

Original Article

Production, Extraction and Characterization of Protein from *Pseudomonas* spp. Habitated in Rhizospheric Soil of Baramati City of Pune District of Maharashtra State, India.

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Abstract

Protein extraction is a fundamental biological process in which cellular proteins are isolated for downstream analysis and characterization. Certain bacterial genera, including *Pseudomonas*, *Erwinia* and *Xanthomonas* are widely studied for their diverse protein profiles and biochemical significance. The present study aimed to extract, purify and characterize proteins from *Pseudomonas* spp. isolated from rhizospheric soil. Bacterial isolation was followed by protein extraction using the TRIzol-based protein extraction method. The extracted protein was purified through dialysis to remove contaminants and residual solvents. Quantitative analysis of protein concentration was performed using UV-Visible NanoDrop spectrophotometry. The protein concentration was determined to be 1.184 mg/mL at 280 nm, with an A260/A280 ratio of 1.50, indicating acceptable protein purity. Further characterization was carried out using SDS-PAGE analysis. The gel electrophoresis results revealed a distinct protein band at approximately 120 kDa after staining with Coomassie Brilliant Blue R-250, confirming the presence of the extracted protein fraction. Overall, this study successfully demonstrated the extraction, purification and characterization of protein from *Pseudomonas* spp. contributing to a better understanding of bacterial protein profiles.

1. Introduction

Proteins are fundamental biomolecules that play essential roles in cellular structure, enzymatic activity, transport and regulation. In microbiological and proteomic research, the extraction of high-quality proteins is a critical step that directly influences the accuracy and reproducibility of downstream analytical techniques such as electrophoresis and mass spectrometry [1,2]. Efficient protein extraction is

particularly important when working with complex biological samples, including environmental bacterial isolates, where variability in cellular composition can significantly impact protein recovery [2]. Advances in microbial proteomics over the past decade have highlighted the importance of optimizing sample preparation methods, especially protein extraction, to achieve comprehensive proteome coverage. Recent studies demonstrate that variations in extraction

protocols can lead to significant differences in protein yield, diversity and detectability, emphasizing the need for standardized and reproducible methodologies [1,3]. This is especially relevant for Gram-negative bacteria, where structural complexity can hinder efficient cell lysis and protein isolation. Soil ecosystems harbor diverse microbial communities, among which *Pseudomonas* spp. are widely recognized for their metabolic versatility and ecological significance. These bacteria play key roles in nutrient cycling, biodegradation of environmental pollutants and plant growth promotion [4,5].

Additionally, *Pseudomonas* species are frequently used as model organisms in studies of stress response, biofilm formation and pathogenicity. Proteomic investigations have revealed complex protein expression patterns in *Pseudomonas* spp., highlighting the importance of reliable extraction methods for accurate characterization [5,6]. However, protein extraction from *Pseudomonas* spp. presents several challenges due to the presence of a complex outer membrane enriched with lipopolysaccharides, which can act as a barrier to cell lysis and contribute to contamination of protein samples [7].

Environmental isolates, particularly those derived from soil, may exhibit additional resistance mechanisms and adaptive responses that further complicate protein recovery [8]. Therefore, the selection of an appropriate extraction method is crucial to ensure efficient lysis and high-quality protein isolation. Various protein extraction techniques have been developed, including mechanical disruption, enzymatic digestion and chemical lysis. While mechanical methods such as sonication and bead beating are effective, they may result in incomplete lysis or protein degradation if not carefully optimized [3]. Chemical extraction methods have gained prominence due to their simplicity and ability to simultaneously isolate multiple biomolecules from a single sample, making them suitable for integrated molecular analyses [9].

Among these, TRIzol-based extraction is widely used due to its ability to sequentially isolate RNA, DNA and proteins from the same biological sample. The method is based on a monophasic solution of phenol and guanidine isothiocyanate, which disrupts cellular structures and denatures proteins. Upon addition of chloroform, phase separation occurs, allowing RNA to partition into the aqueous phase, DNA into the

interphase and proteins into the organic phase [10,11]. Proteins can subsequently be precipitated using organic solvents such as ethanol or isopropanol, facilitating their recovery for downstream applications. Although TRIzol is primarily known for nucleic acid extraction, several studies have demonstrated its applicability for protein isolation, particularly in multi-omics workflows where simultaneous recovery of different biomolecules is required [9,12].

However, protein extraction using TRIzol presents certain limitations, including protein denaturation, difficulties in resolubilization and potential contamination with residual organic solvents [12,13]. These challenges necessitate careful optimization of extraction conditions to improve protein yield and quality. Chloroform plays a critical role in facilitating phase separation and removing lipids and other interfering substances, thereby enhancing protein purity. Ethanol precipitation further contributes to protein recovery by reducing solubility and promoting aggregation into a pellet [13].

Recent studies emphasize that optimization of these steps is essential for achieving reproducible and high-quality results in proteomic analyses [1,3]. Despite its advantages, there is limited research specifically addressing the use of TRIzol-based methods for protein extraction from soil-isolated *Pseudomonas* spp. Most studies have focused on clinical strains or nucleic acid extraction, leaving a gap in understanding its effectiveness for environmental bacterial isolates [8,14]. Addressing this gap is important for expanding the application of proteomic techniques in environmental microbiology.

Therefore, the present study was designed to isolate *Pseudomonas* spp. from rhizospheric soil and to systematically extract, purify, and characterize their proteins using a TRIzol method-based extraction, followed by dialysis, spectrophotometric quantification and SDS-PAGE analysis. This study aims to contribute to improved methodological frameworks for bacterial protein analysis and to enhance our understanding of microbial functional diversity in soil ecosystems.

2. Materials and Methods

2.1 Collection of Samples

Rhizospheric soil samples were collected for the isolation of *Pseudomonas* spp. A total of three soil samples were collected: two samples from Nakshatra

Garden of Vidya Pratishthan's Arts, Science and Commerce College, Baramati and one sample from the fertilizer plant area of the School of Biotechnology, Vidya Pratishthan's Arts, Science and Commerce College, Baramati. Samples were collected aseptically in sterile containers and transported to the laboratory for further microbiological analysis.

2.2 Enrichment and Isolation of Bacterial Isolate

1 gm of rhizospheric soil was aseptically serially diluted up to 10^{-5} using sterile saline solution. From the final dilution, 0.1 mL aliquots were spread onto sterile nutrient agar plates. The plates were incubated at 28°C for 24–48 hours to allow colony development. Subsequently, the sample was inoculated into 100 mL of sterile nutrient broth and incubated at 28–30°C for 24 hours under aerobic conditions.

2.3 Characterization of Bacterial Isolate

After incubation, distinct colonies were examined based on morphological characteristics such as colony size, pigmentation, margin, elevation, and texture (figure 1). Preliminary identification was performed using Gram staining and standard biochemical tests. Representative isolates were submitted to the Pathology Laboratory, Baramati Hospital, Baramati for further biochemical characterization, validation and Confirmation [15] (figure 2).

2.4 Extraction of Protein

Pure cultures of *Pseudomonas* species were inoculated into nutrient broth supplemented with 2% glycerol and incubated at 30°C for 24 hours in a shaker incubator at 150 rpm (figure 4).

2.4.1 Preparation of Bacterial Isolate Sample for protein extraction

Following incubation, bacterial cells were harvested by centrifugation at 12,000 rpm for 10 minutes at 4°C. The supernatant was discarded, and the pellet was washed with sterile phosphate buffer (pH 7.2). The washed pellet was subjected to sonication at 20 kHz for 30 seconds (pulse mode) to obtain the cell lysate. The lysate was centrifuged at 12,000 rpm for 10 minutes at 4°C and the supernatant was transferred to a fresh sterile tube for protein extraction [16].

2.4.2 TRIzol-Based Protein Extraction

To the collected supernatant, 1 mL of TRIzol reagent was added and mixed thoroughly by vortexing. Subsequently, 0.2 mL of chloroform was added and the mixture was incubated on ice for 5 minutes. The sample

was centrifuged at 12,000 rpm for 15 minutes at 4°C, resulting in phase separation. After centrifugation, three layers were observed. The upper aqueous layer (RNA phase) was discarded. To the remaining solution, 1 mL of absolute ethanol was added and mixed gently. The mixture was incubated at room temperature (25°C) for 10 minutes and centrifuged at 12,000 rpm for 10 minutes at 4°C. The resulting supernatant containing protein was carefully transferred to a fresh sterile tube [17] (figure 5).

2.4.3 Protein Precipitation

An equal volume (1 mL) of chilled acetone was added to the protein-containing supernatant, mixed thoroughly by vortexing and incubated at –20°C overnight to facilitate protein precipitation. The following day, the mixture was centrifuged at 12,000 rpm for 10 minutes at 4°C. The protein pellet was collected, air-dried at room temperature and reconstituted in 500 µL of sterile phosphate buffer (pH 7.2). The rehydrated protein sample was stored at –20°C until further analysis [17] (figure 6).

2.5 Protein Purification

The extracted protein sample was purified using dialysis. The sample was loaded into a dialysis membrane with a molecular weight cut-off (MWCO) of 10 kDa and dialyzed overnight against Tris buffer (pH 6.8) at 4°C with gentle stirring to remove low molecular weight impurities [18].

2.6 Protein Quantification

The partially purified protein concentration and purity were determined using a Thermo Scientific NanoDrop UV–Visible spectrophotometer. A small aliquot of the protein sample was loaded directly onto the measurement pedestal and absorbance was recorded at 280 nm. The A260/A280 ratio was calculated to assess protein purity.

2.7 Protein Characterization by SDS–PAGE

The molecular weight of the partially purified protein was determined using SDS–PAGE. A 5% stacking gel and 6% resolving gel were prepared. Protein samples were mixed with loading buffer containing 10% SDS and β-mercaptoethanol and heated at 85°C for 2 minutes prior to loading. Electrophoresis was carried out at constant voltage until proper band separation was achieved. The gel was stained with 0.25% Coomassie Brilliant Blue R-250 and destained using a solution containing 30% methanol, 10% glacial acetic acid and

distilled water until clear protein bands were visualized [2] (figure 7).

3. Results

3.1 Isolation of *Pseudomonas* species

Pseudomonas spp. was successfully isolated from rhizospheric soil sample on sterile nutrient agar plate. 30 colonies were isolated.



Figure 1: Isolation of *Pseudomonas* spp. on sterile nutrient agar plate.

4.1. Identification of *Pseudomonas* spp.

4.2.1. Morphological Characterization

Table 1: Morphological characters of *Pseudomonas* spp.

Size	Medium
Shape	Circular
Colour	Yellow Green
Elevation	Raised
Margin	Smooth
Consistency	Butyrous
Opacity	Opaque
Gram character	Gram negative rods



Figure 2: *Pseudomonas* spp. colonies under Ultraviolet light.

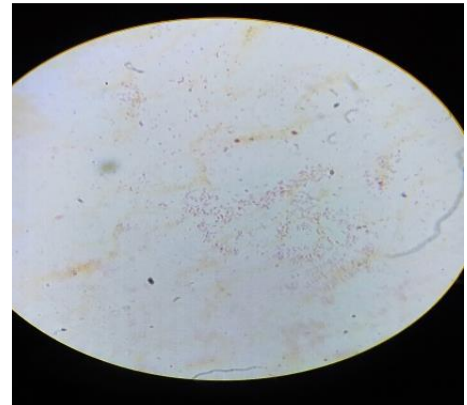


Figure 3: Gram staining result of *Pseudomonas* spp.



Figure 4: 24 hours grown culture of *Pseudomonas* spp. into sterile Nutrient Broth containing 2% glycerol.

4.3 Trizol Extraction

Three layers, top layer contains RNA, middle layer contains protein and bottom layer contains DNA separated successfully from the supernatant of sonicated cells after addition of Trizol reagent and chloroform.

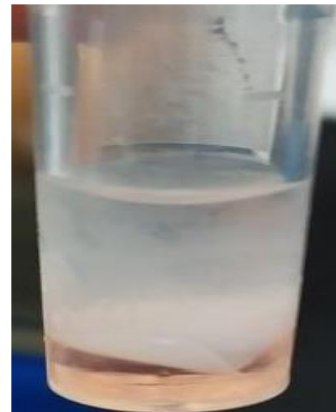


Figure 5: Separation of three layers of RNA, Protein and DNA respectively after addition of Trizol reagent and chloroform.

4.4 Protein Precipitation

Protein was precipitated after addition of chilled acetone and it was stored by adding sterile phosphate buffer at -20°C .



Figure 6: Protein precipitation by chilled acetone

4.6 Protein Quantification

Partially purified protein was accurately measured using Thermo Scientific Nanodrop UV-VIS spectrophotometer.

4.7 Protein Characterization

Partially purified protein was successfully characterized by SDS PAGE analysis. It showed visible protein band (~ 120 kDa) after staining with Coomassie Brilliant Blue R-250 stain and destained with solution containing 30% methanol, 10% glacial acetic acid and water.



Figure 7: SDS PAGE gel showing visible bands of proteins.

Therefore, our results have proved that biological protein can be produced by *Pseudomonas* spp. isolated from rhizospheric soil.

Table 2: A260/280 of proteins under study.

Sample name	Blank	INP
Protein Concentration	0.579	1.184
Unit	mg/ml	mg/ml
A280	0.579	1.184
260/280	1.23	1.50
Sample type	1Abs=1mg/ml	1Abs=1mg/ml

5. Discussion

Protein extraction is a crucial step in microbial research for understanding cellular structure and functional biomolecules. In the present study, successful extraction, purification and characterization of proteins from *Pseudomonas* spp. isolated from rhizospheric soil highlights their metabolic adaptability and biochemical potential. The use of TRIzol-based extraction followed by dialysis proved effective for obtaining relatively pure protein, consistent with recent studies demonstrating that extraction methodology significantly influences protein yield and downstream proteomic profiling [19].

The observed protein concentration (1.184 mg/mL) and A260/A280 ratio (1.50) indicate acceptable purity, although slight nucleic acid contamination may be present, as reported in microbial protein quantification studies [19]. Furthermore, SDS-PAGE analysis revealed a distinct protein band at approximately 120 kDa, confirming successful protein isolation and supporting the continued relevance of electrophoretic techniques in protein characterization [20]. Similar findings have been reported in bacterial systems, where SDS-PAGE remains a reliable method for resolving protein fractions and assessing molecular weight distribution [21]. Overall, the results validate the applied methodology and contribute to the understanding of bacterial protein profiles, though advanced proteomic approaches are recommended for detailed functional analysis.

6. Conclusion

The present study successfully demonstrated the extraction, purification and characterization of proteins from *Pseudomonas* spp. isolated from rhizospheric soil. The application of the TRIzol method proved to be an effective approach for protein isolation, while subsequent dialysis ensured the removal of impurities and residual contaminants. Quantitative analysis using UV-Visible spectrophotometry confirmed an

appreciable protein yield with acceptable purity levels. Furthermore, characterization through SDS–PAGE revealed a distinct protein band at approximately 120 kDa, indicating the presence of a specific protein fraction. These findings highlight the reliability of the adopted methodology and contribute to a better understanding of bacterial protein profiles. The study provides a foundation for future investigations focusing on functional characterization and potential biotechnological applications of proteins derived from rhizospheric microorganisms.

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Appendices

Abbreviations	
TRIzol	Total RNA Isolation reagent
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
°C	Degree Celsius
%	Percentage
G	Gram
Min	Minutes
Sec	Seconds
RPM	Rotation Per minute
ml	Milliliters
AgI	Silver iodide
kD	Kilo dalton
Kb	Kilobytes
A260/A280	Absorbance at light of wavelength 260 nm/ Absorbance at light of wavelength 280 nm

Composition of Trizol Reagent	
Ingredients	ml
Phosphate buffer	1
Trizol reagent	1
Chloroform	0.2
Absolute alcohol	1
Chilled acetone	1

Composition of 6% Resolving Gel	
Ingredients	For 100 ml
Water	5.3ml
Acrylic amidemix(30%)	2ml
Tris (1.5M,pH8.8)	2.5ml
SDS (10%)	0.1ml
Ammonium persulphate	0.1ml
TEMED	0.008ml

Composition of Nutrient Agar	
Ingredients	g/l
Peptone	10
Sodium chloride	5
Yeast extract	3
Agar	20
Distilled water	1000
pH	7.2

Composition of King's B Medium	
Ingredients	g/l
Protease Peptone	20
Dipotassium hydrogen phosphate	1.5
Magnesium sulphate heptahydrate	1.5
Agar	20
Distilled water	1000
pH	7.2

Composition of 5% Stacking Gel	
Ingredients	For 5ml
Water	3.4ml
Acrylic amide mixture (30%)	0.83ml
Tris (1.0M,pH6.8)	0.63ml
SDS (10%)	0.05ml
Ammonium persulphate (10%)	0.05ml
TEMED	0.005ml

Gel Destaining Solution	
Ingredients	In ml
Methanol	30ml
Glacial acetic acid	10ml
Water	Adjust to 100ml