**ADVANCED RESEARCH IN LIFE SCIENCES**
**9, 2025, 48 – 53**

Advanced Research in Life Sciences

**DOI: 10.2478/arls-2026-0003**
Research Article

# Alphafold Modeling and Molecular Docking of *Pseudomonas fluorescens* Cutinase with Agrochemicals

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*Received May, 2025; Revised December, 2025; Accepted December, 2025*

**Abstract**
Cutinases (E.C. 3.1.1.74) are versatile enzymes produced by bacteria and fungi, known for their ability to hydrolyze cutin, a protective plant polyester. These enzymes have gained attention for their potential in agricultural biotechnology, particularly in bioremediation and sustainable pest management. Building upon our previous *in silico* characterization of *Pseudomonas fluorescens* cutinase using homology modeling (Phyre$^2$), this study employed AlphaFold, an AI-driven structure prediction tool, to generate a more accurate 3D model of the enzyme. The refined structure was validated using PROCHECK tool, with 93.8% of residues in favored Ramachandran regions, confirming its reliability for molecular docking studies. To assess the enzyme's potential interactions with agrochemicals, CB-DOCK2 was used to dock the cutinase against eight ligands, including widely used insecticides (chlorpyrifos, malathion, diazinon, cypermethrin, deltamethrin) and herbicides (2,4-D butyl ester, glyphosate, propanil). Comparative analysis revealed strong binding affinities for cypermethrin (−9.8 kcal/mol) and deltamethrin (−9.5 kcal/mol), while moderate interactions were observed with chlorpyrifos (−6.4 kcal/mol), diazinon (−6.5 kcal/mol), and the herbicide propanil (−7.2 kcal/mol). The natural substrate, Cutin-1, exhibited a binding score of −8.0 kcal/mol, providing a reference for evaluating pesticide interactions. These findings suggest that *P. fluorescens* cutinase may play a role in the binding or degradation of certain synthetic pesticides, particularly pyrethroids and organophosphates. Future studies should include molecular dynamics simulations to assess binding stability and enzymatic assays to validate hydrolysis activity. Additionally, exploring cutinase engineering for enhanced pesticide degradation could open new avenues for eco-friendly bioremediation strategies. This work advances our understanding of bacterial cutinases and highlights their potential applications in sustainable agriculture.

**Keywords:** *Pseudomonas fluorescens,* pesticides, herbicides, AlphaFold, enzyme docking, agrochemical degradation.

## Introduction

Cutinases (Cut, EC 3.1.1.74) are inducible extracellular serine hydrolases, secreted by fungi, oomycetes, and bacteria, that degrade cutin, the fatty acid polyester forming the structural backbone of plant cuticles and a key component of cell walls [1]. The plant cuticle, covering aerial organs like leaves, flowers, fruits, and stems, serves as a protective barrier against water loss, pathogen infection, and environmental stresses while regulating gas exchange and solute transport [2].

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Cutinases are utilized in diverse industrial sectors, including paper manufacturing, textile processing, and detergent formulation, as well as environmental biotechnology. Their catalytic superiority over lipases stems from their unique structural features: the absence of a helical lid domain covering the active site, which contains a canonical Ser-His-Asp catalytic triad. This open active site configuration permits continuous solvent accessibility, eliminating the need for interfacial activation [3]. These biochemical characteristics enhance their hydrolytic activity against various polyesters, including synthetic plastics like polyethylene terephthalate (PET). Consequently, cutinases have become increasingly valuable for biocatalytic applications and polymer biodegradation processes, particularly in sustainable material recycling and bioremediation strategies [4].

Cutin-rich agricultural byproducts like apple pomace and tomato peel serve as cost-effective inducers for bacterial cutinase production via fermentation [5]. Although *Pseudomonas* spp. are promising hosts, strain-dependent variability in enzyme activity highlights the need to explore diverse bacterial sources for optimized industrial cutinases. Notably, *Pseudomonas cepacia* [6] and *Pseudomonas mendocina* [7] have attracted particular interest for cutinase expression, given their potential in biodegradation and biocatalytic applications. Cutinases also play key ecological roles, enabling PGPR (plant growth-promoting rhizobacteria) colonization of plant roots [8]. Notably, cutinase-producing *Pseudomonas fluorescens* strains with PGPR activity thrive in rhizosphere soils [9], highlighting their dual industrial-agricultural potential. In addition, cutinases show strong potential in agrochemical applications, particularly in pesticide and insecticide degradation. Their unique ability to hydrolyze organophosphates and carbamates [10] positions them as valuable tools for sustainable agriculture and environmental remediation. Indeed, the production of these proteins is primarily achieved through heterologous expression systems like *Escherichia coli* and *Pichia pastoris*, with ongoing research focusing on enhancing their efficiency through genetic engineering strategies [11]. However, inherent limitations such as rare codon usage [12], low solubility, and costly purification processes frequently constrain large-scale production [13]. This underscores the critical role of bioinformatics tools for structural prediction and structure-function analysis, enabling rational protein design to improve expression yields and stability before experimental implementation.

In this research, the 3D structure of cutinase was predicted using the AI-based tool AlphaFold. The model was then refined and validated, after which it was docked with both insecticide and herbicide ligands for further analysis.

## Material and methods

### *Protein modeling and validation*

The amino acid sequence of *Pseudomonas fluorescens* S613 cutinase (UniProt ID: A0A0P8X0D6) was obtained in FASTA format from the UniProt database (https://www.uniprot.org). The three-dimensional (3D) structural model of the cutinase protein query sequence was predicted using the AlphaFold server (https://alphafold.ebi.ac.uk/) [14], which employs deep neural networks trained on evolutionary patterns from multi-sequence alignments while incorporating physicochemical constraints governing protein folding [14]. This preliminary model was subsequently refined via the GalaxyWEB server (http://galaxy.seoklab.org/refine) to enhance its physicochemical accuracy [15]. The refined model was assessed using Ramachandran plot generated by the PROCHECK tool on the SAVES v6.1 server (https://saves.mbi.ucla.edu/) [16]. The final 3D structure was then visualized and further optimized using the PyMOL Molecular Graphics System (Schrödinger LLC, version 2.3).

### *Molecular docking Analysis*

Molecular docking analysis was performed using CB-DOCK2 (https://cadd.labshare.cn/cb-dock2/index.php) [17], to evaluate interactions between the refined cutinase structure and eight agrochemical ligands, including five insecticides (chlorpyrifos, malathion, diazinon, cypermethrin, deltamethrin) and three herbicides (2,4-D butyl ester, glyphosate, propanil) (Table 1).

The ligands, obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in SDF format, were converted to PDB using PyMol 2.3 and energy-minimized with Chem3D Ultra 8.0. Docking identified five potential binding cavities, with the lowest Vina score model selected for further analysis. Enzyme-ligand interactions were visualized using BIOVIA Discovery Studio.

**Table 1. Ligand properties for *Pseudomonas fluorescens* cutinase docking**

| Ligand | Pubchem CID | Molecular Formula | Molecular Weight (g/mol) | Application |
|---|---|---|---|---|
| Chlorpyrifos | 2730 | $C_9H_{11}Cl_3NO_3PS$ | 350.6 g/mol | Insecticide |
| Malathion | 4004 | $C_{10}H_{19}O_6PS_2$ | 330.4 g/mol | |
| Diazinon | 3017 | $C_{12}H_{21}N_2O_3PS$ | 304.35 g/mol | |
| Cypermethrin | 2912 | $C_{22}H_{19}Cl_2NO_3$ | 416.3 g/mol | |
| Deltamethrin | 40585 | $C_{22}H_{19}Br_2NO_3$ | 505.2 g/mol | |
| 2,4-D butyl ester | 7206 | $C_{12}H_{14}Cl_2O_3$ | 277.14 g/mol | Herbicide |
| Glyphosate | 3496 | $C_3H_8NO_5P$ | 169.07 g/mol | |
| Propanil | 4933 | $C_9H_9Cl_2NO$ | 218.08 g/mol | |
| Cutin-1 (Natural substrate) | 4057195 | $C_{21}H_{19}N_2O_2P$ | 362.4 g/mol | |

## Results and discussion

### Protein modeling and validation

The FASTA sequence of *Pseudomonas. Fluorescens* S613 cutinase protein (UniProt: A0A0P8X0D6) served as input for tertiary structure prediction using AlphaFold. The resulting model was refined (GalaxyWEB) and visualized in PyMOL (Figure 1.A). Structural validation via Ramachandran plot analysis (PROCHECK tool, Figure 1.B) confirmed the model's reliability, with 93.8% of residues in favored regions, 5.8% in additional allowed regions, 0.0% in generously allowed regions, and 0.4% in disallowed regions.

This represents a significant structural refinement compared to our previous characterization of *Pseudomonas fluorescens* S613 cutinase (UniProt: A0A0P8X0D6) using homology modeling (Phyre$^2$) with ModRefiner refinement [18], which yielded 89.2% favored, 7.7% additional allowed, 2.1% generously allowed, and 1.1% disallowed residues. The current AlphaFold-derived model demonstrates three key improvements: (i) a 4.6% increase in favored residues (93.8% vs 89.2%), (ii) complete elimination of generously allowed regions (0.0% vs 2.1%), and (iii) a 63% reduction in disallowed regions (0.4% vs 1.1%). These enhancements validate the superior precision of this AI-based approach, particularly for resolving the catalytic triad geometry (Ser-His-Asp) critical for both cutin hydrolysis and PGPR colonization functions. The refined model of *P. fluorescens* cutinase not only achieves superior structural accuracy but also enables advanced functional studies. Moreover, the optimized catalytic triad geometry permits detailed investigation of substrate binding pockets, while the improved global fold facilitates analysis of plant cell wall interaction networks. Furthermore, this high-resolution model significantly enhances the reliability of molecular docking analyses against diverse agrochemical targets, including insecticides and herbicides, by providing precise atomic coordinates for the active site and potential binding cavities. These advancements address prior limitations in homology-based models, offering new opportunities to explore cutinase's bioremediation potential against synthetic pesticides.

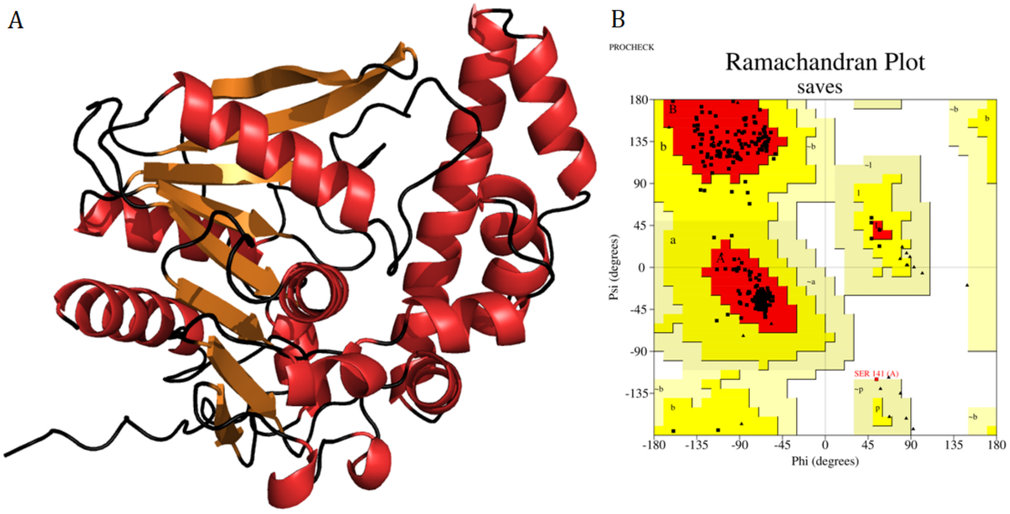

**Figure 1. The 3D structure of *Pseudomonas. fluorescens* cutinase predicted by AlphaFold.** (A) The structural model, generated using AlphaFold, refined with GalaxyWEB server, and visualized with PyMOL, showing the α-helix (red), β-sheet (orange), and loop (black). (B) PROCHECK-validated Ramachandran plot for the refined cutinase. Phi vs. Psi angles categorize residues into favored (red), allowed (yellow), generously allowed (beige), and disallowed (white) regions. Non-Gly/Pro (■), Gly (▲).

## Molecular docking Analysis

The modeled and refined *Pseudomonas fluorescens* cutinase enzyme was subjected to molecular docking analyses using the CB-DOCK2 server to evaluate binding interactions with selected agrochemical ligands. These included five insecticides; chlorpyrifos, malathion, diazinon, cypermethrin, and deltamethrin; along with three herbicides, namely 2,4-D butyl ester, glyphosate, and propanil. For comparative analysis, the natural substrate Cutin-1 was also docked against the enzyme, with results summarized in Table 2.

**Table 2. Minimum binding energies and predicted cavity sizes for cutinase derived from CB-DOCK2 Vina scoring**

| Ligand | Vina score | Cavity volume (Å) |
|---|---|---|
| Chlorpyrifos | -6.4 | 2146 |
| Malathion | -5.5 | 2146 |
| Diazinon | -6.5 | 2146 |
| Cypermethrin | -9.8 | 2146 |
| Deltamethrin | -9.5 | 2146 |
| 2,4-D butyl ester | -6.9 | 2146 |
| Glyphosate | -5.1 | 2146 |
| Propanil | -7.2 | 2146 |
| Cutin-1 (Natural substrate) | -8.0 | 2146 |

Table 2 analysis revealed distinct binding affinities: cypermethrin and deltamethrin showed the strongest interactions (Vina scores: −9.8 and −9.5 kcal/mol, respectively), surpassing even the natural substrate Cutin-1 (−8.0 kcal/mol). Moderate binding was observed for chlorpyrifos (−6.4 kcal/mol), diazinon (−6.5 kcal/mol), and propanil (−7.2 kcal/mol), while glyphosate exhibited the weakest affinity (−5.1 kcal/mol). The consistent cavity volume (2146 Å) across all ligands suggests stable accommodation within the active site. CB-DOCK2 analysis identified the most suitable ligand based on the lowest binding energy (Vina scores) and cavity size parameters. Using the curvature-dependent surface-area model [19], five potential interaction models were generated to characterize the binding affinity between *P. fluorescens* cutinase and the selected ligands. Molecular docking revealed cypermethrin's specific interactions with catalytic residues Pro25, Gly68, Leu69, Ser75, and Val304. These residues formed multiple stabilizing interactions—including carbon-hydrogen bonds, van der Waals forces, amide-Pi stacking, and Pi-alkyl interactions—which collectively contribute to ligand-enzyme complex stabilization and suggest potential catalytic enhancement. These results are illustrated in Figure 2.

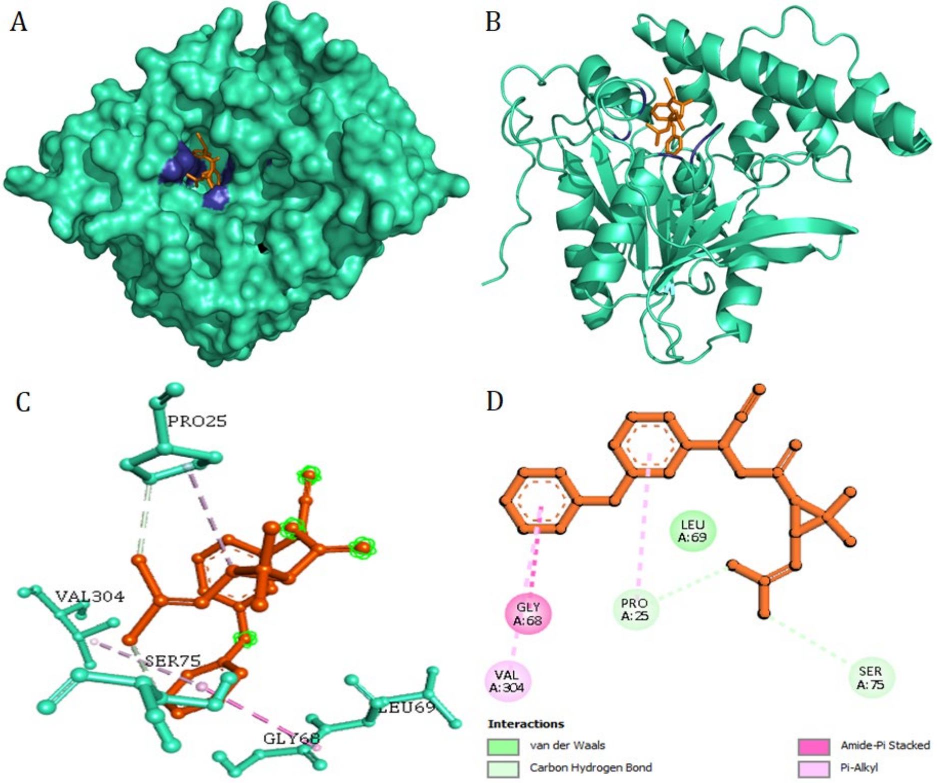

**Figure 2. Molecular docking analysis of Cypermethrin ligand with *Pseudomonas fluorescens* cutinase receptor.** (A) Surface representation of the protein (green) and ligand (orange), with the binding site in blue. (B) Cartoon representation. (C) 3D view of receptor-ligand interacting residues. (D) 2D diagram of residue-ligand interactions.

While previous experimental work identified organophosphate oxons (e.g., chlorpyrifos oxon) as potent cutinase inhibitors ($k = 9.4 \times 10^5$ L/(mol·min)) in [20], our *in silico* study revealed for the first time that intact agrochemicals—particularly pyrethroids (cypermethrin: −9.8 kcal/mol; deltamethrin: −9.5 kcal/mol) and the herbicide propanil (−7.2 kcal/mol)—can bind strongly to *P. fluorescens* cutinase, even exceeding natural substrate affinity (−8.0 kcal/mol). These findings align partially with Walz's inhibition data for organophosphate derivatives but significantly expand the scope to parent compounds and non-organophosphate pesticides. The consistent cavity volume (2146 Å) across all ligands suggests broad substrate adaptability, though the catalytic implications of these interactions require validation. Future work should combine molecular dynamics simulations to assess binding stability with enzymatic assays to verify hydrolysis potential, bridging the gap between computational predictions and biological functionality.

## Conclusions

This study demonstrated the potential of *Pseudomonas fluorescens* cutinase enzyme as a versatile biocatalyst for agrochemical interactions, particularly with synthetic pesticides. The high-resolution structural model, generated using AlphaFold, provided a reliable framework for understanding the enzyme's binding mechanisms, revealing strong interactions with pyrethroid insecticides and certain herbicides. The conserved binding cavity and catalytic residue interactions suggested a broad substrate adaptability, positioning this enzyme as a promising candidate for bioremediation applications in sustainable agriculture.

Looking ahead, this work opened several research avenues. First, experimental validation through enzymatic assays and molecular dynamics simulations would be essential to confirm the predicted catalytic activity against agrochemicals. Second, protein engineering approaches could optimize cutinase for enhanced pesticide degradation efficiency, potentially leading to tailored microbial solutions for environmental detoxification. Finally, exploring the enzyme's dual role in plant growth promotion and agrochemical degradation might offer innovative strategies for eco-friendly farming practices.

By integrating computational insights with practical applications, this study contributed to the development of biocatalytic tools for reducing agrochemical pollution, aligning with global efforts toward sustainable agricultural systems. Future investigations should focus on translating these findings into scalable biotechnological solutions for real-world environmental challenges.

## Acknowledgements

This work was supported by PRFU "Projet de Recherche et Formation Universitaire" Project Research, D01N01UN460120220001.

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