Iranian Journal of Microbiology
Volume 18 Number 4 (August 2026) 629-632
DOI: http://doi.org/10.18502/ijm.v18i4.22206

SHORT COMMUNICATION

Exposure to thermal stress induces overexpression of protein-L-iso aspartate-O-methyl transferase (PIMT) in *Pseudomonas aeruginosa*

Suman Agrawalla[1], Prasanta Kumar Koustasa Mishra[2*], Srinivas Sathapathy[3], Ashok Kumar Sethi[1], Debasis Dash[4], Archana Mahapatra[5], Radhakrishna Sahu[6]

[1]Department of Microbiology, College of Basic Sciences and Humanities, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India
[2]Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India
[3]Department of Veterinary Anatomy and Histology, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India
[4]Department of Botany, College of Basic Sciences and Humanities, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India
[5]Department of Veterinary Anatomy, Faculty of Veterinary and Animal Sciences, Banaras Hindu university, Rajiv Gandhi South Campus, Uttar Pradesh, India
[6]Department of Veterinary Public Health, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India

Received: December 2025**, Accepted:** June 2026

ABSTRACT

Background and Objectives: Apart from spontaneous deamidation and isomerization of Asp/Asn residues, factors like ageing, oxidative stress, salinity and heat stress may also form abnormal isoaspartate (iso-Asp) residues compromising protein structure and function. PIMT helps in restoration of such iso-Asp residues. *Pseudomonas aeruginosa* is a gram-negative bacteria responsible for diseases with varied degree of pathogenesis in humans and animals. They can survive a wide range of temperature between 25°C to 42°C. The objective of the current study was to monitor the expression dynamics of PIMT in *Pseudomonas aeruginosa* upon exposure to different thermal stresses.
Materials and Methods: The mid log phase culture of *Pseudomonas aeruginosa* was exposed to different temperature (37°C, 42°C, 56°C, 80°C) for an hour. The expression levels of PIMT were quantified by Western blot and ELISA.
Results: A relatively higher level of expression of PIMT was observed in the group exposed to 42°C, 56°C, 80°C in comparison to 37°C. The expression of PIMT increased around 1.5 times in the group exposed to 42°C.
Conclusion: A temperature dependent overexpression of PIMT was observed in *Pseudomonas aeruginosa.*

Keywords: Protein D-aspartate-L-isoaspartate methyltransferase; *Pseudomonas aeruginosa*; Heat stress; Deamidation; Enzyme-linked immunosorbent assay; Western blotting

*Corresponding author: Prasanta Kumar Koustasa Mishra, M.V.Sc., Ph.D, Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, Odisha University of Agriculture and Technology, Bhubaneswar, Odisha, India. Tel: +6742397719 Email: pkkmishra@ouat.ac.in

INTRODUCTION

Protein damage is an impulsive process caused by various abiotic and biotic factors and heat stress is one of them. Protein racemization, isomerization and deamidation are provoked due to heat stress, leading to compromised structure and function of the affected proteins (1). This may be challenging for the host organisms in terms of maintaining cellular integrity and to counter this, the native structure of the affected protein needs to be defended. PIMT, otherwise known as PCMT repairs the abnormal Iso-Asp residues formed due to protein deamidation/ isomerization (2). PIMT, transfers methyl group from S-adenosyl methionine (SAM) to the side chain carboxyl group of the damaged Iso-Asp residue forming a methyl ester which undergoes a non-enzymatic reaction to from L-succinimide intermediate which acts as precursor to Asp and Iso-Asp. This cycle continues till the normal Asp residues are restored (3). PIMT is widely reported in eukaryotic organisms like Arabidopsis, Castor, Ricinus, rat, poultry, mice and human but only a few reports are available in microbes such as *Escherichia coli*, *Mycobacterium tuberculosis* and *Salmonella typhimurium*. *Pseudomonas aeruginosa* is a Gram-negative rod-shaped bacterium which has numerous virulence determinants (4). It is a notorious pathogen, responsible for large share of nosocomial infections. Further, multi drug resistant (MDR) strains of this pathogen are constantly evolving which are difficult to control with conventional antibiotics (5). In this study we tried to study the expression pattern of the PIMT in *P. aeruginosa* exposed to different temperature.

A stock culture of *Pseudomonas aeruginosa* (MTCC 9800) was collected from the departmental repository (Department of Microbiology, CBSH, OUAT, Bhubaneswar, Odisha) and revived on King's B Medium. Later, the culture was grown on *Pseudomonas* agar for fluorescein & Pyocyanin respectively. Isolated colony of the organism was picked and inoculated into King's B broth. Genomic DNA was isolated from an overnight grown *P. aeruginosa* culture by using HiPurA® bacterial genomic DNA purification kit following manufacture's instruction. The isolated DNA was used as a template for polymerase chain reaction (PCR) with a PIMT (*P. aeruginosa*) gene (pcmt) specific primer set (Table 1). These primers which were originally used for cloning of the PIMT gene were manually designed by retrieving the gene sequence of PIMT (*P. aeruginosa*) form NCBI gene bank (NZ_CP020560.1). The flanking sequence of the start and stop codons were selected and restriction sites for EcoRI and HindIII were added in the forward and reverse primers respectively. A dinucleotide sequence was also added to the 5' end of each primer to facilitate digestion process. Promega G2 green master mix was used for PCR with reaction volume of 20 µL. The PCR condition followed for amplification is given in the (Table 2). An amplicon of 663 bp can be observed in the agarose gel (Fig. 1) which corresponds to the size of the pcmt gene of the *P. aeruginosa* (later confirmed by sequencing). This ensures the purity of the culture and presence of an intact PIMT expressing gene in the organism.

Table 1. PIMT gene specific primers for *Pseudomonas aeruginosa*.

Name of the primer	Sequence of the primer (5' to 3')
PIMT Forward primer	ATGAATTCATGACCTCCCAGCG-TACCCG
PIMT Reverse Primer	ATAAGCTTTCAGGCGATCGGG-CCGTTG

Table 2. PCR conditions used for amplification of PIMT gene of *Pseudomonas aeruginosa*.

Steps	Temperature	Time	No of Cycles
Initial Denaturation	94ºC	5 min	1
Denaturation	94ºC	30 secs	35
Annealing	60ºC	45 secs	
Extension	72ºC	1 min	
Final extension	72ºC	10 mins	1

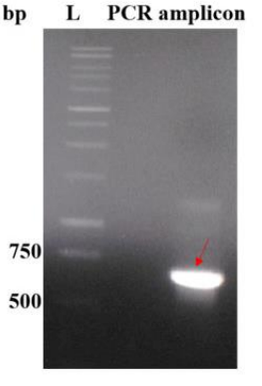

Fig. 1. Agarose gel electrophoresis of (with PIMT gene specific primers) PCR amplicon of *P. aeruginosa*.

A recombinant PIMT (rtPIMT) of *P. aeruginosa* was available in the laboratory. The recombinant protein was analyzed on a 12% SDS-PAGE to check its purity and molecular weight (Fig. 2a). The protein corresponds to 27 kDa. Bradford assay (Cat No: ML178, Himedia) was done to quantify the concentration of the protein which was found to be 0.25 µg/mL. We had a polyclonal antibody, commercially raised against the recombinant PIMT (Anti-PIMT (*P. aeruginosa*) IgG raised in Rabbit). The affinity of the antibody to the protein can be seen in the Fig. 2b where a Western blot was performed by trapping the rtPIMT in a polyacrylamide gel followed by transferring it to nitrocellulose membrane at 200 mA. The anti-PIMT antibody (1:250) was used as a primary antibody and the HRPO conjugated anti-rabbit IgG (1:1000) was used to detect the interaction.

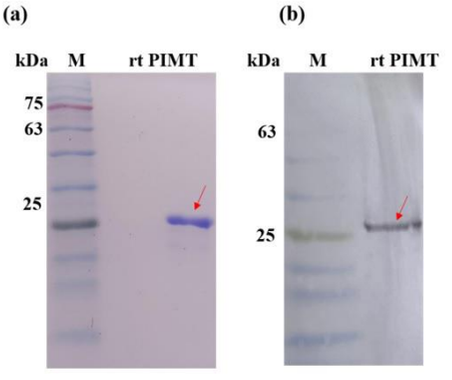

Fig. 2. (a) SDS-PAGE (12%) analysis of recombinant PIMT *P. aeruginosa.* (b) Western blot analysis of recombinant PIMT *P. aeruginosa*. Primary antibody used at 1: 250 dilutions. HRPO Conjugate at 1: 1000.

Early log phase grown pseudomonas culture was used for exposure to thermal stress. For that, an overnight grown culture was sub cultured at 1:100 ratio and grown at 37°C with constant shaking (110 rpm) till the OD_{600} reached 0.4. The culture of *Pseudomonas aeruginosa* (triplicates) was incubated at 4 different temperatures for another 1 hour with constant shaking (37°C, 42°C, 56°C, 80°C). 10 ml of culture from each group was centrifuged at 10,000 rpm at 4°C and the pellets were stored at -20°C till further analysis. The cell pellets were lysed with 5 mL of 8M urea + NaCl (0.85 %) buffer. The lysates were centrifuged at 12,000 rpm for 5 minutes at 4°C. The supernatant was collected from each group and the total protein content was quantified using Bradford Assay with a BSA standard.

The expression levels of PIMT in exposed groups were evaluated by Western blot and ELISA. For Western blot, 10 µg of total protein (supernatant) from each group was loaded into SDS-PAGE and the proteins were transferred to a nitrocellulose membrane at 300 mA for 2 hours followed by blocking with 3% skimmed milk powder. The blot was incubated with Anti-PIMT antibody (1:250) and HRPO conjugate (1: 1000). DAB was used as a substrate to develop the blot. An increase in PIMT expression can be seen (~27 kDa) in the lanes corresponding to 42°C, 56°C, and 80°C, while PIMT expression is relatively low at 37°C (Fig. 3a). However, this did not provide a clear indication of the amount of PIMT expression in the said groups. To quantify the exact amount of PIMT expression in each group, ELISA was performed. A standard curve was prepared by taking different concentration of rtPIMT and the absorbance at 450 nm was recorded due to its interaction with the anti-PIMT and conjugate antibody (Fig. 3b). Hundred nano-grams of total protein from each supernatant was coated to a 96 well plate. Carbonate- bicarbonate buffer (pH 9.6) was used as a coating buffer. The wells were washed and blocked with 1% gelatin. Anti-PIMT was diluted at 1: 500 and conjugate was used at 1: 5000 dilutions. TMB was used as a substrate for the HRPO conjugate. The reaction was stopped with a stop solution (1N sulfuric acid) and the absorbance at 450 nm was recorded with the help of a microplate reader. After subtracting blank, the PIMT quantity was interpolated from the standard curve. The quantity of PIMT expression in different test groups has been given in the Fig. 3b.

A significant difference among the groups can be seen in the expression pattern and over expression in the groups exposed to 42°C, 56°C, 80°C can be noted when compared with 37ºC. This clearly indicates the role of PIMT in thermal stress in *Pseudomonas aeruginosa*. The over-expression might be a combat strategy to repair the overproduction of Iso-Asp in response to thermal stress and resulted deamidation of normal Asp/Asn residues in several proteins. Earlier, it was reported that PIMT helps in survival of *S. typhimurium* in poultry and an increase in expression of PIMT was also observed at 42ºC which is the normal body temperature of poultry (6). Our study corroborates to their finding (overexpression of PIMT upon exposure to heat stress) in *P. aeruginosa*. Earlier, it was also observed in *E. coli* that the overexpression of PIMT is associated with enhanced heat

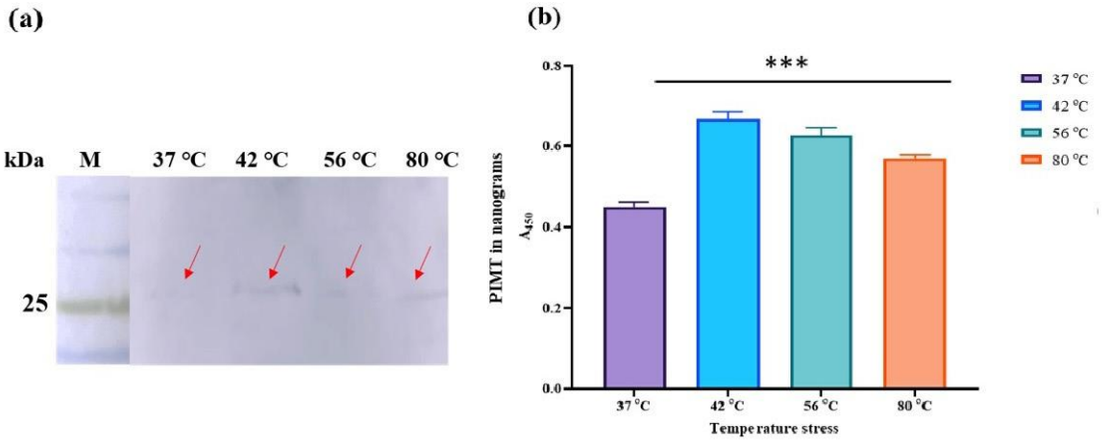

Fig. 3. (a) Western blot analysis of expression pattern of PIMT in *P. aeruginosa* upon exposure to different thermal stress. (b) Quantification of PIMT expression by ELISA. One-way ANOVA was used for the analysis. Values are Mean ± SD of two independent observations. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ns- not significant)

shock survival (7). A similar finding was also made in the eukaryotic counterpart *A. thaliana* suggesting the enzyme repairs anti-oxidant enzymes which maintains the reductive state of the vital proteins in heat stress and oxidative stress (8).

REFERENCES

1. Wakankar AA, Borchardt RT. Formulation considerations for proteins susceptible to asparagine deamidation and aspartate isomerization. *J Pharm Sci* 2006; 95: 2321-2336.
2. Mishra PKK, Mahawar M. PIMT-Mediated Protein Repair: Mechanism and Implications. *Biochemistry (Mosc)* 2019; 84: 453-463.
3. Mishra PKK, Gattani A, Mahawar M. Isolation and Identification of Protein L-Isoaspartate-O-Methyltransferase (PIMT) Interacting Proteins in *Salmonella* Typhimurium. *Curr Microbiol* 2020; 77: 695-701.
4. Gupta A, Mardi P, Mishra PKK, Kumar A, Kumar R, Mahapatra A, et al. Evaluation of supplemented protein-L-isoaspartate-O-methyltransferase (PIMT) gene of *Carica papaya* and *Ricinus communis* in stress survival of *Escherichia coli* BL21(DE3) cells. *Prep Biochem Biotechnol* 2024; 54: 882-895.
5. Wu W, Jin Y, Bai F, Jin S (2015). Pseudomonas aeruginosa. In: Molecular Medical Microbiology. London, United Kingdom, Academic Press, pp. 753-767.
6. Pesingi PK, Kumawat M, Behera P, Dixit SK, Agarwal RK, Goswami TK, et al. Protein-L-Isoaspartyl Methyltransferase (PIMT) Is Required for Survival of *Salmonella* Typhimurium at 42°C and Contributes to the Virulence in Poultry. *Front Microbiol* 2017; 8: 361.
7. Kindrachuk J, Parent J, Davies GF, Dinsmore M, Attah-Poku S, Napper S. Overexpression of L-isoaspartate O-methyltransferase in *Escherichia coli* increases heat shock survival by a mechanism independent of methyltransferase activity. *J Biol Chem* 2003; 278: 50880-50886.
8. Ghosh S, Kamble NU, Verma P, Salvi P, Petla BP, Roy S, et al. *Arabidopsis* protein l-ISOASPARTYL METHYLTRANSFERASE repairs isoaspartyl damage to antioxidant enzymes and increases heat and oxidative stress tolerance. *J Biol Chem* 2020; 295: 783-799.