraction and quality assessment of total RNA, the samples were subjected to reverse transcription to synthesize complementary DNA (cDNA), which serves as a stable template for quantitative gene expression analysis. Reverse transcription was carried out using Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase, an enzyme commonly employed for efficient and high-fidelity conversion of RNA into single-stranded cDNA. The reaction mixture included purified RNA, oligo(dT) primers or random hexamers, dNTPs, and the reverse transcriptase enzyme, incubated under optimized temperature conditions to facilitate complete transcription of mRNA transcripts.

The resulting cDNA was subsequently used as a template in quantitative real-time PCR (RT-qPCR) assays to quantify the expression levels of target genes. RT-qPCR was performed using the GoTaq® qPCR Master Mix kit, which contains SYBR Green I dye—a fluorescent DNA-binding dye that intercalates specifically with double-stranded DNA. As the PCR amplification proceeds,

SYBR Green fluorescence intensity increases proportionally with the accumulation of the amplified product, allowing real-time monitoring of gene expression.

To ensure accurate and reliable quantification, the bacterial 16S ribosomal RNA (16S rRNA) gene was selected as the internal reference or housekeeping gene. The 16S rRNA gene is highly conserved and exhibits stable expression across different experimental conditions and bacterial strains, providing a consistent baseline for normalization of gene expression data. This normalization corrects for any variability in cDNA input amounts or amplification efficiency between samples.

Primers specific to the arsenate reductase gene (arsC) were carefully designed using sequence information retrieved from the GenBank database. Primer design was optimized to achieve high specificity, efficiency, and to avoid the formation of secondary structures or primer-dimers. The primers targeted conserved regions of the arsC gene to accurately detect and quantify its transcript levels in both *Pseudomonas putida* and *Pseudomonas stutzeri* isolates.

The RT-qPCR reactions were carried out in a thermal cycler equipped with real-time fluorescence detection capabilities. Cycling parameters included initial denaturation, followed by repeated cycles of denaturation, annealing at a temperature optimized for the primers, and extension. Melt curve analysis was performed at the end of amplification to confirm the specificity of the PCR products by detecting a single, distinct melting temperature peak corresponding to the target amplicon.

Data obtained from the RT-qPCR experiments were analyzed using the comparative Ct (threshold cycle) method, allowing the calculation of relative gene expression levels of arsC normalized to 16S rRNA and compared to untreated controls. This approach provided quantitative insights into the bacterial response to arsenic stress at the transcriptional level.

Genetic and Statistical Data Analysis:

The relative quantification of arsC gene expression levels in *Pseudomonas putida* and *Pseudomonas stutzeri* under various arsenic treatment conditions was performed using the widely accepted comparative threshold cycle method, also known as the ΔΔCt method. This analytical approach facilitates the determination of fold changes in target gene expression by normalizing the expression level of the gene of interest (arsC) to that of a stable internal reference gene (16S rRNA) within the same sample, thereby correcting for any sample-to-sample variation in RNA input or reverse transcription efficiency.

The ΔCt (delta Ct) value for each sample was calculated by subtracting the Ct value of the reference gene from the Ct value of the target arsC gene. Subsequently, the ΔΔCt (delta delta Ct) value was obtained by comparing the ΔCt of each arsenic-treated sample to the ΔCt of the untreated control sample. The relative fold change in arsC expression was then calculated using the formula 2^(-ΔΔCt), which quantitatively expresses the induction or repression of arsC transcripts in response to arsenic exposure.

To determine whether observed changes in gene expression were statistically significant across different arsenic concentrations, one-way analysis of variance (ANOVA) was employed. This

statistical test evaluates the differences between the means of multiple groups to ascertain if at least one treatment condition leads to a significantly different expression level compared to others. The use of one-way ANOVA is appropriate for assessing dose-dependent responses of gene expression across the gradient of arsenic concentrations tested.

The level of significance was rigorously set at $p < 0.0001$, which imposes a stringent threshold to minimize the likelihood of false-positive results. This high level of confidence strengthens the reliability and robustness of the conclusions drawn regarding the modulation of arsC gene expression under arsenic-induced stress. Post-hoc multiple comparison tests (such as Tukey's HSD or Bonferroni correction), if applied, further delineate which specific treatment groups differ significantly from each other.

All statistical analyses were conducted using appropriate software packages, ensuring accurate calculation of means, standard deviations, and p-values. The data are presented as mean ± standard error (or standard deviation), reflecting the reproducibility and consistency of the experimental results.

Results:

The results of arsC gene expression analysis conducted via RT-qPCR demonstrated a clear and measurable response in both *Pseudomonas putida* and *Pseudomonas stutzeri* upon exposure to increasing concentrations of sodium meta-arsenite ($NaAsO_2$). To quantitatively assess gene expression levels, the relative expression values were calculated using the widely accepted Livak method (2^-ΔΔCT), which provides a reliable means of determining fold changes in target gene expression normalized against a stable reference gene.

In this study, the 16S rRNA gene was employed as the internal control due to its consistent and stable expression across all tested samples, thereby serving as a dependable baseline for normalization. For each bacterial sample subjected to varying arsenic concentrations, the threshold cycle (CT) values were first determined for both the target arsC gene and the reference 16S rRNA gene.

The initial step involved calculating the ΔCT values, which represent the difference between the CT of the arsC gene and that of the reference gene within the same sample. Subsequently, the ΔΔCT values were derived by comparing the ΔCT of each treated sample against the ΔCT of the untreated control group. This comparative approach allows for the evaluation of relative changes in gene expression triggered by arsenic stress.

Using the 2^-ΔΔCT formula, the fold change in gene expression was then computed, providing a quantitative measure of how much the arsC gene was upregulated or downregulated relative to the control condition. The data revealed a concentration-dependent increase in arsC expression for both *P. putida* and *P. stutzeri*, reflecting the activation of genetic detoxification mechanisms in response to arsenic exposure.

arsC gene* expression in *Pseudomonas putida:

The analysis of arsC gene expression in *Pseudomonas putida* revealed a gradual and consistent increase in expression levels corresponding with rising arsenic concentrations, as depicted in

Figure 1. At the lowest tested concentration of 25 ppm, the fold change in gene expression was measured at 9.63 ± 0.78, while at the highest concentration of 200 ppm, expression reached a maximum fold change of 57.47 ± 6.83, as detailed in Table 3-1.

These results reflect a regulatory response of the arsC gene to arsenic-induced toxic stress, demonstrating a graded activation of cellular defense mechanisms. The progressive increase in arsC expression indicates that *P. putida* employs a finely tuned regulatory system that scales the activation of detoxification pathways according to the severity of arsenic exposure. This system likely facilitates the enzymatic conversion of the highly toxic arsenite [As(III)] to the less harmful arsenate [As(V)], thereby mitigating intracellular toxicity.

The observed pattern of gene expression signifies *P. putida*'s ability to adapt to varying levels of arsenic contamination by modulating arsC expression proportionally to the concentration of the toxin. Such a controlled genetic response underscores the existence of an efficient regulatory network that ensures the maintenance of vital cellular functions under toxic stress conditions.

Table (1) Gene expression analysis of the *arsC* Gene in *Pseudomonas putida*

Sample	CT *arsC*	CT (*16SrRNA*)	ΔCT (Test)	ΔCT (control)	ΔΔCT	Fold Change (2^-ΔΔCT)
T25	27.83	28.03	-0.20	3.140	-3.340	10.18
T25	27.98	28.03	-0.05	3.140	-3.190	9.08
T50	27.38	28.40	-1.02	3.140	-4.160	17.81
T50	27.33	28.50	-1.17	3.140	-4.310	19.98
T100	27.14	28.58	-1.44	3.140	-4.580	23.89
T100	25.98	27.79	-1.81	3.140	-4.950	30.83
T150	26.54	28.86	-2.32	3.140	-5.460	44.36
T150	26.19	28.18	-1.99	3.140	-5.130	34.85
T200	25.47	28.03	-2.56	3.140	-5.700	52.64
T200	25.23	28.03	-2.80	3.140	-5.940	62.30
Control (Mean)	31.355	28.215	3.140			

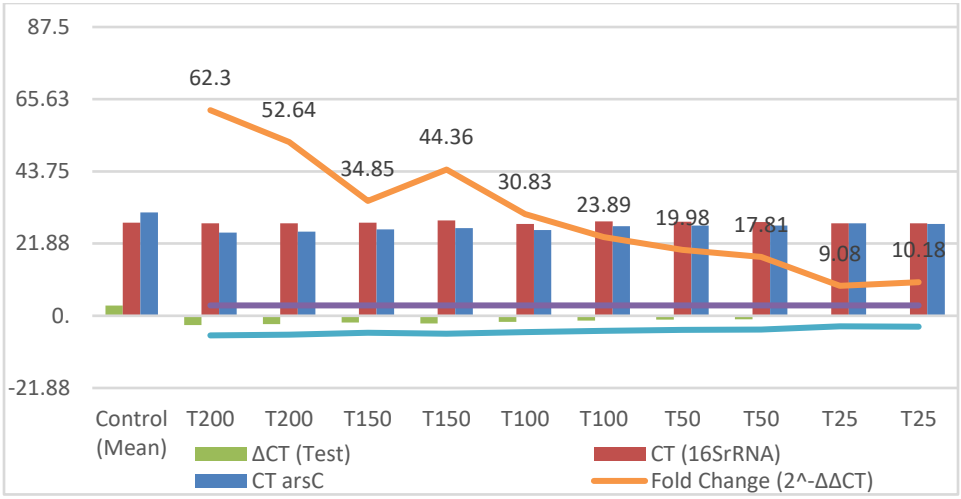

Fig 1: shows Gene Expression Analysis of the *arsC* Gene in *Pseudomonas putida*

***arsC* gene expression in *Pseudomonas stutzeri*:**

The expression analysis of the arsC gene in *Pseudomonas stutzeri* revealed a notably strong and robust response to increasing arsenic concentrations, as illustrated in Figure 2. At the lowest tested concentration of 25 ppm, the fold change in gene expression was already significant, recorded at 15.71 ± 0.48, demonstrating that *P. stutzeri* promptly detects even low levels of arsenic stress. As the concentration of arsenic increased, there was a progressive and substantial upregulation of the arsC gene, culminating in a maximum fold change of 85.91 ± 1.34 at 200 ppm, as detailed in Table 3-2.

This marked induction of arsC expression signifies a highly efficient molecular sensing and detoxification response system within *P. stutzeri*. The pronounced increase in expression suggests that this strain possesses advanced regulatory pathways enabling it to swiftly activate the arsenic resistance operon in response to toxic environmental conditions. Such a response likely facilitates enhanced survival and tolerance by catalyzing the reduction of the more toxic arsenite [As(III)] to the less harmful arsenate [As(V)], thereby mitigating the intracellular toxicity.

These findings imply that *Pseudomonas stutzeri* is particularly well-adapted to arsenic-contaminated environments, with a superior genetic machinery that enables it to respond dynamically and robustly to varying degrees of arsenic stress. This superior responsiveness is critical for its potential application in bioremediation efforts aimed at detoxifying arsenic-polluted soils and waters.

Sample	CT *arsC*	CT (*16SrRNA*)	ΔCT (Test)	ΔCT (control)	ΔΔCT	Fold Change (2^-ΔΔCT)
T25	28.33	28.03	0.30	4.255	-3.955	15.37
T25	28.28	28.03	0.25	4.255	-4.005	16.05
T50	27.12	28.40	-1.28	4.255	-5.535	46.63
T50	27.31	28.50	-1.19	4.255	-5.445	43.38
T100	27.11	28.58	-1.47	4.255	-5.725	53.22
T100	26.38	27.79	-1.41	4.255	-5.665	50.82
T150	26.98	28.86	-1.88	4.255	-6.135	69.82
T150	26.26	28.18	-1.92	4.255	-6.175	71.94
T200	25.87	28.03	-2.16	4.255	-6.415	84.96
T200	25.84	28.03	-2.19	4.255	-6.445	86.86
Control (Mean)	32.470	28.215	4.255			

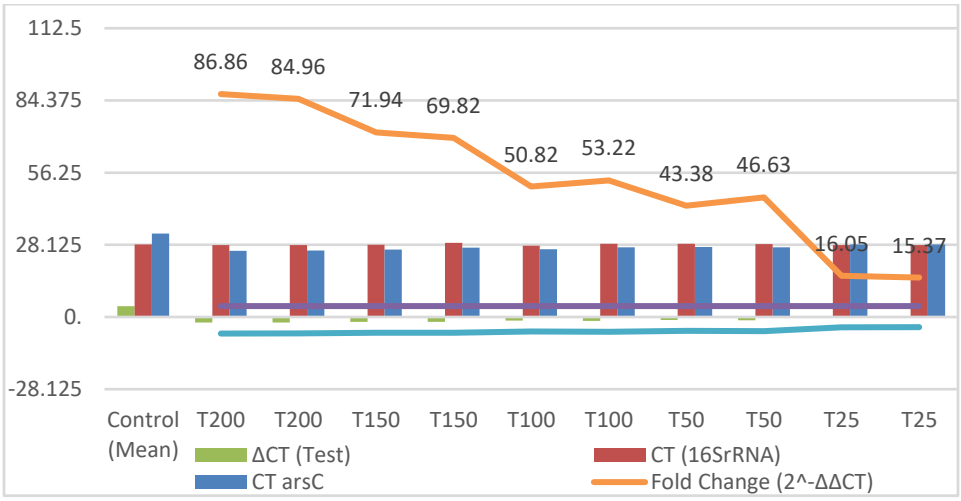

Fig 2 : shows gene expression analysis of the *arsC* gene in *Pseudomonas stutzeri*

Comparison between the two strains:

The results clearly demonstrate that *Pseudomonas stutzeri* consistently exhibits a higher level of arsC gene expression compared to *Pseudomonas putida* across all tested arsenic concentrations, as illustrated in Figure 3. Specifically, the fold change in *arsC* expression for *P. stutzeri* ranged

from 6.08 at 25 ppm to 31.27 at 150 ppm, reflecting a substantial induction of this gene in response to arsenic stress.

When comparing the relative increase in *arsC* expression between the two species, the percentage difference was most pronounced at 50 ppm, where *P. stutzeri* showed an increase of 138.15% compared to *P. putida*. This marked difference suggests that at moderate arsenic concentrations, *P. stutzeri* mounts a significantly stronger genetic response. Interestingly, this percentage difference gradually decreased as the arsenic concentration increased, reaching approximately 49.49% at the highest tested concentration of 200 ppm, as detailed in Table 3-3.

This trend indicates that while *P. stutzeri* generally exhibits a more robust activation of the arsC gene, the relative advantage over *P. putida* diminishes at very high arsenic levels. This could suggest saturation of the regulatory mechanisms or convergence of stress responses at extreme toxin concentrations.

Overall, these findings highlight that *Pseudomonas stutzeri* possesses a greater ability to sense and respond to arsenic exposure at the molecular level, as evidenced by its elevated arsC expression. This enhanced expression likely translates into a stronger capacity to resist or tolerate arsenic toxicity compared to *Pseudomonas putida*. Moreover, the varying percentage differences across concentrations imply possible differences in the underlying genetic regulatory mechanisms or intensities of response between the two species. Such differences could be due to variations in promoter strength, regulatory proteins, gene copy numbers, or post-transcriptional controls that modulate *arsC* expression.

Understanding these species-specific responses provides valuable insights for selecting optimal bacterial strains for bioremediation applications, particularly in environments with fluctuating or high levels of arsenic contamination. It also underscores the importance of examining gene expression dynamics over a range of contaminant concentrations to fully elucidate microbial adaptation and resistance mechanisms.

Table (3): Comparison of *arsC* Gene Fold Change Between *Pseudomonas stutzeri* and *Pseudomonas putida* Under Different $NaAsO_2$ Concentrations

$NaAsO_2$ Concentration (ppm)	*P. putida* Fold Change	*P. stutzeri* Fold Change	Difference (*P.Stutzeri* - *P.Putida)*	% Increase (*P.Stutzeri* over *P.Putida*)
25	9.63	15.71	6.08	63.14%
50	18.9	45.01	26.11	138.15%
100	27.36	52.02	24.66	90.13%
150	39.61	70.88	31.27	78.94%
200	57.47	85.91	28.44	49.49%

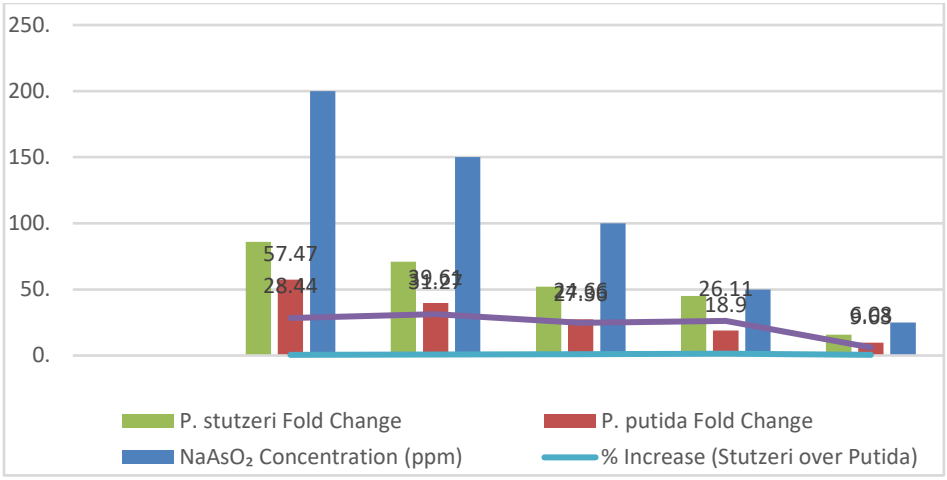

Fig 3: Shows Comparison of *arsC* Gene Fold Change Between *Pseudomonas stutzeri* and *Pseudomonas putida* Under Different $NaAsO_2$ Concentrations

Effect of Arsenic concentration on *arsC* gene expression: ANOVA results:

The results of the analysis of variance (ANOVA) demonstrated highly significant differences in *arsC* gene expression levels among the different concentrations of sodium meta-arsenite ($NaAsO_2$) in both *Pseudomonas putida* and *Pseudomonas stutzeri*. Specifically, the F-value reached 174.63 in *P. putida* and 268.91 in *P. stutzeri*, as shown in Table (3-4), with both results being statistically significant at $p < 0.0001$.

These exceptionally high F-values indicate that the variation in gene expression is not due to random fluctuations but is directly influenced by the applied arsenic concentrations. Such findings highlight a strong and consistent correlation between increased arsenic levels and elevated expression of the arsC gene, reinforcing the concept that this gene is highly responsive to arsenic-induced stress.

Moreover, the more pronounced F-value in *P. stutzeri* suggests a greater sensitivity and regulatory efficiency in this species, which corresponds with earlier observations of its higher fold-change values and possibly more robust genetic or regulatory architecture.

Statistical validation of the relationship between arsenic exposure and *arsC* activation enhances the credibility and reproducibility of the molecular data, affirming that the use of *arsC* expression as a quantitative biomarker is both statistically and biologically sound. This strengthens the utility of arsC-based expression analysis in toxicogenomic studies and supports its integration into biomonitoring frameworks for assessing microbial adaptation and the effectiveness of bioremediation in arsenic-contaminated environments.

Table (4) One-way ANOVA results

Strain	F-Value	p-Value	Conclusion
P. putida	174.63	< 0.0001	Highly significant differences
P. stutzeri	268.91	< 0.0001	Extremely highly significant differences

Discussion:

The gene expression analysis of the *arsC* gene demonstrated a clear and dynamic response pattern in *Pseudomonas putida* and *Pseudomonas stutzeri* under exposure to different concentrations of sodium meta-arsenite ($NaAsO_2$). This response was found to be dose-dependent, indicating that the expression levels of the *arsC* gene increased proportionally with the rise in arsenite concentration. This suggests that both bacterial species possess a finely tuned regulatory mechanism to sense and respond to arsenic-induced stress.

The arsC gene plays a pivotal role within the arsenic resistance operon (ars operon), which is a well-characterized genetic system that enables bacteria to survive in arsenic-contaminated environments. Specifically, the arsC gene encodes the enzyme arsenate reductase, which catalyzes the crucial reduction of arsenite [As(III)], a highly toxic and mobile form of arsenic, into arsenate [As(V)], a less toxic and less bioavailable species. This enzymatic detoxification step is vital, as As(III) readily interferes with cellular processes by binding to thiol-containing proteins and enzymes, thereby impairing metabolic function in both microbial cells and plants.

The upregulation of *arsC* expression observed in this study highlights the adaptive response of *P. putida* and *P. stutzeri* to increasing arsenic stress, and reinforces the role of the ars operon as a key genetic system in microbial arsenic resistance. Moreover, the observed differences in the fold change values between the two species suggest possible variations in regulatory efficiency, enzyme kinetics, or genetic background, which could influence their respective abilities to cope with arsenic-contaminated environments.

These findings align with previous reports (e.g., Bhattacharjee & Rosen, 2021), which describe the *arsC* gene as a central element in the detoxification of arsenic and as a biomarker for assessing microbial resistance and bioremediation potential. Understanding such gene expression dynamics not only provides insights into microbial survival strategies under metal stress but also offers a molecular basis for selecting and optimizing bacterial strains for use in bioremediation of arsenic-contaminated soils and water systems.

In this study, the expression of the *arsC* gene exhibited a clear concentration-dependent increase in response to arsenic exposure, a pattern commonly observed in regulatory genes activated under toxic stress. This progressive upregulation reflects the bacteria's capacity to adapt to increasing levels of environmental contaminants through precise genetic control mechanisms.

In *Pseudomonas putida*, the fold change in arsC expression ranged from 9.63 ± 0.78 at 25 ppm to 57.47 ± 6.83 at 200 ppm, indicating a robust and efficient regulatory response. This upregulation suggests that *P. putida* possesses a well-coordinated system for detecting arsenic and initiating appropriate detoxification processes through the arsenic resistance operon.

In contrast, *Pseudomonas stutzeri* demonstrated a more pronounced genetic response, with the fold change reaching 85.91 ± 1.34 at the highest concentration of 200 ppm. This higher level of expression may reflect enhanced regulatory capacity, potentially due to the presence of strong upstream regulatory sequences, increased copy numbers of the arsC gene, or more efficient

regulation by transcriptional repressors such as *arsR* (Yang et al., 2021). Such genetic features could provide *P. stutzeri* with superior arsenic detoxification capabilities.

The apparent superiority of *P. stutzeri* aligns with findings from previous research (e.g., Vélez et al., 2021; Yu et al., 2023), which have demonstrated the species' advanced regulatory flexibility under multiple forms of environmental stress. Notably, *P. stutzeri* has been shown to activate resistance genes not only in the presence of arsenic but also in response to mixed-metal contamination, making it particularly well-suited for complex polluted environments.

This adaptive genetic response is of critical importance in real-world scenarios, where bacteria are exposed to fluctuating and combined stressors. The genetic plasticity and high regulatory efficiency observed in *P. stutzeri* enhance its potential as a candidate organism for long-term bioremediation strategies. Its ability to sustain gene expression under high levels of arsenic and possibly other metals supports its use in restoring contaminated soils and water systems (Ghosh et al., 2022).

At the molecular level, the expression of the *arsC* gene is primarily regulated by the *ArsR* repressor protein, a transcriptional regulator that plays a central role in the arsenic resistance operon. ArsR functions as a metalloregulatory protein that senses intracellular arsenic levels; upon binding arsenic ions, it undergoes a conformational change that reduces its affinity for the operator region on the DNA. This dissociation from the DNA binding site allows RNA polymerase to access the promoter region, thereby initiating transcription of *arsC* and other operon components.

In the present study, the data revealed that *arsC* activation was notably more sensitive and responsive in *Pseudomonas stutzeri* compared to *P. putida*. This heightened responsiveness could be attributed to enhanced regulatory efficiency at the genetic level or possibly due to additional layers of gene regulation, such as epigenetic modifications, post-transcriptional regulation, or the involvement of small regulatory RNAs (sRNAs), which are emerging as important modulators in bacterial stress responses. However, these alternative regulatory mechanisms remain underexplored in the context of arsenic detoxification and warrant further investigation.

These findings underscore the dual role of the *arsC* gene—not only as a key functional component of the arsenic detoxification pathway, but also as a molecular biomarker for evaluating bacterial responses to arsenic contamination. This perspective aligns with recent trends emphasizing the use of environmentally responsive genes as bioindicators, which offer valuable insights into the microbial adaptation to pollutants and can guide the selection of strains for biotechnological applications (Sharma & Bhattacharya, 2017).

In addition to the molecular findings, statistical analysis using one-way ANOVA revealed highly significant differences ($p < 0.0001$) in *arsC* gene expression across the various concentrations of arsenic ($NaAsO_2$) tested. This level of statistical significance provides robust evidence that the changes in *arsC* expression were not random, but rather proportional to the concentration of arsenic, indicating the presence of a finely tuned and tightly regulated cellular sensing mechanism that responds to arsenic-induced stress.

The strong and statistically validated correlation between arsenic concentration and *arsC* expression levels enhances the credibility of using *arsC* as a molecular biomarker for assessing microbial responses to heavy metal contamination. This supports the hypothesis that *arsC* is not only a functional gene involved in arsenic detoxification but also serves as a sensitive and reliable diagnostic tool for monitoring the impact of arsenic pollution on microbial communities.

From a practical standpoint, quantifying *arsC* expression provides a quantitative molecular assay that can be employed to evaluate the bioremediation potential of bacterial strains. Strains that exhibit elevated and well-regulated *arsC* expression under high arsenic concentrations are likely to possess superior detoxification capabilities, making them ideal candidates for environmental cleanup. Compared to traditional assessment methods that rely solely on reductions in arsenic concentration, molecular indicators like *arsC* offer a more precise and biologically meaningful measure of bacterial activity and adaptation.

Therefore, these findings underscore the value of integrating molecular and statistical approaches to understand bacterial behavior under metal-induced stress. Such integration provides a solid scientific foundation for developing effective, targeted, and sustainable bioremediation strategies, particularly in complex contaminated environments with high levels of arsenic or mixed heavy metals.

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Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conclusions:

The study concludes that the effective role of both *P. putida* and *P. stutzeri* in resisting arsenic through precise gene regulatory mechanisms. This makes them promising strains for use in bioremediation techniques for arsenic-contaminated environments and opens new avenues for employing molecular analysis to evaluate the effectiveness of microorganisms in environmental remediation.

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