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Molecular Cloning and *in silico* Characterization of the *Arabidopsis thaliana* Phytochelatin Synthase 1 (*AtPCS1*) Gene for Potential Phytoremediation Applications

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HIGHLIGHTS

- *AtPCS1* gene was successfully cloned into pBIN61 plant expression vector.
- Recombinant clones were verified via colony PCR and restriction analysis.
- *AtPCS1* was transformed into *Agrobacterium* LBA4404 for plant studies.
- *In silico* analysis revealed structural and functional features of *PCS1*.

Abstract

The *PCS1* gene encodes phytochelatin synthase 1, a member of the phytochelatin synthase family, which catalyzes the biosynthesis of glutathione-derived peptides known as phytochelatins. These peptides bind heavy metal ions and play a crucial role in heavy metal detoxification and tolerance mechanisms in plants. Due to its central role in metal homeostasis, *PCS1* represents an important molecular target for studies related to plant stress responses and phytoremediation. In this study, the *PCS1* gene was isolated and cloned from *Arabidopsis thaliana*. Successful amplification was achieved from *A. thaliana* using high-fidelity *Pfu* polymerase. The amplified *PCS1* gene was ligated into the plant expression vector pBIN61 using T4 DNA ligase and subsequently transformed into *Escherichia coli* JM109 by heat shock. Recombinant clones were selected on kanamycin-containing medium and verified by colony PCR and restriction enzyme analysis. Confirmed plasmids harboring the *PCS1* gene were isolated and preserved as glycerol stocks at −80 °C. Positive constructs were then transferred into *Agrobacterium tumefaciens* strain LBA4404 via electroporation. In addition to experimental cloning, *in silico* analyses were conducted to characterize the physicochemical properties, conserved domain architecture, and sequence conservation of the PCS1 protein, providing complementary insights into its structural and functional features. The resulting *Agrobacterium* strains carrying the *PCS1* gene constitute a valuable resource for future plant transformation studies, and *PCS1*-expressing transgenic plants may serve as promising tools for the phytoremediation of heavy metal–contaminated environments.

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1. Introduction

Environmental pollution has intensified as a consequence of rapid population growth, increasing energy and nutritional demands, unplanned urbanization, excessive consumption, and accelerating technological development (Dzhumashev and Kazakevitch, 2025). Among environmental pollutants, heavy metals represent a particularly serious threat due to their persistence in the environment and their non-degradable nature (Martinez et al., 2006). Heavy metals such as lead, copper, zinc, nickel, cadmium, chromium, arsenic and mercury accumulate in soil, water, and air, posing significant risks to ecosystems and human health (Ali et al., 2013). Conventional physical and chemical remediation methods used to remove heavy metals are often costly and may cause secondary environmental damage. As an alternative, phytoremediation has emerged as an environmentally friendly and cost-effective strategy that utilizes plants to remove, stabilize, or detoxify heavy metals from contaminated environments (Tamma et al., 2025).

Certain plant species are capable of surviving under metal stress due to the presence of intrinsic detoxification and tolerance mechanisms (Ali et al., 2013). Elucidating the molecular basis of these mechanisms is essential for improving phytoremediation efficiency. Advances in omics-based approaches, including transcriptomics, proteomics, and metabolomics, have facilitated the identification and characterization of genes, metabolites, transcription factors, and stress-responsive proteins involved in metal stress responses (Raza et al., 2022). These studies have demonstrated that metal tolerance in plants is largely associated with the differential expression of conserved genes rather than the acquisition of novel genes, and that enhanced expression of such genes can significantly improve metal tolerance (Verbruggen et al., 2009).

One of the key genes implicated in plant heavy metal tolerance is *phytochelatin synthase 1* (*PCS1*), which encodes phytochelatin synthase, a γ-glutamylcysteine dipeptidyl transpeptidase (EC 2.3.2.15) (Li et al., 2020). This enzyme catalyzes the synthesis of phytochelatins from reduced glutathione, a reaction that is activated by metal ions, particularly cadmium (Cd^{2+}) (Grill et al., 1989). Phytochelatins are low-molecular-weight, cysteine-rich peptides that function as metal chelators by forming stable complexes with heavy metal ions. These complexes are subsequently transported from the cytosol into the vacuole, thereby reducing metal toxicity within the cell (Li et al., 2020).

Phytochelatins play a central role in metal and metalloid tolerance across a wide range of organisms. (Iglesia-Turino et al., 2006; Guo et al., 2008). Studies have shown that phytochelatin- and glutathione-deficient mutants of *Schizosaccharomyces pombe* (Glaeser et al., 1991) and *Arabidopsis thaliana* (Cobbett, 2000; Vernoux et al., 2000) exhibit hypersensitivity to cadmium and other heavy metals, highlighting the importance of the phytochelatin pathway in detoxification processes. Moreover, a strong correlation has been reported between phytochelatin accumulation and metal sequestration in plants exposed to mercury (Iglesia-Turino et al. 2006). Overexpression of PCS genes from various plant species, including *Arabidopsis thaliana*, *Triticum aestivum*, *Allium sativum*, and *Brassica juncea*, has been shown to enhance the production of metal-binding peptides and confer increased tolerance to heavy metal stress in both plants and microorganisms. (Clemens et al., 1999; Ha et al., 1999; Heiss et al., 2003; Zhang et al., 2005). Similarly, transgenic plants overexpressing PCS genes, such as wheat *TaPCS1* in *Nicotiana glauca* (Gisbert et al., 2003), and *AtPCS* in *Oryza sativa* (Venkataramaiah et al., 2011), exhibit significantly improved tolerance to cadmium, underscoring the potential application of PCS-based strategies in phytoremediation.

The aim of this study was to isolate and clone the *PCS1* gene from plant source and to characterize the corresponding PCS1 protein through *in silico* analyses. By combining molecular cloning approaches with bioinformatic characterization, this study provides a foundation for future genetic transformation experiments and supports the potential use of *PCS1*-expressing transgenic plants in the phytoremediation of heavy metal-contaminated environments.

2. Materials and Methods

2.1. Plant Material and Genomic DNA Isolation

In this study, *Arabidopsis thaliana* (mouse-ear cress) was used as plant material and obtained from Niğde Ömer Halisdemir University, Faculty of Agricultural Sciences and Technologies, Department of Agricultural Genetic Engineering.

Genomic DNA was isolated from *A. thaliana* for the amplification of the *phytochelatin synthase 1* (*PCS1*) gene. Leaf tissues of A. thaliana were used for DNA extraction. Genomic DNA isolation was performed using the Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit (K0791), following the manufacturer's instructions. The quality and integrity of the isolated DNA were assessed prior to downstream applications.

2.2. Amplification of the Phytochelatin Synthase 1 (PCS1) Gene

Full-length primers containing BamHI restriction enzyme recognition sites were designed using T3 software to amplify the *PCS1* gene. In addition to full-length primers, internal primers were designed to amplify partial fragments of the target gene. Primer sequences used for full-length and partial amplification are listed in Table 1. Polymerase chain reaction (PCR) amplifications of *A. thaliana PCS1* (*AtPCS1*) gene were carried out using both *Taq* DNA Polymerase and high-fidelity *Pfu* DNA Polymerase to evaluate amplification efficiency and accuracy. PCR was carried out in reaction volume of 20 μL containing the genomic DNA (10 ng), forward and reverse primers 0.50 μM each (BamHI site was incorporated), dNTPs 100 μM, 1X PCR Buffer (50 mM KCl, 1.5mM MgSO4 and 10mM Tris-HCl) and Polymerase 1 unit. The PCR was performed at 94 C for 4 minutes, 94°C for 40 seconds, 60°C for 40 seconds and 72°C for 1 minute followed by 35 times.

Table 1. List of primers used for PCR amplification of the *A. thaliana PCS1* (AtPCS1) gene.

Name	Primer sequences (5′-3′)	Annealing temperature	Product size (bp)
AtPCS1-FLF	ACA GGATCC ATGGAAGGATTTTTCAGGTTGATTT	55°C	1350
AtPCS1-FLR	ACA GGATCC CTAATAGGCAGGAGCAGCGAG		
AtPCS1-IF	CAAAGGCTTGTTGCCAAGGA	55°C	378
AtPCS1-IR	GTTGCCGTCGAAGATGCAAT		

PCR products were separated on a 1% agarose gel for 1 h at 90 V and DNA bands were visualized under ultraviolet light using a gel imaging system. DNA fragments were excised from the agarose gel and purified using a commercial gel extraction kit (Thermo Scientific GeneJET Gel Extraction Kit, K0691) according to the manufacturer's protocol.

2.3. Vector Construction and Molecular Cloning

2.3.1. Digestion and Ligation

The plant expression vector pBIN61 was used for cloning the *AtPCS1* gene. Prior to ligation, the pBIN61 plasmid was inoculated into 10 mL of LB (Luria–Bertani) broth supplemented with kanamycin (50 mg/L) and incubated overnight at 37 °C. Plasmid DNA was isolated from the overnight culture using a commercial plasmid isolation kit (Thermo Scientific GeneJET Plasmid Miniprep Kit, K0503) following the manufacturer's instructions.

Both the pBIN61 plasmid vector and the *AtPCS1* gene were digested with the same restriction enzyme (BamHI) prior to ligation. The digested pBIN61 vector and *AtPCS1* insert were analyzed on agarose gel to verify digestion efficiency and estimate DNA concentrations. Vector and insert concentrations were determined based on the relative intensity of the DNA bands observed on the gel.

Ligation is the process of combining the PCR-amplified *AtPCS1* gene with the pBIN61 plasmid vector. The ligation reaction was set up at a 3:1 insert-to-vector molar ratio using 25 ng of *AtPCS1* insert and 75 ng of pBIN61 vector. The reaction mixture contained 1 µL of 10X ligation buffer and 1 µL of T4 DNA ligase (4.0 Weiss units), with the final volume adjusted to 10 µL. The ligation mixture was incubated at 16 °C overnight to allow completion of the ligation reaction.

2.3.2. Bacterial Transformation

The transformation procedure involved the transfer of a portion of the ligation mixture into competent *Escherichia coli* cells. Briefly, 50 µL of chemically competent *E. coli* JM109 cells, stored at −80 °C and thawed on ice, were transferred into a sterile 1.5 mL microcentrifuge tube. A total of 6 µL of the ligation mixture containing the pBIN61 plasmid and *AtPCS1* insert was added to the competent cells and incubated on ice for 20 min. Heat shock was then applied by incubating the mixture at 42 °C for 1 min, followed by immediate incubation on ice for 2 min. Subsequently, 450 µL of LB medium was added, and the transformed cells were incubated at 37 °C for 1 h in a shaking incubator. The cells were then plated onto LB agar medium supplemented with kanamycin (50 mg/L) and incubated overnight at 37 °C. Several bacterial colonies were observed the following day and were screened by colony PCR and restriction enzyme analysis.

2.3.3. Selection and Verification of Recombinant Colonies

Recombinant bacterial colonies were screened by colony PCR using specific internal primers designed to amplify a 378 bp fragment of the *AtPCS1* gene (Table 1). Initially, individual colonies were picked using a sterile pipette tip and streaked onto fresh LB agar plates containing kanamycin (50 mg/L) by scratching an area of approximately 1 cm^2. The plates were incubated at 37 °C for 16 h and subsequently stored at 4 °C for further analysis. Colony PCR was then performed, and the PCR products were analyzed by electrophoresis and visualized.

Following colony PCR analysis, positive colonies harboring the pBIN61 plasmid containing the *AtPCS1* gene were selected and inoculated into LB medium supplemented with kanamycin (50 mg/L). After overnight incubation at 37 °C, plasmid DNA was isolated using a commercial plasmid miniprep kit (Thermo Scientific GeneJET Plasmid Miniprep Kit, K0502) according to the manufacturer's instructions.

The isolated pBIN61-*AtPCS1* plasmids were subjected to restriction analysis using BamHI. The digestion mixture consisted of 10x Cut Buffer (2 µL), BamHI enzyme (1 µL; 1 U/µL), plasmid DNA (1 ng), and sterile distilled water to a final volume of 20 µL. The reaction was incubated at 37 °C for 16 h. Digested products were analyzed by agarose gel electrophoresis to confirm the presence of the expected 1350 bp *AtPCS1* insert.

2.4. Electroporation into Agrobacterium tumefaciens

The recombinant pBIN61-*AtPCS1* plasmid was introduced into competent *Agrobacterium tumefaciens* strain LBA4404 by electroporation using a Bio-Rad electroporation system (#165-2105). Briefly, 2 µL of plasmid DNA (50 ng) was mixed with electrocompetent LBA4404 cells and kept on ice prior to electroporation. Electroporation was performed at 2.2 kV, 25 µF capacitance, and 200 Ω resistance. Immediately after electroporation, 1 mL of SOC medium was added, and the cells were incubated at 28 °C for 3 h with shaking at 200 rpm. The culture was then plated onto LB agar containing kanamycin (50 mg/L) and incubated at 28 °C for 24–48 h. Positive *Agrobacterium* transformants were confirmed by colony PCR.

2.5. In silico and Bioinformatic Analyses

The amino acid sequence of phytochelatin synthase 1 (PCS1) obtained from *A. thaliana* was used for bioinformatic analyses. The reference protein sequence was retrieved from the NCBI database (Accession number: NP_199220.1). Physicochemical properties of the PCS1 protein, including molecular weight, theoretical isoelectric point (pI), amino acid composition, instability index, aliphatic index, and grand average of hydropathicity (GRAVY), were calculated using the ProtParam tool available on the ExPASy server (accessed on January 2026). Conserved domain architecture of the PCS1 protein was analyzed using the NCBI Conserved Domain Database (CDD v3.21, accessed on January 2026). Domain boundaries and functional regions were identified based on Pfam annotations.

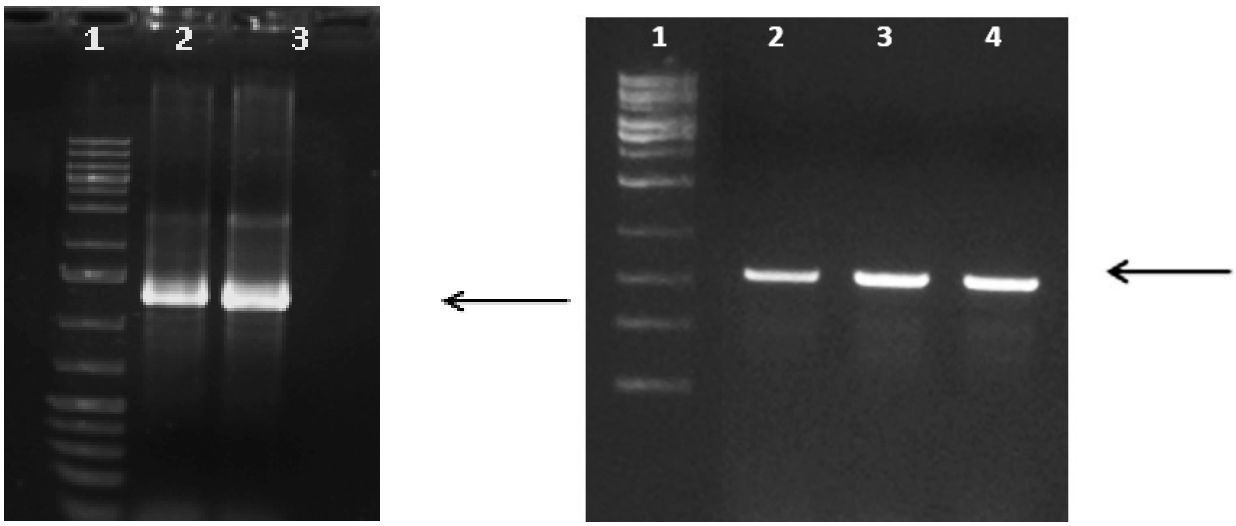
To evaluate sequence similarity and confirm protein identity, BLASTP analysis (version 2.16.0+; accessed on January 2026) was performed against the NCBI non-redundant (nr) protein database. Searches were restricted to the Viridiplantae taxonomic group. Protein sequences showing significant similarity were selected based on query coverage, percentage identity, and E-value. BLAST results were summarized to identify homologous phytochelatin synthase proteins across plant species, and the highest-scoring hit from *A. thaliana* was used as the reference sequence for comparative analysis.

3. Results

3.1. Genomic DNA Isolation and Amplification of the PCS1 Gene

Genomic DNA was successfully isolated from *A. thaliana* and used as template DNA for PCR amplification of the *PCS1* gene. Full-length and partial forward and reverse primers containing BamHI overhangs (Table 1), were employed to amplify the *PCS1* gene.

PCR amplification using high-fidelity *Pfu* DNA Polymerase produced a distinct band corresponding to the full-length *PCS1* gene (1350 bp) (Figure 1a). PCR performed with *Taq* DNA Polymerase using partial primers yielded a specific 378 bp fragment of the *PCS1* gene (Figure 1b).



(**a**) (**b**)

Figure 1. PCR amplification of the *AtPCS1* gene. a: Amplification of the full-length *AtPCS1* gene (1350 bp) using *Pfu* DNA Polymerase. Lane 1: 1 kb Plus DNA ladder; lanes 2–3: amplified *AtPCS1* PCR product. b: Amplification of an internal fragment of the *AtPCS1* gene (378 bp) using Taq DNA Polymerase. Lane 1: 1 kb Plus DNA ladder; lanes 2–4: amplified PCR products.

3.2. Construction of the Recombinant Vector and E. coli Transformation

PCR-amplified *AtPCS1* fragments obtained using *Pfu* DNA polymerase were purified and ligated into the pBIN61 plant expression vector. The resulting recombinant constructs were introduced into *E. coli* JM109 cells by heat shock transformation. Transformants were selected on LB agar plates supplemented with kanamycin (50 mg/L). Multiple kanamycin-resistant colonies were obtained, indicating successful ligation and transformation of the *AtPCS1* gene into the pBIN61 vector.

3.3. Screening and Molecular Validation of Recombinant Clones

Following transformation, multiple kanamycin-resistant *E. coli* colonies were obtained after overnight incubation on selective medium. To confirm the presence of the *AtPCS1* gene, recombinant colonies were screened by colony PCR and restriction analysis.

Colony PCR was performed using *AtPCS1*-specific internal primers, yielding an expected amplicon size of 378 bp. As shown in Figure 2, colonies 4 and 5 produced a distinct 378 bp band, indicating the presence of the *AtPCS1* insert. These colonies were therefore selected as positive recombinants.

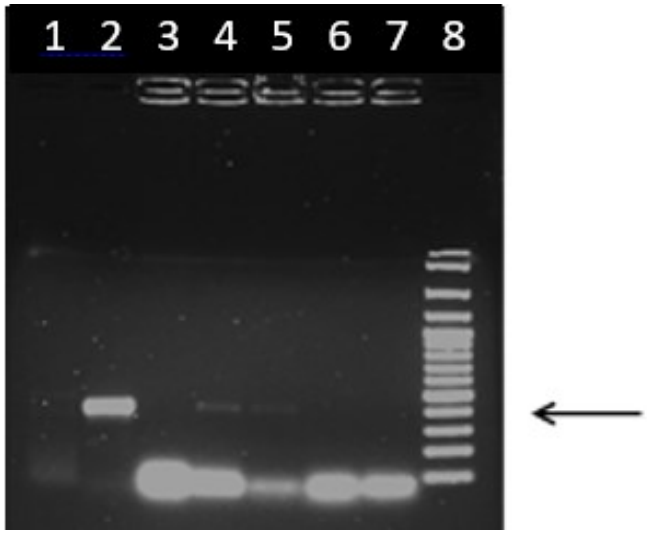


Figure 2. Identification of recombinant *E. coli* colonies by colony PCR using *AtPCS1*-specific partial primers (378 bp). Lane 1: negative control; lane 2: positive control (gel-purified *AtPCS1* fragment); lanes 3–7: screened bacterial colonies; lane 8: 1 kb Plus DNA ladder.

Plasmid DNA isolated from the positive colonies was further verified by restriction digestion with BamHI. Digestion resulted in two fragments corresponding to the pBIN61 vector (~12.939 bp) and the *AtPCS1* insert (1.350 bp), confirming successful cloning of the *AtPCS1* gene (Figure 3). A schematic representation of the recombinant pBIN61-*AtPCS1* construct is shown in Figure 4.

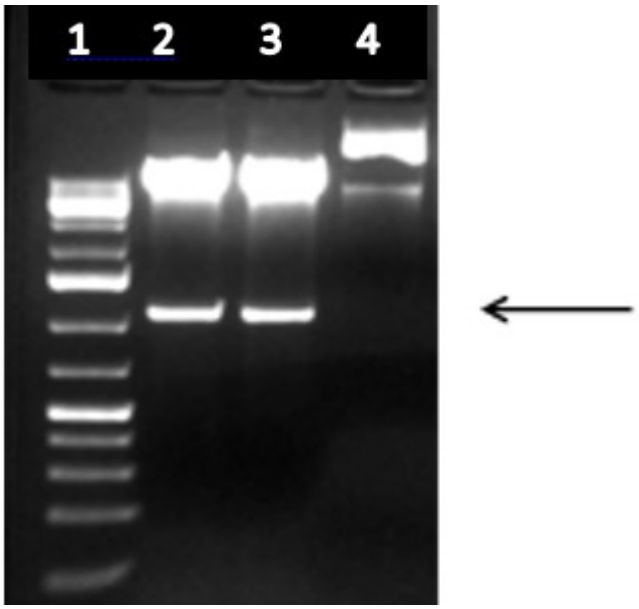


Figure 3. Restriction analysis of recombinant pBIN61-AtPCS1 plasmids following digestion with BamHI. Lane 1: 1 kb DNA ladder; lanes 2–3: BamHI-digested plasmid DNA showing the released AtPCS1 insert (1350 bp); lane 4: undigested plasmid DNA (control).

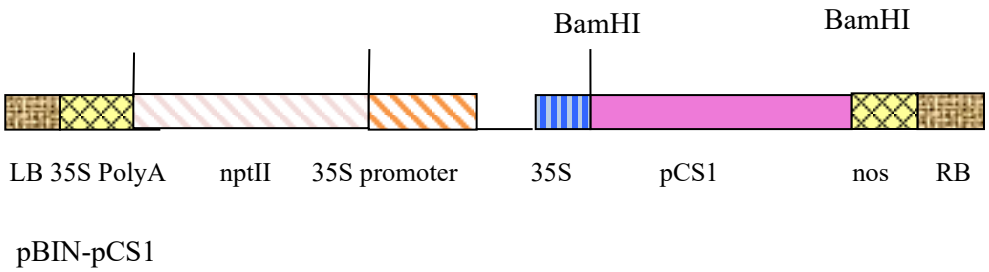


Figure 4. Schematic representation of the recombinant pBIN61-*AtPCS1* construct. The *AtPCS1* gene was cloned into the T-DNA region of the pBIN61 vector, which contains the *nptII* gene conferring kanamycin resistance as a selectable marker.

3.4. Verification of Agrobacterium tumefaciens Transformants

The recombinant pBIN61-*AtPCS1* plasmid was successfully transferred into *A. tumefaciens* strain LBA4404 by electroporation. Following selection on kanamycin-containing medium, several resistant colonies were obtained. The presence of the *AtPCS1* gene in *Agrobacterium* transformants was confirmed by colony PCR, indicating successful introduction of the recombinant construct into the bacterial host.

3.5. In silico Characterization of PCS1 Protein

The amino acid sequence of phytochelatin synthase 1 (PCS1) from *A. thaliana* was subjected to bioinformatic analyses to evaluate its physicochemical properties, conserved domain architecture, and sequence similarity with homologous plant proteins.

ProtParam analysis revealed that the PCS1 protein consists of 485 amino acids, with a predicted molecular weight of 54.47 kDa and a theoretical isoelectric point (pI) of 6.18. The instability index was calculated as 51.53, indicating that the protein is unstable under *in vitro* conditions. The aliphatic index was determined to be 84.43, suggesting a relatively high thermostability. The grand average of hydropathicity (GRAVY) value was -0.237, indicating that the protein is hydrophilic in nature.

Conserved domain analysis using the NCBI Conserved Domain Database (CDD) revealed that the PCS1 protein contains two conserved domains. The N-terminal region harbors a phytochelatin synthase catalytic domain spanning amino acid positions 7–213, corresponding to the PFAM PCS domain (Pfam ID: PFAM05023). In addition, a C-terminal domain annotated as Phytochelatin_C was identified between amino acid positions 231–458 (Pfam ID: PFAM09328). The domain architecture of PCS1 is illustrated in Figure 5.

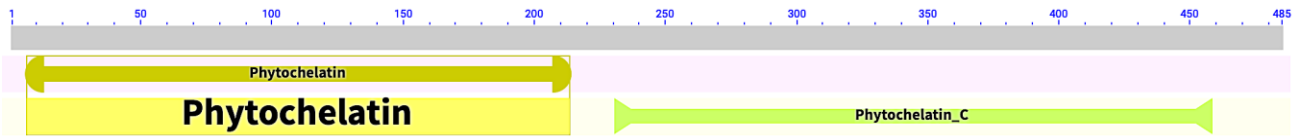


Figure 5. Conserved domain architecture of the *A. thaliana* PCS1 protein identified using the NCBI Conserved Domain Database (CDD). The 485 amino acid-long full-length protein contains two distinct structural regions: a highly conserved N-terminal phytochelatin synthase domain (spanning amino acids ~1 to 220, PFAM05023) responsible for catalytic enzyme activity, and a variable C-terminal domain (Phytochelatin_C) (spanning amino acids ~240 to 455, PFAM09328), which is involved in heavy metal sensing and protein stability. The interval between the domains represents a linker region.

To confirm the identity of the PCS1 protein and assess its evolutionary conservation, BLASTP analysis was performed against the NCBI non-redundant protein database, restricted to the Viridiplantae taxonomic group. The analysis revealed a high degree of sequence similarity between *A. thaliana* PCS1 and phytochelatin synthase proteins from various plant species. The top BLAST hit corresponded to *A. thaliana* PCS1 (Accession no: NP_199220.1), exhibiting 100% sequence identity, complete query coverage, and an E-value of 0.0. In addition, all selected homologous PCS proteins showed complete query coverage (100%) and high sequence

identity ranging from 91% to 96%, with E-values of 0.0, indicating strong evolutionary conservation of PCS proteins among plant species. A summary of selected BLASTP results is provided in Table 2.

Table 2. Summary of selected BLASTP hits for *A. thaliana* PCS1 against the Viridiplantae database.

Accession No.	Organism	Protein	Identity (%)	Query Coverage (%)	E-value
NP_199220.1	*Arabidopsis thaliana*	Phytochelatin synthase 1	100	100	0.0
AAS45236.1	*Arabidopsis halleri*	Phytochelatin synthase 1	96	100	0.0
ACL00594.3	*Leucaena leucocephala*	Phytochelatin synthase	96	100	0.0
AEW23125.1	*Amaranthus tricolor*	Phytochelatin synthase	95	100	0.0
XP_006280379.1	*Capsella rubella*	Glutathione gamma-glutamylcysteinyltransferase 1	93	100	0.0
XP_006403146.1	*Eutrema salsugineum*	Glutathione gamma-glutamylcysteinyltransferase 1	93	100	0.0
XP_013706429.1	*Brassica napus*	Glutathione gamma-glutamylcysteinyltransferase 1	92	100	0.0
XP_010481782.1	*Camelina sativa*	Glutathione gamma-glutamylcysteinyltransferase 1 like	92	100	0.0
BAB85602.1	*Brassica juncea*	Phytochelatin synthase	91	100	0.0
KAJ0262635.1	*Hirschfeldia incana*	Glutathione gamma-glutamylcysteinyltransferase 1	91	100	0.0

4. Discussion

Heavy metal stress represents a major abiotic constraint for plant growth and productivity, and a wide range of detoxification mechanisms have evolved to mitigate toxic effects. Among these, the synthesis of phytochelatins (PCs) via phytochelatin synthase (PCS) is a conserved strategy in many plant species and other organisms for chelating and sequestering heavy metals such as cadmium and arsenic (Goncharuk and Zagoskina, 2023). Phytochelatins bind heavy metal ions and facilitate their compartmentalization, thereby reducing cellular toxicity and contributing to stress tolerance (Seregin and Kozhevnikova 2023). Studies in yeasts and higher plants have demonstrated that PCS genes from diverse taxa are involved in heavy metal detoxification and that manipulation of PCS expression can influence metal tolerance and accumulation profiles in heterologous systems (Clemens et al., 1999).

Successful amplification of the *A. thaliana PCS1* (*AtPCS1*) gene using high-fidelity *Pfu* DNA polymerase provided the template required for cloning and further manipulation. Pfu polymerase is widely recognized for its low error rate and proofreading activity, which enhance the accuracy of PCR products used in cloning workflows, reducing the risk of sequence errors compared to *Taq* DNA polymerase alone (Fiala and Setter, 1986; Slater, 1998). The subsequent ligation of *AtPCS1* into the pBIN61 vector, transformation into *E. coli*, and verification of recombinant constructs demonstrate a robust cloning pipeline suitable for generating expression constructs for functional studies.

Although overexpression of PCS genes in plants has been shown to confer enhanced metal tolerance or altered accumulation in some contexts (Venkataramaiah et al., 2011; Gisbert et al., 2003), the outcomes are not universally consistent. For example, overexpression of certain PCS homologs has been associated with increased cadmium sensitivity in transgenic plants under some conditions, indicating that the net stress response may depend on complex interactions with glutathione availability and cellular thiol metabolism (Zheng et al., 2022). Moreover, heterologous expression of PCS genes from different sources can lead to variable effects on metal accumulation and tolerance depending on host species, promoter context, and

underlying regulatory networks (Shri et al., 2014). These observations highlight the importance of both gene sequence and host background in determining the functional outcomes of PCS expression.

The *in silico* characterization of the *AtPCS1* protein provided additional insight into its structural and evolutionary context. Conserved domain analysis confirmed the expected PCS catalytic domains, and comparative sequence similarity supported strong conservation of PCS proteins across plant species. Furthermore, BLASTP results indicated high sequence identity with known PCS sequences, reinforcing the conclusion that the cloned gene encodes the canonical PCS1 protein. Such bioinformatic analyses are increasingly used to complement experimental molecular biology studies and strengthen functional inferences when direct biochemical assays are not immediately available.

Taken together, the results of this study establish a foundation for future research aimed at employing PCS1 expression constructs in transgenic plants. The generation of *A. tumefaciens* strains carrying the pBIN61-*AtPCS1* construct sets the stage for plant transformation experiments. Future work can explore *in planta* expression under metal stress conditions, quantify PC production and heavy metal accumulation, and assess phenotypic effects in model and non-model species. Integrating PCS1 with other components of metal detoxification pathways, such as glutathione biosynthetic enzymes or metallothioneins, may also provide synergistic benefits for phytoremediation applications, as suggested by recent transgenic studies examining combinatorial gene approaches (Sunitha et al., 2013).

Overall, this study contributes to the molecular toolkit available for investigating and manipulating plant heavy metal responses and supports the continued exploration of phytochelatin synthases as targets for biotechnological solutions to environmental pollution.

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