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Research Paper

3D Structure Prediction and Repairing Protein of *Pseudomonas stutzeri* A1501 lipase

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ABSTRACT: *Proteins fold until they obtain their native functional 3D structure and take their ultimate shape. Every protein is made up of a linear sequence of building blocks called amino acids (typically has 300 amino acids). These amino acids determine how the protein chain will fold up. The aim of this study was to predict the 3D structure of the lipase Pseudomonas stutzeri A150 and to evaluate the impact of improving protein structure on the structure's modeling. This research is based on bioinformatics using in silico analysis to determine the three-dimensional structure of the target protein.*

KEYWORDS: *3D Structure, Prediction, Repair, Folding*

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I. INTRODUCTION

Putative lipase from *Pseudomonas stutzeri* strain A1501 has been successfully cloned and expressed as a functional protein in *E. coli*. The enzyme can be classified as an esterase. This enzyme is capable of a kinetic resolution of racemic esters and therefore can be a candidate for chiral synthesis. This enzyme is alkaline and optimum at a temperature of 50˚C(1). Through the hydrolysis of α-methylbenzyl butyrate, stereospecificity was examined. The enzyme can kinetically resolve chiral esters and is R-specific.
Enantioselective or asymmetric synthesis are some different types of chemical synthesis. According to the IUPAC, it is a chemical reaction that produces one or more extra chiral elements in a substrate molecule and causes the stereoisomeric (enantiomeric or diastereomeric) products to form in unequal amounts.

Lipases have been widely used in the synthesis of chiral compounds for food and drug industry applications(2),(3),(4),(5). It is known that several sources of lipase from bacteria and yeast have enantioselective properties towards certain enantiomers (6),(7),(8). Utilization of enzymes as chiral selectors has many benefits including saving time, relatively lower costs, and being environmentally friendly.

Understanding the 3D structure of a protein is very important because it can provide important information in understanding the biochemical properties and functions of these proteins at the molecular level in detail. Determination of the 3D structure of proteins in laboratory research is relatively difficult because it requires advanced instrumentation, consumes much time, and is expensive. Research on determining the dimensional 3D structure of proteins encouraged the ideas to scientifically predict the 3D structure of proteins from existing sequences based on known 3D structure data in the laboratory. This is based on the relatively limited group of proteins found in nature so that the structural similarities between the two proteins can be inferred from the sequence similarities . This technique is known as protein modeling. The aim of this study was to predict the 3D structure of the lipase *Pseudomonas stutzeri* A150 and to evaluate the impact of improving protein structure on the structure's modeling.

II. METHOD

This research is based on bioinformatics using in silico analysis to determine the three-dimensional structure of the target protein. Enantioselective sequences of protein lipase *Pseudomonas stutzeri* A1501 were obtained from NCBI with access code YP_001174476.1.

Template Determination

The template was determined using the SWISS-MODEL expansion program (http://swissmodel.expasy.org/) by entering the target protein in the uploaded target sequence file. Menu "search" template was used to get the template to be used. The template was selected based on the highest identity value to model the model structure.

Model Evaluation

Model evaluation is carried out by assessing the structure of the target protein modeling results by Ramachandran Plots.

Structure repairing

Furthermore, structural repairs were carried out using galaxy web for the next model to be re-evaluated

III. RESULTS AND DISCUSSION

Protein sequences were obtained from NCBI with access code YP_001174476.1 (picture 1). The protein lipase sequence showed 282 amino acid residues.

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>YP_001174476.1 lipase [Pseudomonas stutzeri A1501]
MRKFTLGCLSATALVALSVSATAAPLPDTPGAPFPSVSSFDNDGPYAVTSQSEGPNCRVYRPRTLGQGGV
RHPIILWGNGTGTGPTTYSGLLTHWASHGFVVAAAETSNAGTGREMLACLDYLVQESNRTYGTYVGVLNT
GRVGTSGHSQGGGGSIMAGQDDRVKATAPIQPYTIGLGHDSSSQRNQRGPMFLMSGGADTIAIPYLNAQP
VFTRANVPIFWGERRYVSHFEPVGNGGEYRGPSTAWFRYQLMDDQSARSTFYGRLCRLCTSLLWSVERKG
IE
```

Figure 1. Target lipase protein sequences

The amino acid sequences of the *Pseudomonas stutzeri* A1501 lipase were prepared with BioEdit® software. These sequences were then used for the identification of templates that will be used in the building of enzyme models. The data was submitted to the server https://swissmodel.expasy.org/interactive and generates several templates. Templates were selected based on the appropriate parameters. The parameters were identity, QMEAN, coverage, GMQE. The Swiss-Model® web server was used to predict the 3D structure of the lipase. In silico method of modeling the 3D protein structure is the best choice for building 3D protein structures because it is easier and faster than other methods but the drawback is that a protein template must be available.

Homology modeling is modeling the 3D structure of a protein based on the alignment of the target protein's amino acid sequence with another protein. A template is a protein with a known three-dimensional structure. This research applied the 2fx5.1 lipase template from *Pseudomonas mendocina* lipase with a sequence identity of 82.68%, GMQE 0.87, and QMEAN 0.91. This value is quite good because it's close to 1. Protein sequences with similar amino acid sequences are also similar in structure(9).

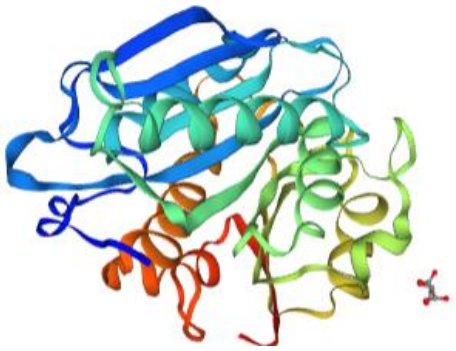

Figure 2. Protein model

Evaluation of the model structure was carried out after the model was obtained. Evaluation of the model structure was carried out using Ramachandran plots on the web http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html. The results of the Ramachandran plot showed that the most favored region was 87.5%. A good quality model would be expected to have over 90% in the most favored regions. For this reason, it is necessary to improve the 3D structure to obtain better quality 3D structures. Structural improvements were made using the web server https://galaxy.seoklab.org/. After repairing the structure with the galaxy web, the quality of the protein structure was checked again using Ramachandran and the results obtained were "most favored regions" of 93.5%. Based on the Ramachandran plot, the structural quality of a protein is

good if there are generally more amino acid residues in the "favoured" region. So the results of structural improvements in this study are quite good(10).

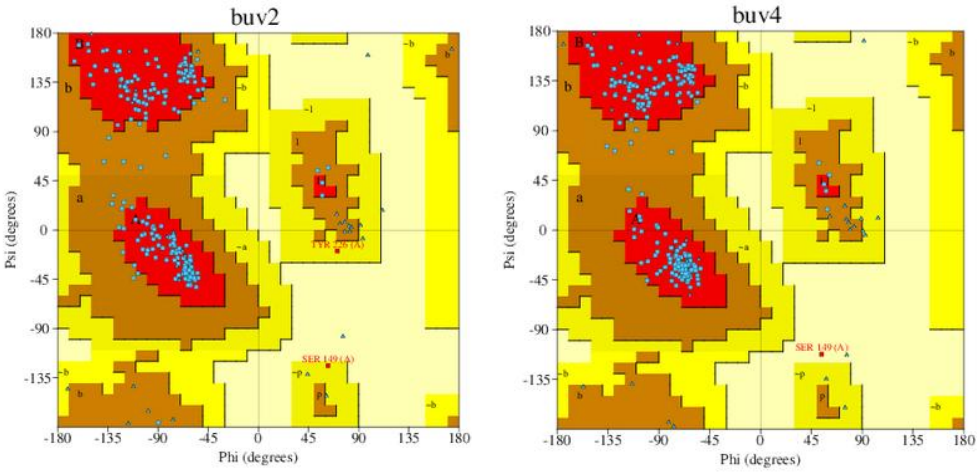

Figure 3. Ramachandran Plot (left) before repairing (right) after repair

Repairing structure made changes in the secondary structure of the protein. This can be seen in table 1 below. A protein takes on a variety of conformations during the folding process before settling into its ultimate, stable, and distinctive form. The final shape of a protein is typically the most energetically favorable one(11).

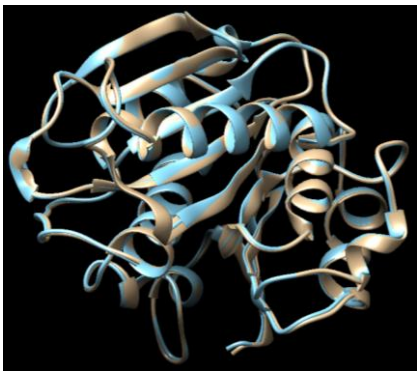

Figure 4. Superimpose before (blue) and after repair (gold)

Table 1 Secondary structure before and after repair

	Strand	Alpha helix	3-10 helix	Other	Total residue
Before	61 (24%)	61 (24%)	16 (6.3%)	116 (45.7%)	254
After	60 (23.6%)	63 (24.8%)	13 (5.1%)	118 (46.5%)	254

Secondary structure	Refinement	Strand 1			Strand 2			No. of helices	No. of residues	
		Start	End	Length	Start	End	Length		Loop	Helix
Beta-alpha-beta-motifs	Before	His72	Gly78	7	Val101	Ala105	5	1	22	**12**
		Leu138	**Ser149**	**12**	**Ala166**	**Pro172**	7	1	**16**	**10**
		Met191	Gly196	6	Ile219	Arg224	6	1	22	13
	After	His72	Gly78	7	Val101	Ala105	5	1	22	**11**
		Leu138	**His148**	**11**	**Val164**	**Ile170**	7	1	**15**	**11**
		Met191	Gly196	6	Ile219	Arg224	6	2	22	13

Secondary structure	Refinement	Start	End	No resid
Strands	Before	Leu138	**Ser149**	**12**
		Ala166	**Pro172**	7

	After	Leu138	**His148**	**11**
		Val164	**Ile170**	7
Helices	Before	Ser89	**His98**	**10**
		Arg240	**Met252**	13
		Arg258	**Thr260**	**3**
	After	Ser89	**Ser97**	**9**
		Arg240	**Leu251**	12
		Gln255	**Arg258**	**4**
Disulphides	Before	**Cysteine 57 and 119**		**Cysteine 266 and 269**
	After	-		**-**

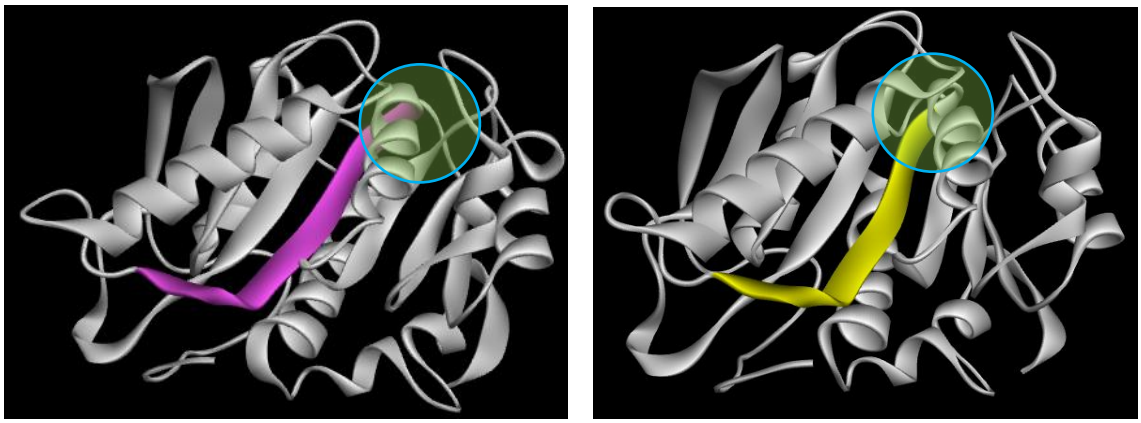

Figure 5. Beta-alpha-beta-motifs : before (fuchia) **Leu138-Ser149** ; after (yellow) **Leu138-His148**

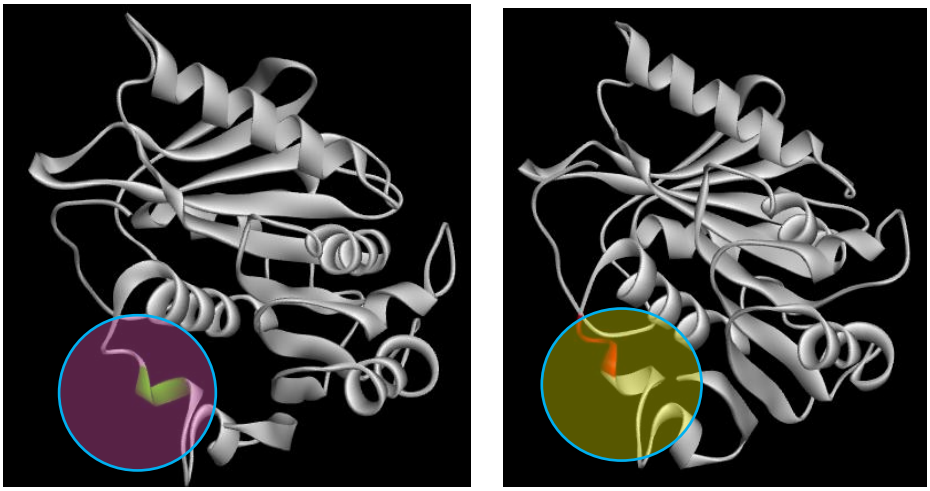

Figure 6. Helices : before (green) **Arg258- Thr260** ; after (red) **Gln255- Arg258**

27 beta turns

Turn	Sequence	Turn type	H-bond
Val37-Phe40	VSSF	IV	
Ser39-Asn42	SFDN	I	
Glu53-Asn56	EGPN	IV	
Gly54-Cys57	GPNC	IV	
Pro62-Leu65	PRTL	VIII	
Thr64-Gln67	TLGQ	II	Yes
Gly80-Thr83	GTGT	II	Yes
Thr107-Ala110	TSNA	IV	
Ala110-Gly113	AGTG	I	Yes
Arg129-Gly132	RTYG	IV	Yes
Gly132-Val135	GTYV	I	
Tyr134-Val137	YVGV	II	Yes
Asp161-Val164	DDRV	I	Yes
Gly176-His179	GLGH	I'	Yes
Gly196-Asp199	GGAD	I	Yes
Asp199-Ala202	DTIA	IV	
Thr200-Ile203	TIAI	IV	
Arg224-Val227	RRYV	II	Yes
Ser228-Glu231	SHFE	I	Yes
Phe230-Val233	FEPV	I	Yes
Pro232-Asn235	PVGN	IV	
Val233-Gly236	VGNG	II'	
Asp254-Ala257	DQSA	I	Yes
Gln255-Arg258	QSAR	I	
Gly263-Cys266	GRLC	I	Yes
Ser271-Trp274	SLLW	I	Yes
Arg278-Ile281	RKGI	IV	

25 beta turns

Turn	Sequence	Turn type	H-bond
Ser39-Asn42	SFDN	I	
Glu53-Asn56	EGPN	IV	
Gly54-Cys57	GPNC	IV	
Pro62-Leu65	PRTL	VIII	
Thr64-Gln67	TLGQ	II	Yes
Gly80-Thr83	GTGT	II	Yes
Thr107-Ala110	TSNA	VIII	
Ala110-Gly113	AGTG	I	Yes
Arg129-Gly132	RTYG	I	
Gly132-Val135	GTYV	I	
Tyr134-Val137	YVGV	II	Yes
Asp161-Val164	DDRV	I	Yes
Pro169-Pro172	PIQP	IV	
Gly176-His179	GLGH	I'	Yes
Gly196-Asp199	GGAD	I	Yes
Asp199-Ala202	DTIA	IV	
Thr200-Ile203	TIAI	IV	
Arg224-Val227	RRYV	II	Yes
Ser228-Glu231	SHFE	I	Yes
Phe230-Val233	FEPV	I	Yes
Pro232-Asn235	PVGN	IV	
Val233-Gly236	VGNG	II'	Yes
Gly263-Cys266	GRLC	I	Yes
Ser271-Trp274	SLLW	I	
Arg278-Ile281	RKGI	IV	

IV. CONCLUSION

Prediction of the 3D structure of *Pseudomonas stutzeri* A150 lipase was carried out using the Swiss Model web and evaluation results suggest improvements to the 3D structure. This aims to obtain a better impact on the 3D structure of the protein.

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