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CHARACTERIZATION OF PLASTIC DEGRADATION ASSOCIATED BACTERIAL ENZYME CUTINASE THROUGH IN-*SILICO* TOOLS

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Abstract

Plastic pollution is a crucial problem globally. It exerts negative impact on aquatic as well as human life. Bioremediation based on bacterial enzymes is an emerging and sustainable mode of plastic degradation. In current study, two plastic degrading enzymes G8GER6 and E9LVH8 from bacteria bacteria Thermobifida fusca and Thermobifida cellulosilytica were targeted for analysis. UNIPROT database was accessed to retrieve the sequences of enzymes. Followed this, enzymes were analyzed via PROPARAM and SOPMA tool and ALPHAFOLD web server. Analysis revealed the number of amino acids (319 and 262), theoretical pI (9.65 and 6.30), extinction coefficients (38390 and 36900), instability index (41.75 and 36.39), aliphatic index (79.06 and 80.50) and GRAVY values (-0.247 and -0.221) for these two enzymes. Alpha helix, extended strand and random coil content was found in the range of 64-88, 36-44 and 154-195, respectively. Both proteins exhibited tertiary structure with complex folding. Current study findings will be helpful in simulating mutations via site directed mutations that might increase binding affinity of enzyme with plastic and its degradation efficiency.

1. Introduction

Several conventional strategies like landfilling, incineration, recycling, thermos oxidative degradation and photodegradation have been employed for plastic degradation since decades. But these are costly, time taking, emit greenhouse gas and leads to deterioration of land. Landfill and photodegradation breaks down plastic in 125 days and 50-100 years, respectively (Udekwu et al. 2024). These shortcomings led to the replacement of these techniques with emerging approach of bioremediation (Agarwal et al. 2024).

Biodegradation being a microbial driven process largely depends on enzymes from bacteria which transform plastic toxins into innocuous compounds. Plastic remediation is attained by PETases, MHETases, cutinases, lipases, esterases, laccases, proteases and peroxidases (Suresh et al. 2025). Cutinases are the serine hydrolases which breakdown cutin. Cutin is composed of epoxy and hydroxy fatty acids (n-C_{16} and n-C_{18}) and ester, peroxides and ether bonds (Dutta and Veeranki 2009). Cutinases act on variety of substrates including polyethylene terephthalate plastic, polylactic acid and polycaprolactone polymers (Martinez and Maicas 2021). Bacteria being the key producers are the major source of cutinases. Literature documents wide variety of cutinase producing bacteria like *Thermobifida cellulosilytica* (ThcCut1) and *Thermobifida fusca* (TfCut2) (Zhang et al. 2022), *Pseudomonas cepacia* NRRL B 2320 (Dutta et al. 2013), *Pseudomonas* sp. MIS38 (Chen et al. 2008), *Pseudomonas nitroreducens* S8 and *Pseudomonas monteilii* S17 (Yen et al. 2023), P. putida (Sebastian et al. 1987; Liang and Zou 2022), *P. pseudoalcaligenes* (Inglis et al. 2011), *P. aeruginosa* S3 (Din et al. 2023), *P. cepacia* NRRL B-2320 (Dutta et al. 2013), *P. mandocino* (Martinez and Maicas 2021), *P. fluorescens* (Bakli et al. 2021), *P. syringae* (Fett et al. 1992), *Bacillus* sp. KY0701 (Adiguzel and Tuncer 2017) and *Acenatobacter baumannii* AU10 (Gururaj et al. 2021).

Considering the importance of cutinases in plastic bioremediation, we initiated current research work to get insight into its properties and structures. These findings might help in engineering of these enzymes.

2. Methodology

2.1 UNIPROT DATABASE

UNIPROT database (https://www.uniprot.org) was accessed on September_2025 to retrieve the sequences of two cutinase enzymes. One enzymes with Accession ID G8GER6 was associated with *Thermobifida fusca*. Second enzyme with Accession ID E9LVH8 is found in *Thermobifida cellulosilytica* (Table 1). UNIPROT database has been developed by UNIPROT consortium which facilitates scientists community by providing information related to protein sequences and functions (Pundir et al. 2016). Its three database layers are; UNIPROT Archive (UniParc), UNIPROT knowledgebase (UniProt) and UniProt NREF database (UniRef) (UniProt Consortium 2018).

Table 1: ***Accession IDs, bacterium and sequences of cutinases documented in current study, retrieved from UNIPROT database***

#	Accession ID	Bacterium	Sequence
1	• G8GER6	*Thermobifida fusca*	MPPHAARPGPAQNRRGRAMAVITPRRERSSLLSRALRFTAAAA TALVTAVSLAAPAHAANPYERGPNPTDALLEARSGPFSVSEER ASRFGADGFGGGTIYYPRENNTYGAVAISPGYTGTQASVAWL

			GERIASHGFVVITIDTNTTLDQPDSRARQLNAALDYMINDASS AVRSRIDSSRLAVMGHSMGGGGTLRLASQRPDLKAAIPLTPW HLNKNWSSVRVPTLIIGADLDTIAPVLTHARPFYNSLPTSISKAY LELDGATHFAPNIPNKIIGKYSVAWLKRFVDNDTRYTQFLCPG PRDGLFGEVEEYRSTCPF
2	E9LVH8	*Thermobifida cellulosilytica*	MANPYERGPNPTDALLEASSGPFSVSEENVSRLSASGFGGGTIY YPRENNTYGAVAISPGYTGTEASIAWLGERIASHGFVVITIDTIT TLDQPDSRAEQLNAALNHMINRASSTVRSRIDSSRLAVMGHS MGGGGTLRLASQRPDLKAAIPLTPWHLNKNWSSVTVPTLIIG ADLDTIAPVATHAKPFYNSLPSSISKAYLELDGATHFAPNIPNKII GKYSVAWLKRFVDNDTRYTQFLCPGPRDGLFGEVEEYRSTCP F

2.2 PROTPARAM TOOL

To predict the physical and chemical aspects of protein structure, EXPASY PROTPARAM tool (https://web.expasy.org/protparam/) was consulted on September 2025. Input was entered in the form of protein sequence comprising of one letter code. The output obtained included the molecular weight, chemical formula, number of amino acids, isoelectric point (I), number of atoms, instability index, aliphatic index, extinction coefficients, half-life, aliphatic index and Grand Average of Hydropathicity (GRAVY) (Gasteiger et al. 2005).

2.3 SOPMA TOOL

To compute the structural features at secondary (2D) level, web server NPS@ (Network Protein Sequence Analysis) SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) was accessed on September 2025. Autocalculated and DSSP were choosen as Garnier and PREDATOR parameters, respectively. Prediction of structures was based on 8 similarity threshold, 17 window width and 4 conformational states (Angamuthu and Piramanayagam 2017).

2.4 ALPHAFOLD DATABASE

To predict the structure at three-dimensional (3D) level, ALPHAFOLD web service (https://alphafold.ebi.ac.uk)was consulted on September 2025. This server is a platform that covers structures of 214 million proteins sequences. It stores predicted structures in mmCIF, binaryCIF and PDB formats. The metadata is presented as JSON format. It represents confidence of structure in terms of pLDDT score ranging in 0 to 100. Value above 90, 70-90, 50-70 and 50 show regions of structure computed with high accuracy, well-modelled with reliable backbone, regions of lower confidence and disordered regions, respectively (Varadi et al. 2024; Brookes and Rocco 2022).

3. Result

3.1 Prediction of physicochemical properties

Physicochemical properties analyzed are shown in Table 2. Cutinase enzymes with IDs G8GER6 and E9LVH8 exhibited 319 and 262 amino acids, respectively. The isoelectric point (I) was high for G8GER6 (9.65) and low for E9LVH8 (6.30). Molecular weight, total number of atoms, extinction coefficients, instability index, aliphatic index and GRAVY values found for G8GER6 were 34421.90, 4822, 38390, 41.75, 79.06 and -0.247, respectively. In case of E9LVH8, values observed

for these properties were 28305.81, 3951, 36900, 36.39, 80.50 and -0.221, respectively.

Table 2: ***Prediction of physicochemical properties of cutinase enzymes via PROTPARAM tool***

#	Physicochemical property	G8GER6	E9LVH8
1	No. of amino acids	319	262
2	Theoretical pI	9.65	6.30
3	Molecular weight	34421.90	28305.81
4	Formula	$C_{1523}H_{2393}N_{447}O_{452}S_7$	$C_{1258}H_{1954}N_{348}O_{385}S_6$
5	Total number of atoms	4822	3951
6	Extinction coefficients	38390	36900
7	Estimated half life	>10 hr	>10 hr
8	Instability index	41.75	36.39
9	Aliphatic index	79.06	80.50
10	GRAVY	-0.247	-0.221

3.2 Prediction of 2D Configuration

The 2D configuration predicted for G8GER6 showed 88, 36 and 195 amino acids, taking part in the formation of alpha helix, extended strand and random coil, respectively. In case of E9LVH8, alpha helix contained 64 amino acids, extended strand comprised of 44 and random coil was made up of 154 amino acids (Table 3).

Table 3: ***Prediction of 2D configuration of cutinase enzymes via SOPMA tool***

#	Enzyme ID	Alpha helix	Extended strand	Random coil
1	G8GER6	88 (27.59 %)	36 (11.29 %)	195 (61.13 %)
2	E9LVH8	64 (24.43 %)	44 (16.79 %)	154 (58.78 %)

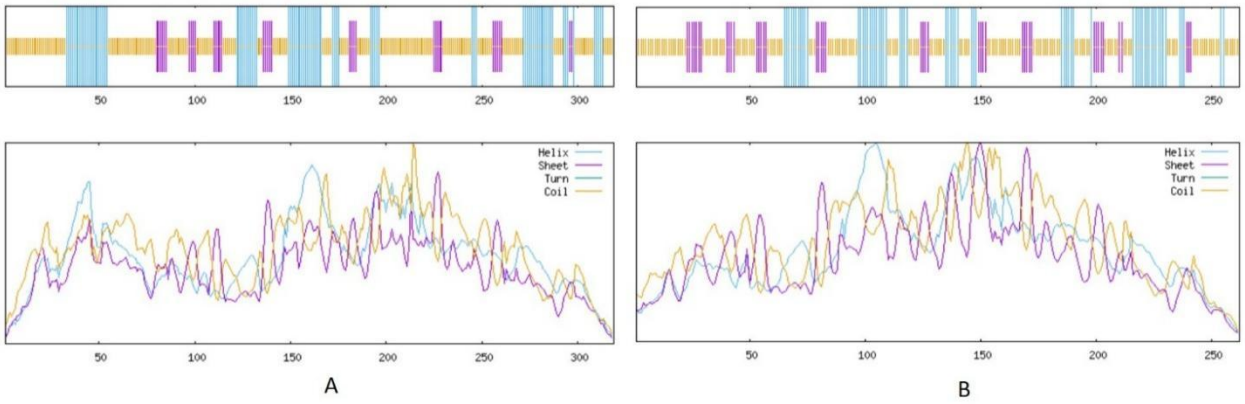

Figure 1: Prediction of 2D configuration of cutinase enzymes via SOPMA tool

(A) G8GER6 (B) E9LVH8

3.3 Prediction of 3D Configuration

The 3D structures of both enzymes demonstrated comparable level of folding as shown in Figure 2. Regions with dark blue color showed very high confidence level with pLDDT score greater than 90. Light blue regions of structure confident regions with pLDDT score lesser than 90 and greater than 70. Yellow regions demonstrated structures with

low confidence with pLDDT score lesser than 70 and greater than 50. Orange colored regions revealed part of structure with very low confidence and exhibited pLDDT value below 50. Structure of E9LVH8 did not show any orange colored region.

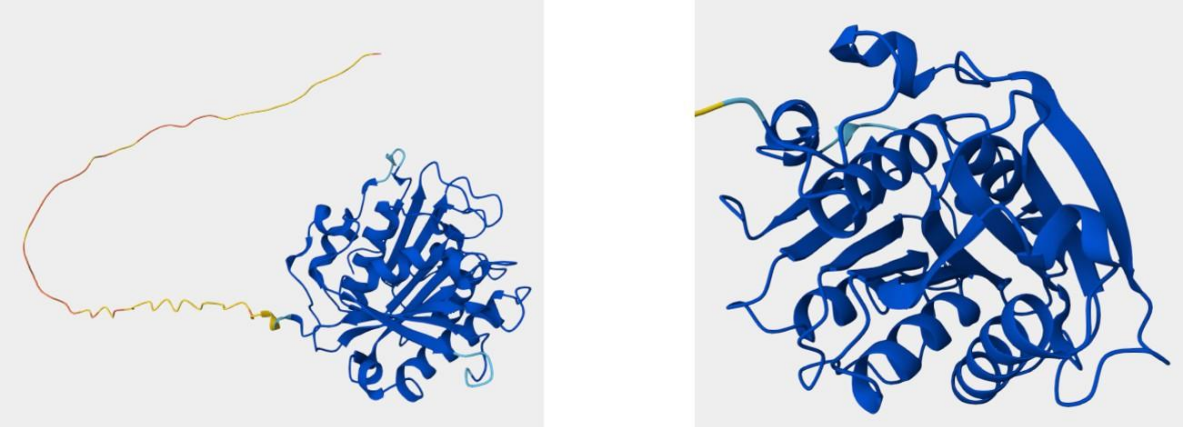

Figure 2: Prediction of 3D configuration of cutinases documented in current study via ALPHAFOLD database
(A) G8GER6 (B) E9LVH8

4. Discussion

Earlier studies have documented characterization of cutinases via *in-silico* tools in Microbispora, Nonomuraea and Micromonospora (Tiong et al. 2023), *Aspergillus tubingensis* (Azarudeen et al. 2025), *Pseudomonas fluorescens* (Bakli et al. 2021), *Thermobifida fusca* (Rajmohan et al. 2025) and *Aspergillus nidulans* (Castro-Rodríguez et al. 2025).

The 2D configurations found in current analysis demonstrated four to five alpha helices linked via five extended sheets which is in accordance with previous literature (Brinch-Pedersen et al. 2024). The 3D structural analysis indicated monomeric configuration in current study documented enzymes which is consistent with earlier findings (Longhi and Cambillau 1999; VL et al 2025).

Calculations of protein properties via PROTPARAM revealed alkaline nature of G8GER6 with pI 9.65 and slightly alkaline nature of E9LVH8 with pI 6.30. The aliphatic index values of 79.06 and 80.50 were observed for two sequences of cutinases which are indicating thermostability of these enzymes. In-vitro stability was higher in E9LVH8 as compared to G8GER6 due to the instability index value of below 40. These indexes might be used to guide the researchers regarding possible amino acid substitution in order to achieve enzymes with greater stability. The engineered form of these enzymes might be destined for sustainable plastic mitigation.

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