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IN-SILICO DEVELOPMENT OF EFFICIENT L-ASPARAGINASE ENZYME FOR ACUTE LYMPHOBLASTIC LEUKAEMIA THERAPY

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ABSTRACT: L-asparaginase has been accepted clinically as an anti-tumour agent for the effective treatment of acute lymphoblastic leukaemia and lymphosarcoma. This enzyme also possesses L-glutaminase activity and causes immunological problems. Hence, efforts have been made to develop mutants with lower or no glutaminase activity. In the present study, a homology model of L-asparaginase obtained from *Pectobacterium carotovorum* was docked and compared with its mutants for activities, analysed for molecular dynamics and structural stability. A total of five *in-silico* mutants were developed using single amino acid mutagenesis and evaluated for ligand binding for both L-asparagine and L-glutamine. One of the mutants, Y306L, showed -5.89 and -5.04 while the wild L-asparaginase revealed -5.50 and -5.12 binding energies for L-asparagine and L-glutamine, respectively. Further, substrate-enzyme interaction analysis indicated that the wild L-asparaginase showed five interactions for L-asparagine as well as for L-glutamine, whereas, the mutant, Y306L depicted eight and three interactions for L-asparagine and L-glutamine. Molecular dynamics analysis denoted that mutant protein is more stable in RMSD, RMSF and radius of gyration to that of wild-type protein. Higher binding affinity was noticed with L-asparagine and lower with L-glutamine along with higher interactions with L-asparagine and lower with L-glutamine in mutant Y306L, compared to wild L-asparaginase. This suggests that this could be a possible potential candidate for the treatment of Acute Lymphoblastic Leukaemia (ALL) with less induced side effects.

INTRODUCTION: Proteins are the building blocks of body and asparagine is a major component required for the synthesis of protein molecules. These can be synthesised by body itself, within the cell by an enzyme called asparagine synthetase or can be taken from extracellular pools [1].

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Tumor cells require high amount of asparagine for rapid proliferation whereas normal cell growth is independent of its requirement. The interest in L-asparaginase arose of its anti-tumour activity.

Unlike normal cells, malignant cells can synthesize L-asparagine slowly, due to their deficiency in L-asparagine synthetase. Thus, depletion of L-asparagine by L-asparaginase leads to the destruction of the tumour cells, since they are unable to complete protein synthesis. In contrast, normal cells are protected from asparagine - starvation due to their innate ability to produce this amino acid [2].

L-asparaginase catalyses the conversion of L-asparagine to aspartic acid and ammonia. In view of the difference between normal and malignant cells, L-asparaginase from *E. coli* and *Erwinia chrysanthemi* is presently used in the treatment of acute lymphoblastic leukaemia [3]. L-asparaginase also catalyses to a lesser extent the hydrolysis of L-glutamine (L-Gln) to L-glutamate (L-Glu). In general, two types of bacterial L-asparaginases have been identified: type I and type II. While type I L-asparaginases are expressed constitutively in the cytoplasm and catalyse the hydrolysis of both L-Asn and L-Gln, type II L-asparaginases are expressed under anaerobic conditions in the periplasmic space of the bacterial membranes and display higher specificity for L-Asn hydrolysis [4].

L-asparaginases from *Pectobacterium carotovorum* were found to be clinically much more beneficial as they possess less glutaminase activity (2.6%) [5] when compared with other L-asparaginases (for example *E. coli* L-asparaginase). Some of the bottle necks in the form of side effects for this enzymatic drug include acute pancreatitis, serious liver disorders, hyperglycemia, immunosuppression, and other dysfunctions, which are purely related to its glutaminase activity. Considering the above, the enzymes suited for treating ALL should be with less Km value and should have strong affinity towards L-asparagine rather than L-glutamine. This could be possible by modifications in the substrate-binding site of the enzyme which may lead to obtain reduced glutaminase activity without any change in the asparaginase activity. Few attempts have been made to nullify the L-glutaminase activity of the *E. coli* L-asparaginase by site-directed mutagenesis [6]. Keeping the potential of the enzyme in cancer treatment, an attempt is made in the present study to design an *in-silico* mutated L-asparaginase enzyme model sourced particularly from *P. carotovorum* and to carry out docking studies to know which of the replacements in the substrate binding site will help to have significantly further suppressed L-glutaminase activity along with enhanced L-asparaginase activity.

MATERIALS AND METHODS:

Homology Modelling: The amino acid sequence of *Pectobacterium carotovorum* L-asparaginase (Accession ID AFA36653.1) was retrieved from NCBI. A sequence similarity search was performed individually by selecting Protein Data Bank (PDB) (https://www.rcsb.org/pdb/home/home.do) using Protein BLAST tool for identifying a template for homology model building for *P. carotovorum* L-asparaginase and sequence was searched for their structural similarity with the query. The resultant template was subjected to multiple sequence alignment using "ClustalW" to identify the conserved region to mutate the protein. MODELLER 9.14 was employed for modelling the three-dimensional structure of L-asparaginase.

***In-silico* Mutagenesis:** Multiple mutations were performed to modelled protein on the basis of multiple sequence analysis and conserved regions. MODELLER 9.14 was used along with an automated approach to comparative modelling by satisfaction of spatial restrains. Three-dimensional models of the target were calculated based on automodel class MODELLER 9.14. The best model was selected on the basis of smallest value of normalised Discrete Optimized Molecule Energy (DOPE) score. These models were then checked for protein structure stereochemistry including Ramachandran plot and Psi/Phi angles using PROCHECK.

Molecular Docking Studies: Docking of ligands, L-asparagine **Fig. 3a** and L-glutamine **Fig. 3b** to both the enzymes of L-asparaginases (wild and mutated version) were performed by using auto dock tool (ADT) 4.2 (http://autodock.scripps.edu/resources/adt). The ADT graphics interface was employed for manual preparation of the protein by adding polar hydrogens and merging non-polar hydrogens [7]. In the case of ligand, both the ligands were sketched in Tripo's Sybyl6.7 and Gasteiger-Huckel charges were added to minimise the molecules and non-polar hydrogens were merged to give stability. Seven rotatable bonds were set to L-asparagine, whereas 8 for L-glutamine. GPF (grid parameter file) and DPF (docking parameter file) files were prepared and the grid points for auto grid calculations were set as 60 × 60 × 60 Å with the active site residues at the centre of the grid box [8]. Lamarckian genetic algorithm method was used to calculate protein-fixed, ligand-flexible calculations [9].

Molecular Dynamics Simulations: Molecular dynamics simulations were carried out for independent wild-type protein as well as mutant

Y306L using GROMACS 5.0.4 package along with GROMOS96 54a7 force field after solvation by simple point charge (SPC) keeping water model embedded in cubic simulation boxes with minimum edge distance of 10 Å.[10] The particle mesh Ewald method was used to treat long-range Coulomb interactions. During this, the bond lengths were constrained using the linear constraint solver (LINCS) algorithm. Van der Waals force and Coulomb interactions cut-off distances were maintained at 1.0 nm. A minimum of 50ns MD simulations of each system was performed. The analysis was performed by GROMACS analysis programs.

RESULTS AND DISCUSSION:

Homology Modelling and *in-silico* Mutagenesis: The L-asparaginase template for homology modelling was identified on the basis of criteria such as maximum score, smaller the e-value, >30% identity with other L-asparaginases (from *Escherichia coli* [11], *Wolinella succinogenes* [12], *Erwinia chrysanthemi* [13], *Erwinia carotovora* [14]) reported in the literature. It is also reported in the literature that the L-asparaginase from above bacterial strains shows high similarity with respect to tertiary and quaternary structures. However, these enzymes differ in quantum of L-glutaminase activity which is known to be the responsible factor for L-asparaginase-mediated ALL treatment side effects including acute pancreatitis, liver disorders, hyperglycemia, immunosuppression. In view of the above, the crystal structure (1HFJ) was selected as template for modelling of L-asparaginase of *P. carotovorum*. This template depicted 53% sequence identity and 92% query coverage with the query sequence of L-asparaginase of *Pectobacterium carotovorum* **Fig. 1**.

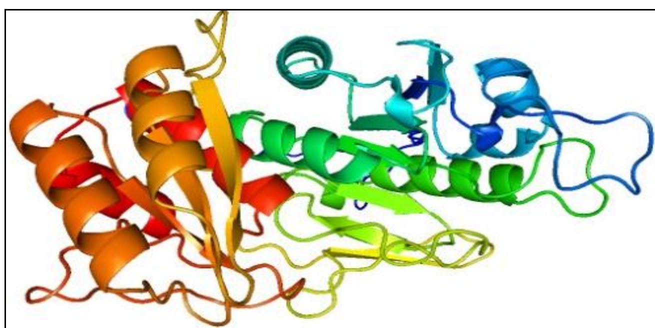

FIG. 1: CARTOON MODEL OF L-ASPARAGINASE SECONDARY STRUCTURE OBTAINED FROM *PECTOBACTERIUM CAROTOVORUM* BY PYMOL VISUALIZING SOFTWARE

Hence, 1HFJ protein was selected as a template for further modelling using MODELLER 9.14 software which uses an inbuilt discrete optimized protein energy (DOPE) function to assess the quality for all the predicted models. DOPE function is used for assessing the homology models by calculating their statistical potential which helps in selection of the best model on the basis of lowest DOPE score. The modelling data generated 20 predicted models which were further analyzed for DOPE score and the homology model which showed minimum DOPE score was validated using Ramachandran plot. Ramachandran plot of selected modelled protein phi and psi angles revealed the stereo chemical quality of the model, and showed that 94.7% core, 5.0% allowed, 0.3% generously allowed, 0.0% disallowed region **Fig. 4a**. The presence of more than 99% of the amino acids in the allowed region, further confirms the reliability of the homology model generated.

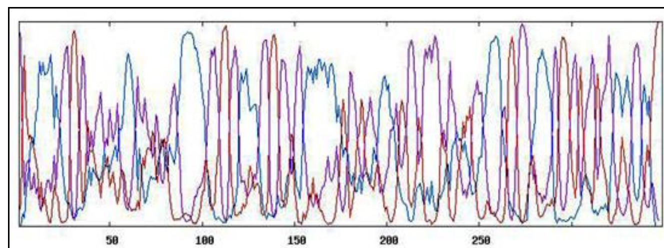

FIG. 2: GOR 4 ANALYSIS FOR PREDICTING SECONDARY STRUCTURE OF ORIGINAL PROTEIN

The sequence analysis of the obtained homology model was compared with L-asparaginase sequences reported in the literature and noticed that Lys43, Ile70, Ile101 and Tyr306 were conserved adjacent to active site. These four residues were mutated to obtain Lys43Ser (K43S), Ile70Leu (I70L), Ile101Val (I101V) and Tyr306Leu (Y306L). Selection of these amino acids (valine, serine, and leucine) was based on their structural simplicity and in order to have limited effect on the nativity of the protein even after mutagenesis. Structural validation of above mutants based on Ramachandran plot revealed presence of 94.7% of amino acids in core region for the first three mutant proteins (Lys43Ser **Fig. 4b1**, Ile70Leu **Fig. 4b2** and Ile101Val **Fig. 4b3**, whereas the mutant protein, Tyr306Leu indicated only 95.3%, amino acids with 0% of amino acid residues in disallowed region, **Fig. 4b4**. Secondary structure prediction was performed by GOR 4 tool from Expasy.

It clearly showed that 33.81% alpha helix (Hh), 20.63% beta strands (Ee) and 45.56% random coils (Ce) in overall sequence for both native and mutated proteins **Fig. 2**.

The observed similarity in secondary structures further suggested that these two (wild and mutated) proteins are structurally similar.

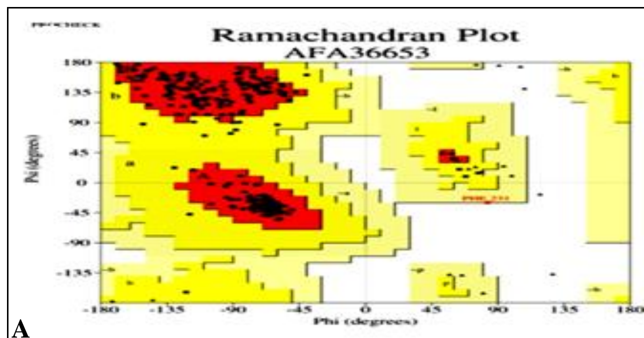

FIG. 4A: RAMACHANDRAN PLOT FOR NATIVE PROTEIN AFA36653

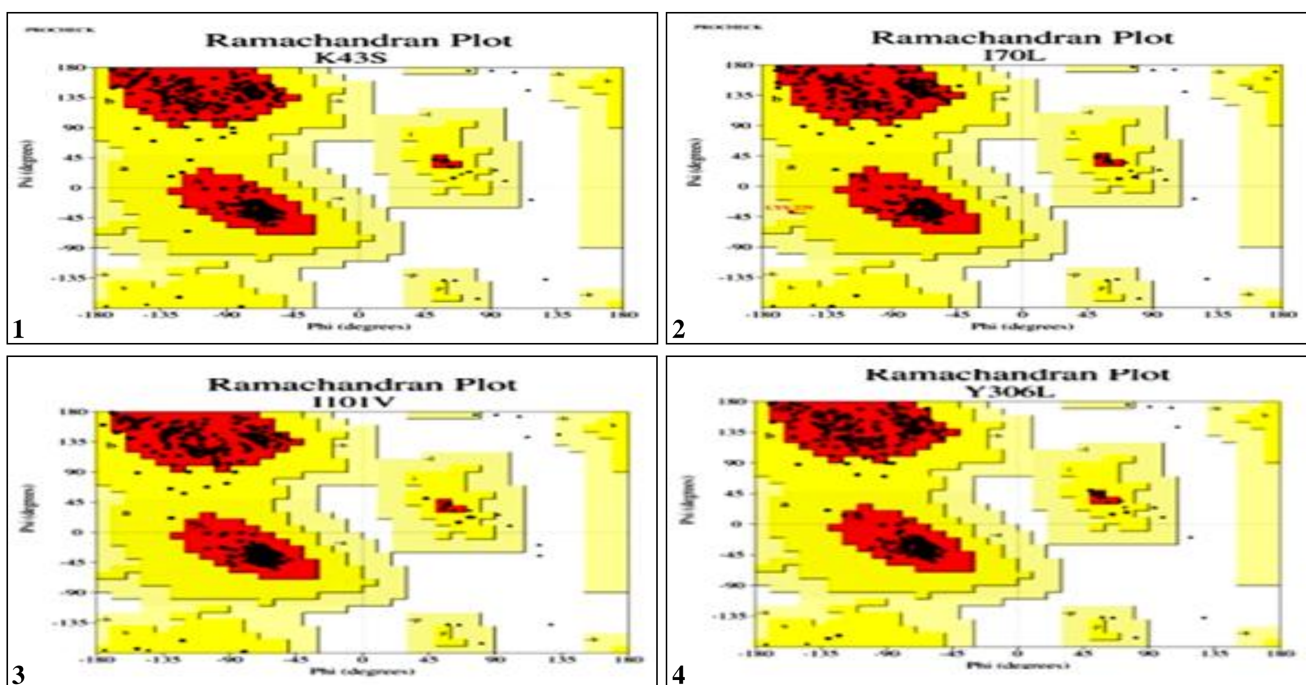

FIG. 4B: RAMACHANDRAN PLOT FOR MUTATED PROTEINS 1. (K43S), 2. (I70L), 3. (I101V), 4. (Y306L)

Molecular Docking Studies: Molecular docking studies were performed for all four mutant proteins in order to understand the substrate binding affinity. Both ligand molecules, L-asparagine (PubChem id 6267) and L-glutamine (PubChem id 5961), whose molecular masses are 132.12 and 146.14 g/mol are retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and sketched by using ISIS Draw **Fig. 3** and used for docking the native and mutant proteins. Rigid docking was performed for the purpose of comparing the binding energies, and flexible dockings were performed by keeping each of the active site residues as flexible and checked for the binding affinities with respect to the substrate binding site and the ligand.

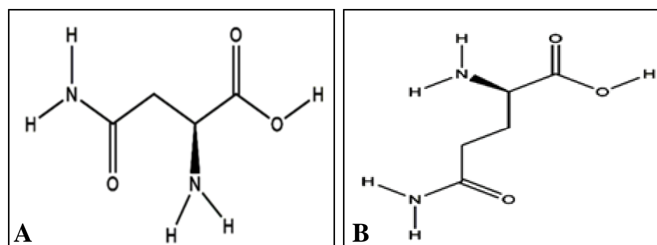

FIG. 3: LIGANDS A. L- ASPARAGINE AND B. L-GLUTAMINE

Molecular docking provided information on enzyme-ligand interactions and binding energies for wild and mutant proteins; the obtained data helped in selection of most populated cluster with the lowest binding energy. It was noticed that the binding energy values for native protein with both

ligands L-asparagine and L-glutamine were observed to be -5.51 and -5.12 Kcal/mol, respectively **Table 1** and **Fig. 5a** and **5b** suggesting that this *P.caratovorum* L-asparaginase has higher affinity towards L-asparagine and lower towards L-glutamine.

These results are in contrast to the docking data reported by Ramya *et al*., [8] where the authors report binding energy of -5.59 kcal/mol towards L-asparagine and -8.08 kcal/mol towards L-glutamine for *P. carotovorum* L-asparaginase (PDB ID: 2JK0).

TABLE 1: BINDING ENERGY OF WILD TYPE PROTEIN WITH L- ASPARAGINE AND L- GLUTAMINE RESPECTIVELY

S. no.	Protein	Substrate	Binding Energy (kcal/mol)	Interacting amino acid	Hydrogen bond Length
1	Wild type	L-Asparagine	-5.51	Asn204	2.214
				Glu205	2.020
				Gln206	1.721
				Gly305	1.994
				Gly305	1.954
		L- Glutamine	-5.12	Tyr189	1.819
				Asn204	1.718
				Gln206	1.744
				Gly305	2.039

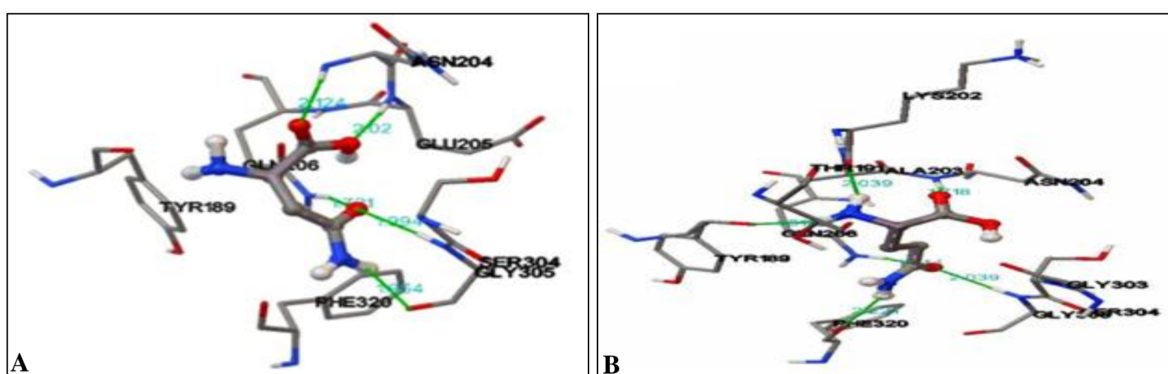

FIG. 5: DOCKING RESULTS OF WILD TYPE PROTEIN A. WITH L- ASPARAGINE; B. WITH L-GLUTAMINE

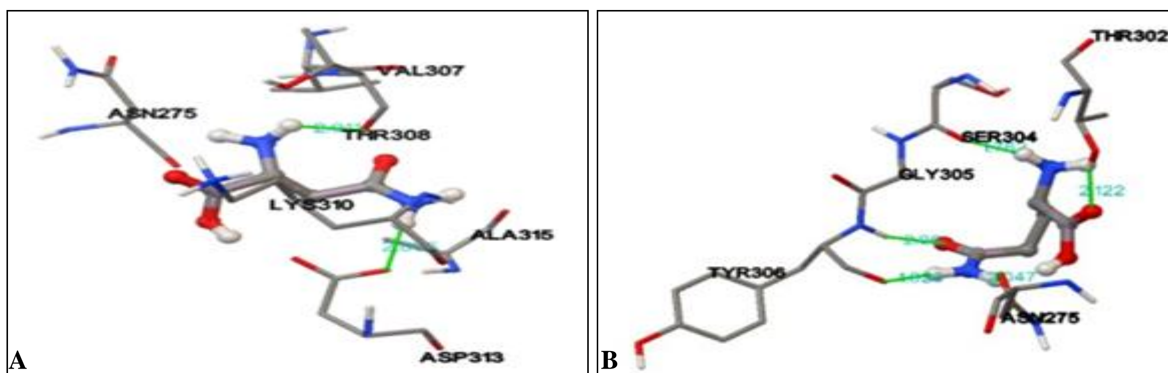

FIG. 6: DOCKING RESULTS OF MUTANT PROTEIN K43S A. WITH L- ASPARAGINE; B. WITH L-GLUTAMINE

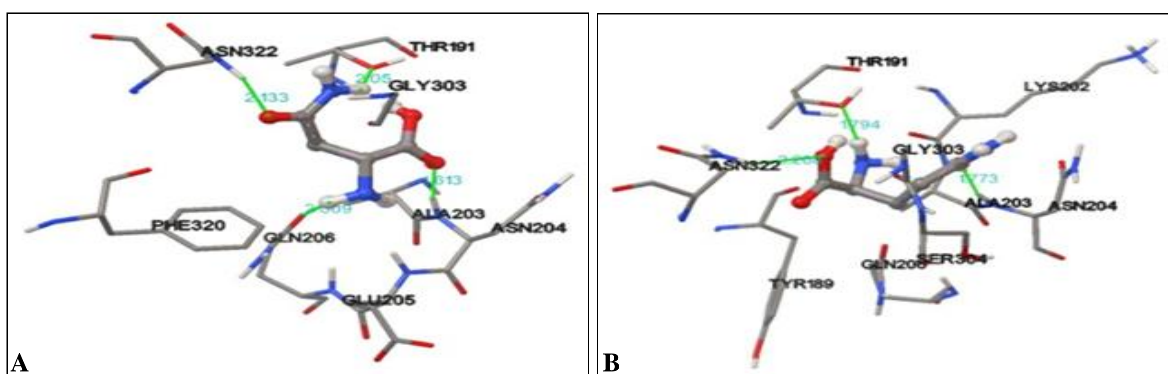

FIG. 7: DOCKING RESULTS OF MUTANT PROTEIN I70L A. WITH L- ASPARAGINE; B. WITH L-GLUTAMINE

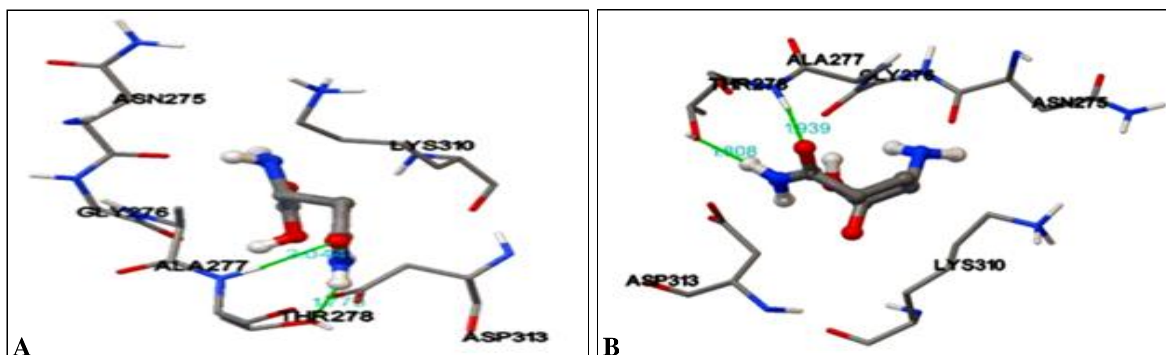

FIG. 8: DOCKING RESULTS OF MUTANT PROTEIN I101V A. WITH L- ASPARAGINE; B. WITH L-GLUTAMINE

An overview of the binding energies of *P. caratovorum* L-asparaginase four mutated proteins (Lys43Ser, Ile70Leu, Ile101Val and Tyr306Leu) for both substrates indicated the lowest binding energies for Tyr306Leu for L-asparagine and L-glutamine. The observed binding energies for mutated model Tyr306Leu is -5.89 and -5.04 kcal/mol for L-asparagine and L-glutamine, respectively indicating this mutant protein has low glutaminase activity in comparison with that of wild-type protein **Table 2** and **Fig. 9a** and **9b**. Critical analysis of the wild-type protein and Tyr306Leu further denoted that the selective mutant protein has >6% higher affinity towards L-asparagine and >9% lower affinity towards L-glutamine.

The binding energy analysis of all mutant proteins further indicated lower affinities for both substrates with mutant proteins Lys43Ser, Ile70Leu and Ile101Val compared to native protein. This can be evidenced from the tables **Table 1** and **2** that 28, 20 and 37% lower binding energies for L-asparagine for Lys43Ser, Ile70Leu and Ile101Val, respectively. Reddy *et al.*, [10] and Oza *et al.*, [15] reported similar trend of binding energies for both substrates with PDB ID: 1NNS and L-asparaginase of *Withania sominefera* respectively, however, these energy values are much lower compared to the selected model protein in the present study. This observation further confirms that selected mutant protein can be a promising candidate for ALL treatment.

TABLE 2: BINDING ENERGIES OF MUTATED PROTEINS USING AUTODOCK 4.2

S. no.	Mutated proteins	Substrate	Binding Energy (kcal/mol)	Interacting amino acid	Hydrogen bond Length
1	K43S	L-Asparagine	-3.93	Thr308	2.011
				Asp313	2.005
		L- Glutamine	-2.81	Asn275	2.047
				Thr302	2.122
				Ser304	1.762
				Gly305	2.060
				Tyr306	1.826
2	I70L	L-Asparagine	-4.11	Thr191	2.05
				Asn204	1.613
				Gln206	2.009
				Asn322	2.133
		L- Glutamine	-4.13	Thr191	1.794
				Asn204	1.773
				Asn322	2.208
3	I101V	L-Asparagine	-3.46	Ala277	2.044
				Thr278	1.775
		L- Glutamine	-3.26	Ala277	1.939
				Thr278	1.808
4	Y306L	L-Asparagine	-5.89	Tyr189	2.124
				Tyr191	1.850
				Lys202	1.899
				Asn204	1.740
				Glu205	2.048
				Gln206	1.675
				Gly305	2.005
		L- Glutamine	-5.04	Gln206	1.904
				Gly305	2.169
				Ser304	2.136

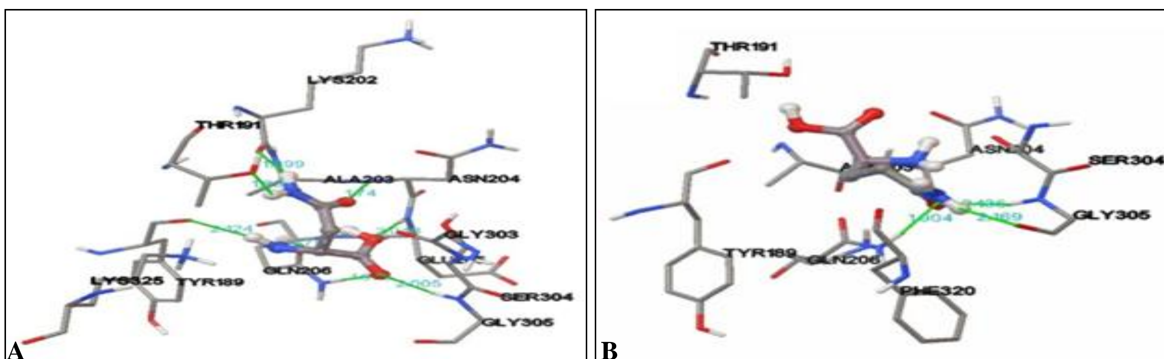

FIG. 9: DOCKING RESULTS OF MUTANT PROTEIN Y306L A. WITH L- ASPARAGINE; B. WITH L-GLUTAMINE

Enzyme-ligand interaction analysis revealed that one of the mutated proteins Tyr306Leu, depicted the highest amino acid interaction with L-asparagine compared to native and other mutated proteins. This can be evidenced from the **Table 2** where Tyr306Leu with L-asparagine made inter molecular hydrogen bonds with seven amino acids (Tyr189, Tyr191, Lys202, Asn204, Glu205, Glu206 and Gly305) while with L-glutamine, only three amino acids (Gln206, Gly305 and Ser304) **Table 2**. In case of native protein, L-asparagine made hydrogen interactions with five amino acids positioned at Asn204, Glu205, Gln206, Gly305 and Gly305. The observed two molecular hydrogen interaction with the ligand L-asparagine at Gly305 of enzyme could be evidenced from **Fig. 5A** where one hydrogen interaction was with carboxyl group and another with amine group. With L-glutamine as ligand, it showed four interactions (Tyr189, Asn204, Gln206 and Gly305) in case of native protein. All other mutant proteins showed less molecular hydrogen interactions either of ligands (L-asparagine and L-glutamine) **Table 2**. Similar docking studies on *E. coli* L-asparaginase using PDB ID: 1NNS revealed two hydrogen bond interactions with ligand molecule, L-asparagine and five with L-glutamine. These data further confirm that the mutant protein Tyr306Leu has more stable structure to that of native as well as other mutant proteins simulated in this study.

Molecular Dynamics and Simulations: To observe the structural and functional behaviour of the wild-type as well as mutant protein, molecular dynamics simulation study was performed for L-asparaginase wild-type and mutant protein [16]. Authors performed the RMSD, RMSF and Radius of gyration (Rg) analysis between wild-type and mutant Y306L protein structures for their stability. Wild protein RMSD values revealed an initial increase for first 5 ns of simulation followed by a more or less constant value for the rest of experimental period (till 50 ns). The initial gradual increase in RMSD value was noticed from 0.2 to 0.32 nm and subsequent second phase the value was ranging from 0.32 to 0.45 nm with L-asparagine as substrate **Fig. 10a**. More or less similar trend was noticed with mutated protein with L-asparagine as substrate for initial rapid phase and more deviations were observed in second phase (5-50 ns) where the observed deviation was in the range of 0.3 to 0.42 nm. Further, analysis suggested that mutated protein deviated less in the simulation period of 5 to 30 ns and further increase of scan time did show similar RMSD value denoting both mutated and wild proteins behave in same manner after 30 ns simulation. Critical evaluation of this data with L-asparagine substrate indicated that the mutated protein showed less RMSD value in the entire study range compared to wild protein suggesting mutated protein is more stable compared to wild-type.

Simulation of wild and mutant protein with L-glutamine as substrate depicted similar trend of initial increase of RMSD value for first 10 ns (0.35 to 0.40 nm) followed by constant value in the entire simulation period (up to 50 ns) **Fig. 10b**. Over all, it was observed that with L-glutamine as substrate, higher RMSD values were observed for mutant protein in the entire period of study unlike L-asparagine as substrate. Critical understanding of simulation pattern indicated that mutant and wild-type protein differed in RMSD value of ~0.05 nm at two regions; one in the simulation range of 14 to 25 ns and another from 40 to 50 ns. The observed significant variation in the average RMSD values of wild-type and mutant structures after the

relaxation period (~0.15 nm) may lead to the conclusion that the mutation performed at Tyr306Leu could increase the protein stability that might enhance the functional ability of the mutant protein when docked with L-asparagine and showed decreased stability with L-glutamine, thus providing a suitable basis for developing a stable protein with more affinity towards L-asparagine.

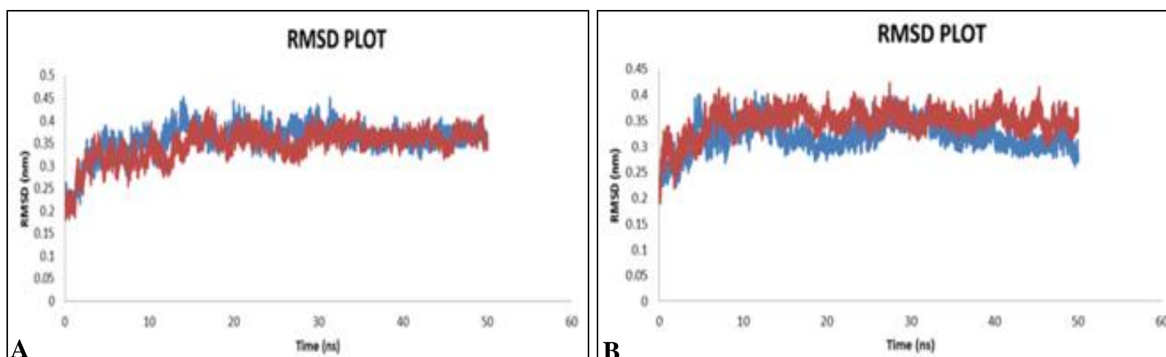

FIG. 10: STRUCTURE MODEL REFINEMENT ANALYSIS (RMSD) OF WILD PROTEIN VERSUS MUTANT Y306L WITH (A) ASPARAGINE AND (B) GLUTAMINE

For determining the mutation effect on dynamic behaviour of residues, the C-α RMSF values of mutant and native structures were calculated. RMSF value of wild-type protein, with asparagine as substrate fluctuated within a range between 0.45 to 0.65 nm **Fig. 11a** in the entire simulation period (without considering a few N-terminal amino acids). However, mutant model (Tyr306Leu) with asparagine as substrate fluctuated at ~0.42 nm only. With glutamine as substrate, wild-type protein fluctuated more at ~0.42 nm only **Fig. 11b**. The initial large peak in RMSF plot was noticed in mutant and wild-type models and this might be due to the presence of small α-helical conformation held by a long loop at the N-terminal part of each model while other peaks represent the inter-connecting loops of β-sheets. Comparative evaluation of RMSF plot denoted that all the residues were observed to fluctuate in the same manner in both proteins (wild and mutated versions) and both substrates (L-asparagine and L-glutamine) **Fig. 11a** and **11b**, however, the C-terminal region (position ~180 to 190 amino acid residues) of wild-type fluctuated more in comparison with mutant with L-asparagine as substrate **Fig. 11a**. With L-glutamine as substrate, mutant protein fluctuated more in the entire protein while at the position ~180 to 190 amino acid residues mutant protein revealed higher fluctuation whereas at position ~160 to170 amino acid residues wild-type protein depicted more fluctuation.

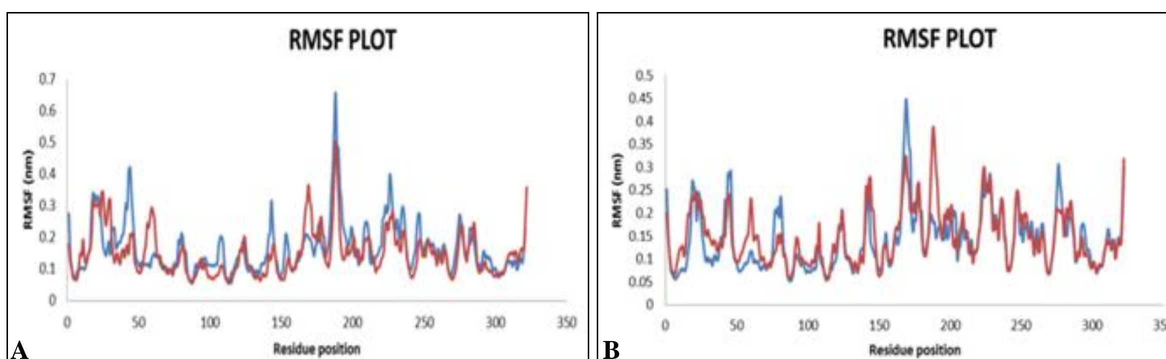

FIG. 11: STRUCTURE MODEL REFINEMENT ANALYSIS (RMSF) OF WILD PROTEIN VERSUS MUTANT Y306L WITH (A) ASPARAGINE AND (B) GLUTAMINE

The radius of gyration (Rg) is the mass-weighted root mean square distance of group of atoms from their common centre of mass, hence it provides an observation into global dimension of protein. The radius of gyration graph for alpha-carbon atoms of protein versus time at 300 K was depicted in **Fig. 12**. A major change has been observed in Rg value after 30 ns with respect to wild and mutant proteins with L-asparagine as substrate **Fig. 12a**. This can be evidenced by the fact that a similar Rg value

was found for first 30 ns and further increase in simulation time beyond 30 ns resulted in higher Rg value for mutant and decreased Rg for wild-type protein **Fig. 12a**. Such a trend suggest that indicating more at time periods of 0 to 50 ns in both native and mutants with L-asparagine **Fig. 12a** and L-glutamine **Fig. 12b**. Based on Rg plot, mutant structures were found more stable than the native.

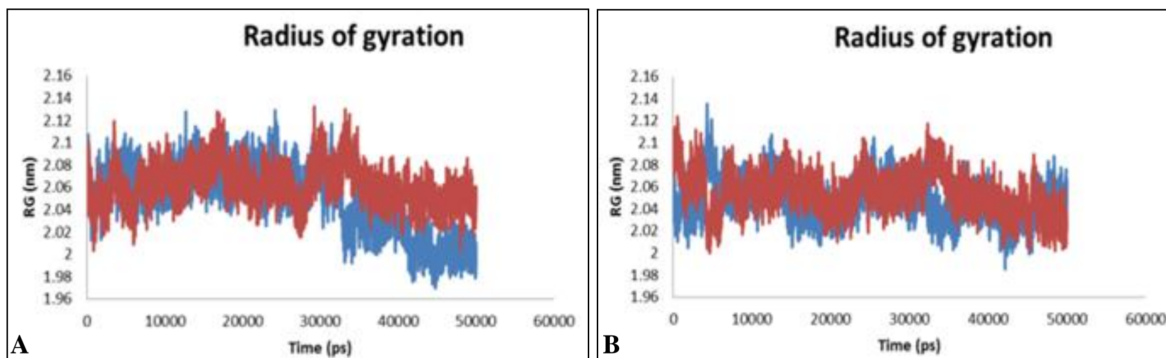

FIG. 12: STRUCTURE MODEL REFINEMENT ANALYSIS (RADIUS OF GYRATION) OF WILD PROTEIN VERSUS MUTANT Y306L WITH (A) ASPARAGINE AND (B) GLUTAMINE

CONCLUSION: The present work was focussed on the application of *in-silico* mutagenesis for making the protein drug L-asparaginase from *Pectobacterium carotovorum* to have reduced glutaminase activity. Tyr306Leu (Y306L) showed more binding efficiency towards L-asparagine compared with L-glutamine resulting in low glutaminase activity. Further, molecular dynamics study was also revealed that mutant is more stable when compared to wild-type L-asparaginase. The future work is directed toward the *in-vitro* site directed mutagenesis for verification of predictions in the laboratory and thus obtaining an enzyme with reduced side effects which in turn will be a possible drug for treating ALL.

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CONFLICT OF INTEREST: Authors declare no conflict of interest.

REFERENCES:

1. Offman MN, Krol M and Patel N: Rational engineering of L-asparaginase reveals importance of dual activity for cancer cell toxicity. Blood 2011; 117(5): 1614-1621.
2. Nguyen HA, Su Y, Zhang JY, Antanasijevicm A and Arnon Lavie A: A novel L-asparaginase with low L-glutaminase coactivity is highly efficacious against both T and B cell acute lymphoblastic leukemias *in-vivo*. Cancer Res 2018; 78(6): 1549-1560. doi: 10.1158/0008-5472. CAN-17-2106.
3. Ehsanipour EA, Sheng X and Behan JW: Adipocytes cause leukemia cell resistance to L-asparaginase via release of glutamine. Cancer Res 2013; 73(10): 2998-3006.
4. Labrou NE, Papageorgiou AC and Avramis VI: Structure-function relationships and clinical applications of L-asparaginases. Curr Med Chem 2010; 17(20): 2183-2195.
5. Kumar S, Dasu VV and Pakshirajan K: Localization and production of novel L-asparaginase from *Pectobacterium carotovorum* MTCC 1428. Process Biochemistry 2010; 45: 223-229.
6. Derst C, Henseling J and Rohm KH: Engineering the substrate specificity of *Escherichia coli* asparaginase.II. Selective reduction of glutaminase activity by amino acid replacements at position 248. Protein Sci 2000; 9(10): 2009-2017.
7. Kravchenko OV, Kislitsin YA, Popov AN, Nikonov SV and Kuranova IP: Three dimensional structure of L-asparaginase from *Erwinia carotovora* complexed with aspartate and glutamat. Acta Cryst 2008; 64: 248-256.
8. Ramya LN, Mukesh Doble, Rekha PB and Pulicherla KK: *In-silico* engineering of L-asparaginase to have reduced glutaminase side activity for effective treatment of acute lymphoblastic leukemia. J Pediatr Hematol Oncol 2011; 33: 617-621.
9. Sousa SF, Fernandes PA and Ramos MJ: Protein-ligand docking: Current status and future challenges. Proteins 2006; 65: 15. [PMID: 16862531].
10. Reddy R, Rajulapati SB, Durthi CP, Bhatia M and Pola M: Molecular dynamic simulations of *Escherichia coli* L-asparaginase to illuminate its role in deamination of asparagine and glutamine residues. 3 Biotech 2016; 6: 2. Doi: 10.1007/s13205-015-0339-9.
11. Yun MK, Nourse A, White SW, Rock C and Richard J: Crystal structure and allosteric regulation of the cytoplasmic *Escherichia coli* L-asparaginase I. J. Mol. Biol 2007; 369: 794-811.

12. Lubkowski J, Palm GJ, Gilliland GL, Derst C, Röhm KH and Wlodawer A: Crystal structure and amino acid sequence of *Wolinella succinogenes* L-asparaginase. Eur. J. Biochem 1996; 241: 201-207
13. Aghaiypour K, Wlodawer A and Lubkowski J: Structural basis for the activity and substrate specificity of *Erwinia chrysanthemi* Lasparaginase. Biochemistry 2001; 40: 5655-5664.
14. Kravchenko OV, Kislitsin YA, Popov AN, Nikonov SV and Kuranova IP: Three-dimensional structures of L-asparaginase from *Erwinia carotovora* complexed with aspartate and glutamate. Acta Crystallogr D Biol Crystallogr 2008; 64: 248-256.
15. Oza VP, Parmar PP, Patel P, Singh R, Trivedi U and Subramanian RB: Homology modelling of plant l-asparaginase: characterization of its ligand binding efficiency. Journal of Advanced Bioinformatics Applications and Research 2011; 2: 100-107.
16. Huey R, Morris GM, Olson AJ and Goodsell DS: A Semi-empirical free energy force field with charge-based desolvation. J Comput Chem 2007; 28: 1145-1152.

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