J. Soils and Crops 35 (2) 295-299, December 2025 ISSN 0971-2836 (Print)
ISSN 2582-2756 (Online)

IN SILICO ANALYSIS OF PIN4, PARVULIN TYPE PPIASE FROM SOIL AMOEBA (*Dictyostelium discoideum*)

Khanchuila Shingnaisui[1] and Aruna Naorem[2]

ABSTRACT

The study was carried out to perform *in silico* analysis of Pin4, parvulin type PPIase in soil amoeba, *D. discoideum* at the Department of Genetics, University of Delhi in 2024. Parvulin belongs to the Peptidyl-prolyl*cis/trans* isomerases (PPIases) that catalyze the *cis/trans* isomerization of peptide bonds N-terminal to proline residues regulating various cellular processes such as protein folding. *D. discoideum* is a soil-dwelling amoeba that plays a pivotal role in maintaining soil fertility and the environment. In this study, the protein sequence and structural analysis of Pin4 was performed using the available bioinformatic tools to understand the role of Pin4 in *D. discoideum.* Results have shown that Pin4 had conserved PPIase domain and positively charged amino acid residues at its N-terminus which may play a crucial role in regulating Pin4 functions. Structural analysis also revealed significant homology with other parvulin. Additionally, Pin4 exhibited high unstable protein biochemical nature in the cell. This study highlights the need to further investigate Pin4 in *D. discoideum* to understand its impact on soil nutrient cycling and ecosystem balance.

(Key words: *Dictyostelium discoideum*,soil amoeba,Parvulin,Pin4, PPIases)

INTRODUCTION

Peptidyl-prolyl*cis/trans* isomerases (PPIases) are ubiquitous enzymes that are essential in protein folding affecting various protein functions (Gurung *et al.,* 2023). Parvulin belongs to the third family of PPIase that accelerates *cis*/*trans* interconversion by recognizing proteins/peptides with non-phosphorylated or phosphorylated Ser/Thr-Pro moieties. It further emerged into two sub-families based on structure and functional differences. Pin1 type belongs to the first family and is the most widely studied parvulin including human Pin1, *S. cerevisiae (*ESS1*),* and *D. melanogaster* (Dodo) (Lu *et al.,* 1996; Maleszka *et al.,* 1996). The second family belongs to the non-Pin1 type parvulin commonly known as Pin4 encoding Par14 and Par17 in humans (Uchida *et al.,* 1999). Par14 has been reported in diverse cellular processes such as transcription, gene expression, disease pathogenesis, etc. (Saeed and Piracha, 2023; Saningong and Bayer, 2015; Zhang *et al.,* 2013). Despite its conservation across species, non-Pin1 types are less explored.

D. discoideum is a soil-dwelling amoeba that has a distinctive life cycle of both unicellular and multicellular stages. During growth they feed on bacteria and upon starvation, they undergo development and form multicellular structures. Its unique life cycle plays a pivotal role in maintaining the soil environment and its fertility. *D. discoideum* regulates nutrient recycling, soil fertility, and plant pathogen control by consuming bacteria and other microorganisms as food sources (Kessin, 2001; Raper, 1935).

Recently, two parvulin-type PPIases, PinA and Pin4 have been reported in *D. discoideum* (Haokip and Naorem, 2017). PinA is a true homolog of human Pin1 and can complement the function of yeast Ess1. However, no further study has been reported on Pin4. With the above background, it was worthwhile to analyze the protein sequences, structure, and biochemical nature of Pin4.

MATERIALS AND METHODS

In silico analysis

The study was carried out to perform *in silico* analysis of Pin4, parvulin type PPIase in soil amoeba, *D. discoideum* at the Department of Genetics, University of Delhi in 2024.

The protein sequence of Pin4 (DDB_G0277775) from *D. discoideum* were obtained from the Dicty genome database, Dictybase(Anonymous, 2009). The FASTA sequence of human Par14 and Par17 were derived from the National Center for Biotechnology Information (NCBI) database (Anonymous, 2024). The sequences were aligned against human Par14 and Par17 to identify the sequence similarities using CLUSTAL OMEGA. The 3D protein structure modeling was predicted using AlphaFold and the

1. Ph.D. Scholar, Dept. of Genetics, University of Delhi South Campus, Benito Juarez Road, New Delhi-110021, India
2. Asst. Professor, Dept. of Genetics, University of Delhi South Campus, Benito Juarez Road, New Delhi-110021, India (Corresponding author)

RMSD (Root Mean Square Deviation) was calculated using PyMOL. Post-translation modifications (PTMs) were predicted through Phosphos Site Plus (Hornbeck *et al.,* 2015). Protein sequences of Pin4 were analyzed for any cleavage sites by using an ExPasy Peptide cutter (Gasteiger, 2005).

RESULTS AND DISCUSSION

Protein sequence analysis of Pin4

Firstly, the protein sequence of *D. discoideum* Pin4 (DDB_G0277775) was analyzed to identify the sequence similarity with the known Pin4 parvulin-type of humans. For this analysis, the protein sequence of Pin4 having 123 amino acids were aligned against human Par14 having 131 amino acids along with Par17 having 140 amino acids using CLUSTAL OMEGA. It revealed a high sequence conservation between Pin4 and human Par14 and Par17, specifically within the positively charged amino acid residues at the N-terminus and PPIase sequence at the C-terminus (Figure 1). The N-terminus of Pin4 shares 27% of sequence identity with its human counterpart, implying functional similarities. Similarly, the predicted acetylation sites in human Par14 at the N-terminus were found to be conserved in *D. discoideum* Pin4 as predicted using Phosphosite Plus (Figure 2). In contrast, the phosphorylation site on serine 19 (S19) residue in Par14, which is responsible for its nuclear localization and DNA binding activity (Reimer *et al.,* 2003), is absent in the corresponding position or at its nearby positions in Pin4 (Figure 2B). This differences in protein modifications suggest the unique functional role of Pin4 in *D. discoideum*. Thus, by exploring the signaling pathways involving Pin4, we can gain insight into its distinct functional role and expand the knowledge of parvulin-type PPIase in maintaining soil health and fertility.

Domain and structural organization of Pin4 in *D. discoideum*

The structural domain architecture analysis of Pin4 using SMART software with the Pfam database showed conservation among the parvulin type of Pin4 in humans and *D. discoideum* (Figure 3). The N-terminus of Pin4 lacks the WW domain or FHA domain of other parvulin-type which is required for protein-protein interactions. Instead, it has an N-terminal extension similar to human Par14. The protein encoded by *D. discoideum* Pin4 and human Par14 has a catalytic domain spanning 101 amino acids (Pin4) and 108 amino acids (Par14/Par17) respectively. In this, the catalytic domain is presented as rotamase which may be essential for PPIase activity. Further, the overlapping protein structure of Pin4 and human Par14 is also reflected by its predicted secondary protein structures using AlphaFold with an RMSD (Root mean square deviation) value of 0.708 (Figure 4). From this structural analysis and together with the previous report of Pin4 as a PPIase (Haokip and Naorem, 2017), we, demonstrated that Pin4 is a putative homolog of human Par14, sharing potentially conserved function. Therefore, Pin4 may regulate various cellular processes of transcription, chromosome remodeling, cell signaling, etc as reported in humans (Saeed and Piracha, 2023; Saningong and Bayer, 2015). Further research is necessary to elucidate the regulatory functions of Pin4 in *D. discoideum* and its impact on the soil ecosystem.

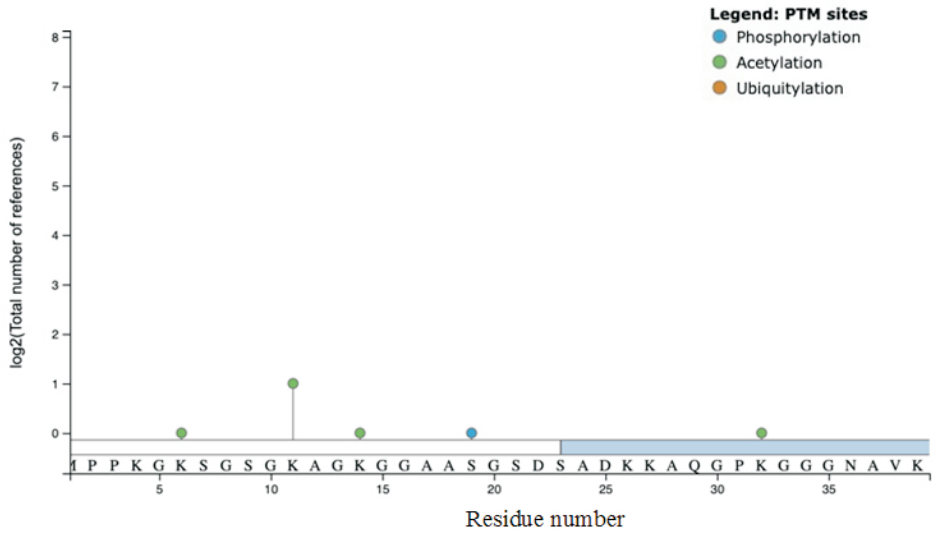
Analysis of Pin4 protein stability

The stability of a protein is a critical aspect in influencing its function. To analyse the potential proteolytic processing and stability of the Pin4 protein, we utilized ExPasy Peptide Cutter to predict proteolytic cleavage sites. Our result reveals multiple protease sites, including one thrombin site in the Pin4 sequence. The Pin4 sequence contain protease site such as proteinase K, trypsin, pepsin, chymotrypsin and various endopeptidase. Table 1 shows the detailed enzyme sites and number of cleavage sites in Pin4 sequence. The presence of numerous protease sites suggests high susceptible to protein degradation similar to its human counterpart Par14, which is reportedly prone to degradation by chymotrypsin and protease due to the absence of an N-terminal extension of Par17 (Mueller *et al.,* 2006). The high instability of the Pin4 protein may significantly affect ecological implications of *D. discoideum* and the soil ecosystem. This warrants further investigation to elucidate the underlying mechanism of protein instability that may directly or indirectly impact on soil ecosystem.

```
Par17           ----------------------------MPMAGLLKGLVRQLERFSVQQQASKMPPKGK
Par14           ------------------------------------------------------MPPKGK
Pin4            ------------------------------------------------------MPPKKP

Par17           SGSGKAGKGGAASGSDSADKKAQGPKGGGNAVKVRHILCEKHGKIMEAMEKLKSGMFNEV
Par14           SGSGKAGKGGAASGSDSADKKAQGPKGGGNAVKVRHILCEKHGKIMEAMEKLKSGMFNEV
Pin4            AE--KNAKKPAAGGKADKKGKGDDAKGGITQVKVRHILCEKEAKLMEAVNLIKSGKFNEV

Par14           AAQYSEDKARQGGDLGWMTRGSMVGPFQEAAFALPVSGMDKPVFTDPPVKTKFGYHIIMV
Par17           AAQYSEDKARQGGDLGWMTRGSMVGPFQEAAFALPVSGMDKPVFTDPPVKTKFGYHIIMV
Pin4            AQSHSEDKARASGLLGYIGRGDMVQEFTDRAFNQPIG------VVSEPFRTQFGYHIILV

Par14           EGRK
Par17           EGRK
Pin4            EDKK
```

Figure 1. Protein sequence alignment of *D. discoideum* Pin4 against human Par14 and Par17 using CLUSTAL OMEGA. The colored residues at the N terminal indicate the positively charged amino acids. PPIase domain sequences are depicted within the box.

A



B

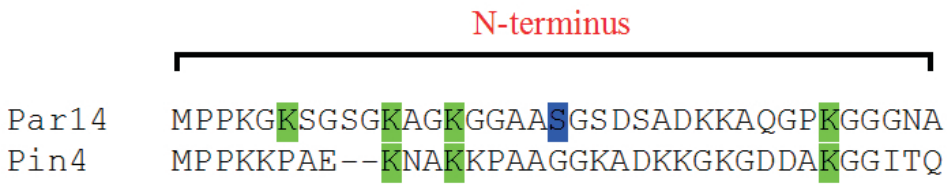


Figure 2. Putative post-translational modifications in Pin4. A) The protein post-modification sites predicted by Phosphosite Plus using human Par14. The sites of modifications such as phosphorylation, acetylation, and ubiquitylation were indicated with different colors. B) The acetylation site in human Par14 and *D. discoideum* Pin4 are highlighted in green. Phosphorylation at Serine (S19) is absent in *D. discoideum* Pin4 (highlighted in blue).

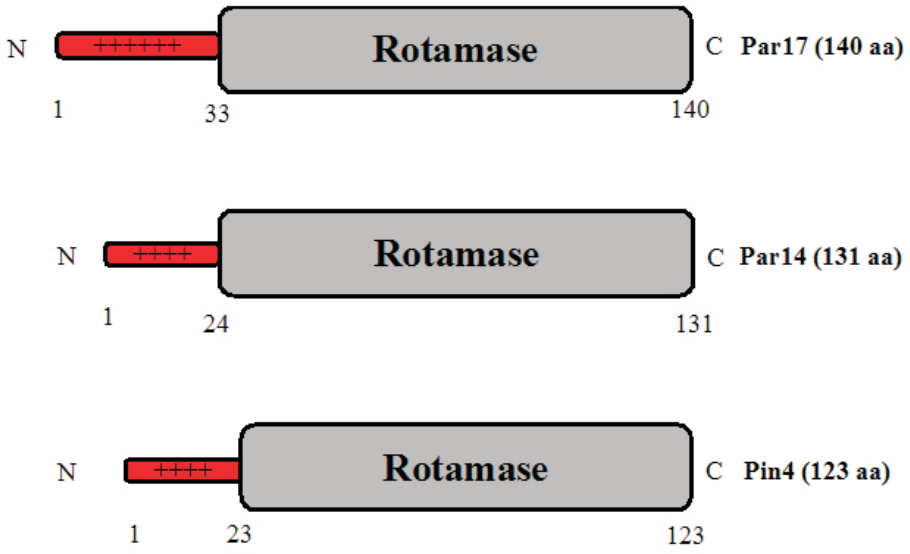


Figure 3. Domain architecture of *D. discoideum* Pin4 and human Par14. The domain architecture was derived using SMART software with the Pfam database. The domains presented are shown with their respective positions. The number of amino acids was indicated on the right side.

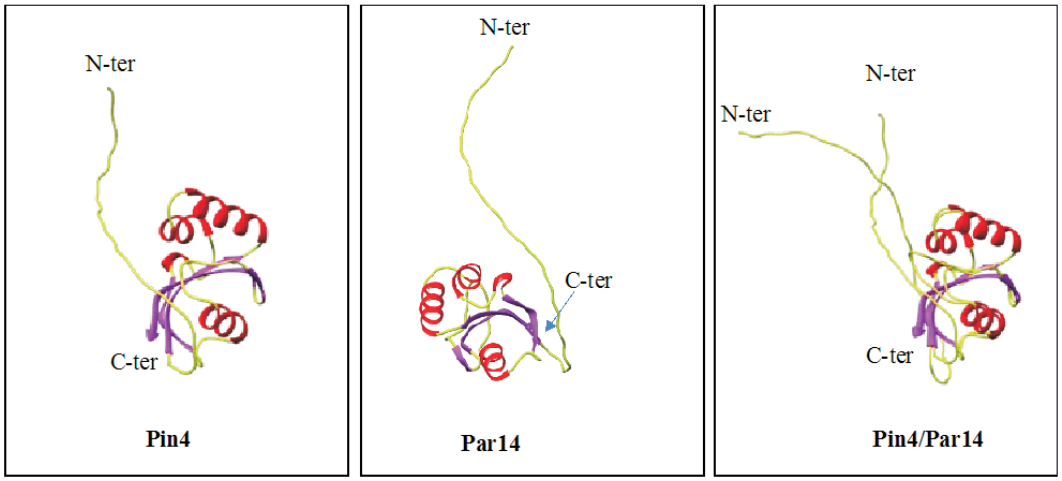


Figure 4. Predicted protein structure of Pin4 and its overlapping structures with human Par14. The secondary structures of Pin4 and Par14 were predicted using Alpha Fold and analyzed through Chimera. N and C terminus were indicated in the protein structures. The overlapping structures are shown on the right side having an RMSD value of 0.708.

Table 1. The list of enzymes, number of the cleavage site(s) and their position in the Pin4 sequence

Enzyme	No. of cleavage	Positions of cleavage sites
Arg-C proteinase	5	39, 75, 68, 96, 10
Asp-N endopeptidase	7	20, 26, 27, 71, 87, 94, 121
Asp-N endopeptidase + N-terminal Glu	15	7, 20, 26, 27, 43, 45, 50, 70, 71, 87, 91, 94, 106, 120, 121
Chymotrypsin	18	40, 42, 49, 50, 62, 69, 80, 81, 83, 89, 93, 98, 109, 113, 115, 116, 119
Glutamyl endopeptidase	8	8, 44, 46, 51, 71, 92, 107, 121
LysC	18	4, 5, 9, 12, 13, 19, 22, 23, 25, 30, 37, 45, 48, 57, 60, 73, 123, 124
Pepsin	18	48, 49, 54, 55, 61, 79, 80, 81, 82, 83, 92, 93, 97, 108, 113, 114, 115, 119
Proline endopeptidase	2	6, 14
Proteinase K	52	7, 8, 11, 15, 16, 20, 29, 33, 34, 36, 38, 41, 42, 44, 46, 47, 49, 51, 53, 55, 56, 61, 62, 65, 66, 71, 74, 76, 80, 81, 83, 84, 90, 92, 93, 94, 97, 98, 102, 104, 105, 107, 109, 111, 113, 115, 117, 118, 119, 120, 121
Staphylococcal peptidase I	8	8, 44, 46, 51, 71, 92, 107, 121
Thermolysin	35	6, 10, 14, 15, 19, 32, 35, 37, 40, 41, 48, 49, 52, 54, 55, 61, 64, 65, 73, 75, 79, 80, 83, 89, 96, 97, 101, 103, 104, 108, 112, 116, 117, 118, 119
Thrombin	1	86
Trypsin	21	4, 9,12, 19, 22, 23, 25, 30, 37, 39, 45, 48, 57, 60, 73, 75, 86, 96, 110, 123, 124

Note: The enzymes listed in the table plays a vital role in protein processing and degradation influencing the protein stability and its function in various cellular processes. There is limited report on these specific enzymes in soil amoeba, *D. discoideum.*

REFERENCES

Anonymous, 2009. Protein sequence of Pin4 (DDB_G0277775) from *D. discoideum.* Dicty genome database, Dictybase.

Anonymous, 2024. The FASTA sequence of human Par14 and Par17. National Center for Biotechnology Information (NCBI) database.

Gasteiger, E., C. Hoogland, A. Gattiker, S.E. Duvaud, M.R. Wilkins, R.D. Appel and A. Bairoch, 2005. Protein identification and analysis tools on the ExPASy server. Humana press: pp. 571-607.

Gurung, D., J. A. Danielson, A. Tasnim, J. T. Zhang, Y. Zou and J. Y. Liu, 2023. Proline Isomerization: From the Chemistry and Biology to Therapeutic Opportunities. Biol. **12**(7): 1008.

Haokip, N. and A. Naorem, 2017. Functional characterisation of parvulin-type peptidy lprolyl *cis-trans* isomerase, PinA in *Dictyostelium discoideum*. BBRC, **482**(2): 208-214.

Hornbeck, P. V., B. Zhang, B. Murray, J.M. Kornhauser, V. Latham and E. Skrzypek, 2015. PhosphoSitePlus, 2014: mutations, PTMs and recalibrations. NAR, **43**(D1): D512-D520.

Kessin, R. H. 2001. *Dictyostelium:* evolution, cell biology, and the development of multicellularity. Cambridge University Press. Vol. 38.

Maleszka, R., S.D. Hanes, R.L. Hackett, H.G. De Couet and G.L. Miklos, 1996. The *Drosophila melanogaster dodo* (dod) gene, conserved in humans, is functionally interchangeable with the ESS1 cell division gene of *Saccharomyces cerevisiae*. PNAS, **93**(1): 447-451.

Mueller, J. W., D. Kessler, D. Neumann, T. Stratmann, P. Papatheodorou, C. Hartmann-Fatu and P. Bayer, 2006. Characterization of novel elongated Parvulin isoforms that are ubiquitously expressed in human tissues and originate from alternative transcription initiation. BMC Mo.l Biol. **7**: 1-9.

Lu, P. K., S.D. Hanes and T. Hunter, 1996. A human peptidyl–prolyl isomerase essential for regulation of mitosis. Nat. **380**(6574): 544-547.

Raper, K. B. 1935. *Dictyostelium discoideum*, A new species of slime mold from decaying forest leaves. J. Agric. Res. **50**. 135.

Reimer, T., M. Weiwad, A. Schierhorn, P. K. Ruecknagel, J. U. Rahfeld, P. Bayer and G. Fischer, 2003. Phosphorylation of the N-terminal domain regulates subcellular localization and DNA binding properties of the peptidyl-prolyl *cis/trans* isomerase hPar14. J. Mol. Biol. **330**(5): 955-966.

Saeed, U. and Z. Z. Piracha, 2023. PIN1 and PIN4 inhibition via parvulin impeders Juglone, PiB, ATRA, 6,7,4'-THIF, KPT6566, and EGCG thwarted hepatitis B virus replication. Front Microbiol. **14**: 921653.

Saningong, A. D. and P. Bayer, 2015. Human DNA-binding peptidyl-prolyl *cis/trans* isomerase Par14 is cell cycle dependently expressed and associates with chromatin in vivo. BMC Biochem. **16**: 1-9.

Uchida, T., F. Fujimori, T. Tradler, G. Fischer and J. U. Rahfeld, 1999. Identification and characterization of a 14 kDa human protein as a novel parvulin-like peptidyl prolyl *cis/trans* isomerase. FEBS Lett. **446**(2-3): 278-282.

Zhang, J., Y. Nakatsu, T. Shinjo, Y. Guo, H. Sakoda, T. Yamamotoya, Y. Otani, H. Okubo, A. Kushiyama, M. Fujishiro, T. Fukushima, Y. Tsuchiya, H. Kamata, M. Iwashita, F. Nishimura, H. Katagiri, S. Takahashi, H. Kurihara, T. Uchida and T. Asano, 2013. Par14 protein associates with insulin receptor substrate 1 (IRS-1), thereby enhancing insulin-induced IRS-1 phosphorylation and metabolic actions. J. Biol. Chem. **288**(28): 20692-20701.

Rec. on 15.01.2025 & Acc. on 30.01.2025