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AlphaFold modeling and molecular docking of *Pseudomonas fluorescens* cutinase with agrochemicals

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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 extracellular serine hydrolases produced by fungi, bacteria, and oomycetes to degrade cutin, the protective polyester matrix of plant cuticles. These enzymes are distinguished from lipases by their open active site architecture (featuring a canonical Ser-His-Asp triad) and lack of a helical lid, enabling direct solvent access and enhanced hydrolytic activity against diverse substrates, including synthetic polyesters like PET. This versatility has spurred their industrial use in textiles, detergents, and plastic biodegradation, as well as emerging applications in sustainable agriculture and environmental remediation.

Pseudomonas species, particularly *P. fluorescens*, are promising cutinase producers due to their natural role as plant-growth-promoting rhizobacteria (PGPR) and their ability to utilize cutin-rich agricultural waste as induction substrates. Notably, *Pseudomonas*-derived cutinases demonstrate dual agro-biotechnological potential, facilitating root colonization while exhibiting pesticide-degrading capabilities against organophosphates and carbamates. However, industrial-scale production faces challenges such as heterologous expression bottlenecks, solubility issues, and costly purification—prompting the need for computational tools to guide protein optimization.

In this study, we leverage AlphaFold to predict the 3D structure of *P. fluorescens* cutinase, followed by model refinement and molecular docking with agrochemical ligands. This in silico approach aims to elucidate structure-function relationships for targeted biocatalyst design, bridging the gap between computational prediction and bioremediation applications.

• Materials and methods

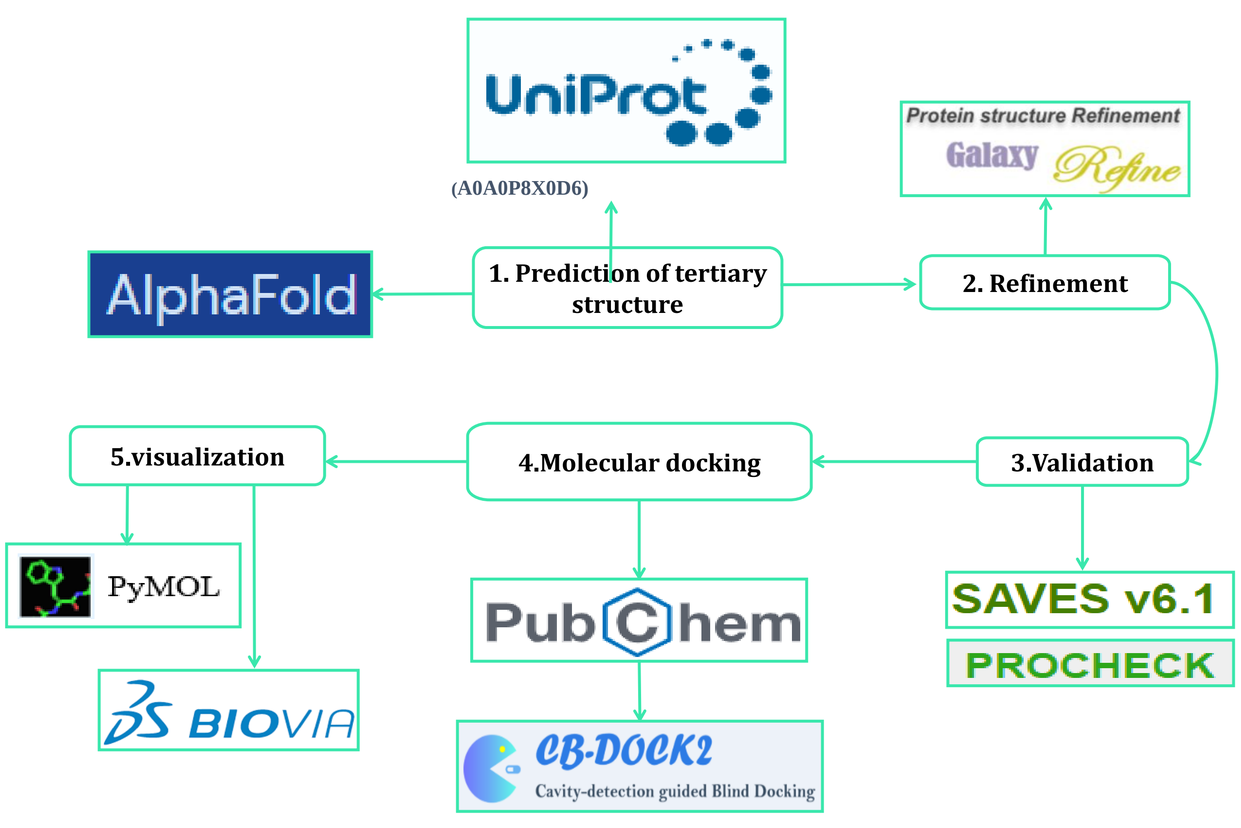

Figure 1. *In silico* Workflow for structural and functional analysis of *Pseudomonas fluorescens* cutinase.

Table1. 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 discussions

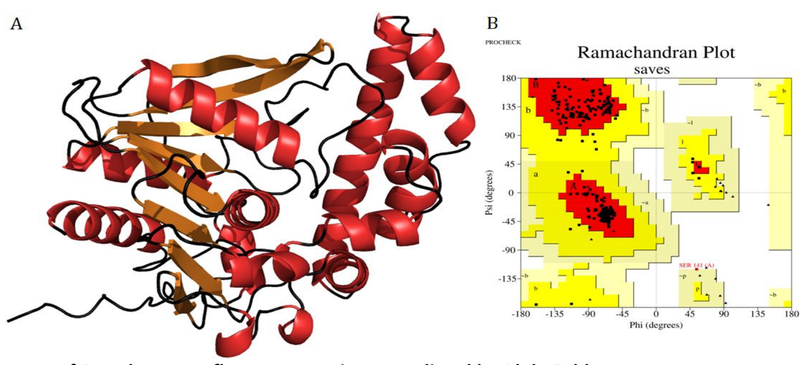

Figure 2. The 3D structure of *Pseudomonas. fluorescens* cutinase predicted by AlphaFold.
(A) The structural model, generated using AlphaFold, refined with GalaxyWEB server, and visualized with PyMOL, showing the α-helix (red), β-sheet (orange), and loop (black). **(B)** PROCHECK-validated Ramachandran 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 (▲).

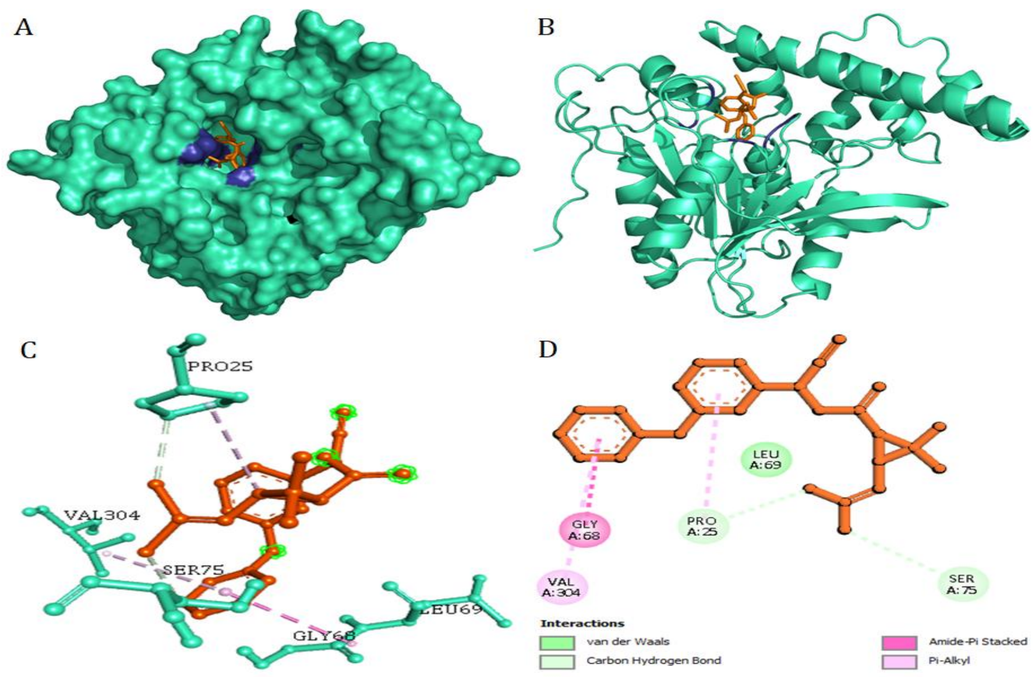

Figure 3. Molecular docking analysis of Cypermethrin ligand with *Pseudomonas fluorescens* cutinase receptor. (A) Surface representation of the protein (green) and ligand (orange), with the binding site in blue. **(B)** Cartoon representation. **(C)** 3D view of receptor-ligand interacting residues.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

Table2. 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

• Conclusions

✓ *In silico* analysis predicted that *Pseudomonas fluorescens* cutinase could degrade insecticides and herbicides.

✓ Structural modeling revealed versatile degradation capacity.

✓ Molecular modeling identified key residues for biocatalyst optimization.

✓ *In vitro* and *in vivo* studies are required to validate the predicted agrochemical degradation capabilities.

✓ Protein engineering approaches should be explored to enhance the enzyme's bioremediation potential.

• References

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