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# Detection of Novel L-arginase Gene Sequences from *Pseudomonas aeruginosa* in Soil and Sewage Samples

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## Abstract

**Background:** *Pseudomonas aeruginosa* is known for its flexibility and importance as both a pathogen and a model organism for genetic studies. This investigation was conducted to detect L-arginase enzyme gene sequences from *P. aeruginosa* in soil and sewage samples.

**Methods:** Soil and sewage samples were collected from different regions in Iraq for six months, from November 2022 to April 2023. *Pseudomonas aeruginosa* was isolated by culturing samples on nutrient medium and McConkey agar medium. Gram stains and biochemical tests were performed to identify the isolates, and the VITEK 2 system was used to confirm the identity of *P. aeruginosa*. DNA was extracted from the *P. aeruginosa* isolates and used for molecular identification by amplifying and sequencing the 16S rRNA gene. Also, the L-arginase gene sequences were amplified using the PARG1 and PARG2 primers.

**Results:** Out of 52 soil and sewage samples, 33 isolates (63.5%) of *P. aeruginosa* were identified, including 15 (28.8%) from soil and 18 (34.6%) from sewage. Among these, 9 (60.0%) of the soil isolates and 12 (66.7%) of the sewage isolates produced L-arginase.

**Conclusion:** The present study's findings revealed presence of the L-arginase enzyme from *P. aeruginosa* isolates derived from soil and wastewater samples. This research is considered a crucial step toward understanding the genetic structure and functions of L-arginase in *P. aeruginosa*, providing insights for future scientific investigations.

## Introduction

*Pseudomonas aeruginosa* is a versatile Gram-negative bacterium known for its adaptability and significance as both a pathogen and a model organism for genetic studies. Its genetic manipulation has provided valuable insights into bacterial gene expression and regulation. One important aspect of *P. aeruginosa* gene construction is identifying and isolating the target gene of interest. Once the gene is isolated, various molecular biology techniques can be employed to manipulate it [1]. Selecting the right genetic components is essential for gene expression in *P. aeruginosa* gene construction. This includes choosing appropriate promoters, ribosome binding sites, and transcriptional terminators to control gene expression levels and ensure proper regulation.

Researchers often optimize these elements to achieve the desired gene expression patterns. Once the gene is successfully integrated or introduced into *P. aeruginosa*, further characterization and analysis are performed to verify its functionality and assess the phenotypic effects. This involves applying techniques, such as PCR, gene expression profiling, protein analysis, or functional assays to verify gene functionality and assess phenotypic effects [2, 3].

L-arginase is an enzyme that catalyzes the hydrolysis of L-arginine into L-ornithine and urea. While L-arginase is commonly found in many organisms, including bacteria, its presence and role in *P. aeruginosa* has been the subject of investigation. This bacterium has been reported to possess L-arginase activity. It is known that L-arginase in *P. aeruginosa* is involved in numerous physiological activities, including nitrogen metabolism and adaptation to different environmental conditions. Also, it has been suggested that L-arginase activity in *P. aeruginosa* contributes to its virulence and pathogenesis [4].

L-arginase activity in *P. aeruginosa* has been associated with regulating the production of secondary metabolites and quorum-sensing molecules. The expression of several virulence factors in *P. aeruginosa* has been proposed to be influenced by L-arginase [5]. *Pseudomonas aeruginosa* has been detected in sewage treatment plants. Studies have investigated its prevalence, antibiotic resistance, and virulence factors in this context. Poursina *et al.*, [6] carried out an investigation on *P. aeruginosa* in sewage treatment plants and reported on the prevalence, antibiotic resistance patterns, and virulence factors of *P. aeruginosa* strains isolated from these environments.

This investigation aimed to detect L-arginase enzyme gene sequences from *P. aeruginosa* in soil and sewage samples obtained from various locations in Iraq.

## Methods

### Collection of samples

Soil and sewage samples were collected for six months, from November 2022 to April 4, 2023, from various locations in Iraq. The samples were carefully transported to the laboratory for further analysis.

### Laboratory examination of collected samples

The samples were cultured on nutrient rich media, such as McConkey agar medium to isolate *Pseudomonas aeruginosa*. After that, the samples were stained with Gram stain, then biochemical tests were performed to identify the isolates. The identity of all the *P. aeruginosa* isolates was confirmed using VITEK 2 (BioMérieux) kit.

### Identification of *Pseudomonas aeruginosa* using the polymerase chain reaction

DNA was extracted from the *P. aeruginosa* isolates and subjected to PCR-amplification to detect the PA-SS (16S rRNA) gene to identify *P. aeruginosa* species.

The PCR mix was made in a total volume of 25  $\mu$ L comprising oligonucleotide primers (forward and reverse) and Quick-Load® Taq 2X Master Mix, which was liquefied at room temperature. Table 1 shows the nucleotide sequences of the oligonucleotide primers used for amplification.

Primer ID	Primer sequence (5'-3')	Target	Product size (bp)	Reference
PA-SS	F: GGGGGATCTTCGGACCTCA R: CCTTAGAGTGCCACCCG	16S rRNA	956	[7]
PARG1	F: ATCGGGCAGTTGTGTTCAAG R: CATCACGCCAGGGTAATCA	Detection of L-arginase gene	300	Designed in this study
PARG2	F: CTCGATGTGATCGGCCAGTT R: AGGGTTTAAATCACCAACGTGC	Detection of L-arginase gene	300	Designed in this study

**Table 1:** Oligonucleotide primer sequences for detecting *aeruginosa* and L-arginase gene.

### Detection of L-arginase gene

The design of primers (Table 1) to detect the gene encoding the L-arginase enzyme was carried out in a scientific laboratory at Al-Mustansiriyah University, Iraq.

The amplification conditions for the primers were optimized. The PCR to amplify the L-arginase gene was performed in a thermal cycler with the following conditions: 95 °C for 2 min (initial denaturation), followed by 35 cycles of 94 °C for 20 sec (denaturation), 58 °C for 20 sec (annealing), 72 °C for 40 sec (extension), and one cycle of 72 °C for 1 min (final extension).

## Results

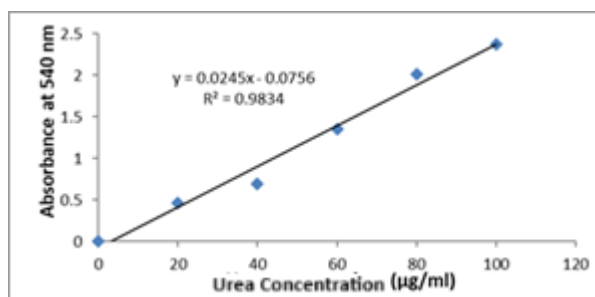
Fifty-two bacterial isolates were obtained from different agricultural soils and sewage water, with a distribution of 28 (53.8%) from soil samples and 24 (46.2%) from sewage samples, as presented in Table2.

Source	No.	Percentage (%)	<i>Pseudomonas aeruginosa</i> / %
Soil	28	53.8	15 (28.8%)
Sewage	24	46.2	18 (34.6%)
Total	52	100%	33 (63.46%)
Chi-Square ( $\chi^2$ )	-	0.307 NS	-
P-value	-	0.579	-

NS: Non-significant

**Table 2:** Distribution of bacteria isolated from soil and sewage.

The regular curve of urea in the present study showed that urea production by L-arginase was very high with a value of approx. 110  $\mu\text{g/mL}$  (Figure 1).



**Figure 1:** Urea concentration gradients (20, 40, 60, 80, 100  $\mu\text{g/mL}$ ) were prepared by adding appropriate amounts of distilled water to specific urea solutions. Each concentration was tested in triplicate by mixing 0.5 mL of urea with 0.1 mL of indicator and 0.5 mL of perchloric acid.

Out of 33 (63.46%) isolates of *P. aeruginosa* from soil and sewage samples, only 9 (60.0%) of the soil isolates produced the L-arginase in M9 media, while 12 (66.66%) isolates from the sewage water produced the L-arginase in M9 media (Table 3 and Figure 2).

Site	Production of L-arginase enzyme (%)	Non-production of L-arginase enzyme (%)	P-value
Soil, n=15	9 (60.0%)	6 (40%)	0.438 NS
Sewage, n=18	12 (66.7%)	6 (33.33%)	0.133 NS

NS: Non-significant

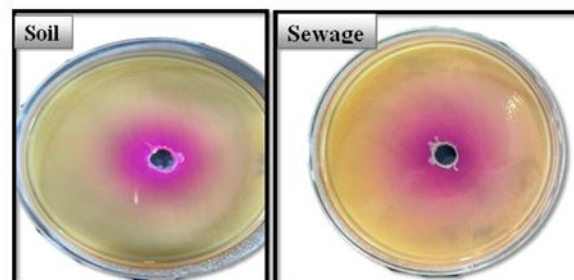
**Table 3:** Distribution of *Pseudomonas aeruginosa* obtained from soil and Sewage samples concerning production of enzymes on M9 agar medium.

The results obtained for the molecular identification of *P. aeruginosa*, and detection of L-arginase gene sequences are shown in Figures 3 and 4, respectively. PCR-product fragment sizes of 956 and 300 bp were obtained after the amplification of the 16S rRNA and L-arginase genes, respectively.

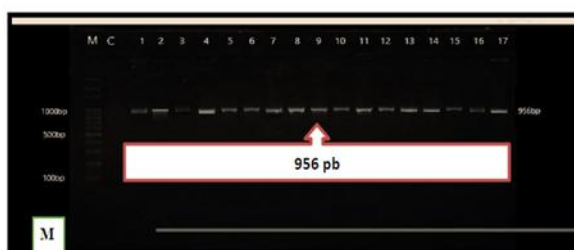
## Discussion

The study found a number of *Pseudomonas aeruginosa* isolates in soil and sewage. While the total number of initial bacterial isolates was higher in soil samples (28) than in sewage samples (24), a greater number of *P. aeruginosa* isolates were ultimately identified from sewage (18) compared to soil (15). This is because bacteria in the soil are more than other microorganisms due to their ability to replicate easily. The observations made in the present study are consistent with previous findings[8,9]. In addition, sewage water provides an

ideal environment for the growth of pathogenic and non-pathogenic bacteria, because it contains many elements necessary for the growth and reproduction of these bacterial species, as reported by Mohammed [10].



**Figure 2:** L-arginase *P. aeruginosa* cultured on modified M9 agar by wells, with the addition of phenol red according to the diffusion agar, where the diffusion zone about the well of the soil isolates was (ps18-38.3) mm, while the isolates from sewage had the highest diffusion zone (ps20m-40.4) mm.



**Figure 3:** Polymerase chain reaction products (956 bp) from *Pseudomonas aeruginosa* 16S rRNA gene amplification on gel electrophoresis. Lanes 1–17: Positive bands. M: DNA ladder (1000 bp).



**Figure 4:** Polymerase chain reaction products (300 bp) from *Pseudomonas aeruginosa* L-arginase gene amplification on gel electrophoresis. M: DNA ladder (1000 bp).

In the present study, L-arginase produced a relatively high amount of urea (approx. 110  $\mu\text{g/mL}$ ). The high production of urea observed during the assay of the L-arginase enzyme by M9 enhanced the color change from yellow to pink (Figure 2). This is crucial in the conversion of ammonia to urea in the urea cycle. Furthermore, the activity of the enzyme can be stimulated by measuring the volume of the enzyme (mL) and the amount of urea released during the enzyme's action. This finding highlights the significance of arginase in maintaining ammonia homeostasis and suggests that enzymatic activity is

functioning efficiently in the given context, which is in agreement with previous reports that have demonstrated the significance of arginase in the regulation of levels of urea production [11]. The measurement of urea concentration provides a valuable indicator of arginase activity and the proper functioning of the urea cycle. Also, Lee and coworkers found that an increased concentration of urea was associated with increased metabolism of arginine [12].

The use of the PCR method in the identification of *P. aeruginosa* is a commonly employed molecular technique in research and clinical settings. The procedure allows for the amplification of specific regions of the bacterial DNA, enabling sensitive and specific detection of the test organism. This procedure was used by Lee *et al.*, [13], whose study focused on the rapid detection of *P. aeruginosa* in medical samples utilizing real-time PCR. The researchers highlighted the benefits of the RT-PCR, including its sensitivity, specificity, and quick turnaround time for detecting *P. aeruginosa* infections in a clinical laboratory setting.

The arginase gene is made up of a 300 bp DNA fragment and encodes the enzyme arginase. The enzyme catalyzes the hydrolysis of arginine to ornithine and urea, contributing to various physiological processes and metabolic pathways in the cells [11]. *Pseudomonas aeruginosa* is a versatile and adaptable bacterium known for its metabolic diversity and ability to thrive in various environments [14]. The identification and characterization of the arginase gene in *P. aeruginosa* provides insights into its potential role in arginine metabolism and the adaptability of this bacterium to different ecological niches.

The discovery of the arginase gene in *P. aeruginosa* has significant implications. It opens avenues for further research into the functional properties of the enzyme and its potential applications. Understanding the regulation and expression of the arginase gene can provide insights into the metabolic pathways involved in arginine utilization by *P. aeruginosa*. Furthermore, the arginase gene may have implications for pathogenicity and virulence. *Pseudomonas aeruginosa* is an opportunistic pathogen associated with numerous infections, particularly in immune-compromised individuals [15]. The arginase enzyme can influence the availability of arginine, an essential amino acid for immune cells, and potentially impact host-pathogen interactions and disease progression.

The study's findings revealed an opportunity for scientific exploration and the potential for leveraging this enzyme's capabilities in bacterial growth research. Also, the present study highlights the possibility of harnessing arginase from natural sources, offering a new approach to inquiring about its applications in scientific investigations.

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## Author Contributions

The conceptualization of this study was done by ZA, AM carried out soil and sewage sample collection. ZA conducted the laboratory analysis. ZA prepared the draft manuscript, which AM and ZA proofread. Both authors agreed to send the manuscript to Advancements in Life Science journal for publication.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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