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Research Article

PREDICTION AND CHARACTERIZATION OF SUBSTRATE SPECIFICITY AND THERMAL STABILITY FOR THERMOSTABLE ALIPHATIC AMIDASES: AN *IN-SILICO* APPROCH

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ABSTRACT

Molecular modeling is a rapidly growing field that uses new data and techniques to control access for trading with computational biology. To decipher the structural integrity of thermophilic amidases, an *in silico* approach was studied for 14 thermostable amidases from selected different thermophiles to perform the homology modeling. Results of the sequence based characterization showed the presence of higher glutamate and histidine in thermophilic aliphatic amidases as compared to mesophilic aliphatic bacterial amidase and they are conserved at specific position of 205-283. Presence of asparagine, glutamine and histidine along with cysteine in a hydrophobic core region provide more stability. These amino acids were found to be higher in amidase from *Geobacillus* sp. Catalytic triad was found at various helix, which is present in both the aliphatic amidases. These developed models have been considered for the molecular docking simulations to identify the binding affinity of amidases with selected targets (substrates). Malonamide, lactamide and valeramide possessed the minimum binding energy with all the thermophilic targets, which were in the range of 3 to 5 kcal.mol^{-1}. Malonamide demonstrated higher specificity as compared to rest of the docked substrates and was further used for molecular dynamics (MD) simulations to evaluate the binding stability and conformational changes. After 1 nano second (ns) MD study with pre-defined parameters, the structural arrangements of individual atoms have been survived at higher temperature specifically for the *Geobacillus* sp.

Keywords: Computational biology, Thermophiles, Thermostable amidase, HOMOLOGY modeling, Molecular docking, Molecular dynamics.

1. INTRODUCTION

Various types of amides are present in nature and play a very critical role in biosynthesis and degradation processes. Nitrilases and amidases are key enzymes in the degradation of amides like nicotinamide, acrylamide, benzoic acid, etc. Amidases are classified under class EC 3.5.1 and EC 3.5.2 as aliphatic and cyclic amidases based on the action on substrate. Various mesophilic and thermophilic bacterial genera like *Rhodococcus, Corynebacterium, Brevibacterium, Geobacillus, Blastobacter, Arthrobacter, Alcaligenes, Helicobacter, Lactobacillus, Mycobacterium, Pseudomonas, Bacillus, Micrococcus* and *Methylophilus* are reported for the amidases production. Thermostable amidase are also reported from many bacterial genera but the exploration of its sequence and structural features with respect to characteristics are scanty [1]. Due to the technology advancements and innovations in the field of chemical, biological and pharmaceutical industries, amidases are applied in bioproduction and biotransformation and also in the treatment of waste water and bioremediation as well. Thermostable amidases have potential value for the improvement of large scale biological processes [2]. Some of the amides showed detrimental effect on environment and on living flora and fauna, which directs the way to the development of unique enzyme production or its modification from extremophilic prokaryotes [3].

Computational tools are now essential for *in silico* analysis of enzymes like amidases, which can be differentiated based on amino acid sequences present in the phylogenetically unrelated bacterial families. Moreover, the information can be used to know the metabolic versatility for microbial mediated transformations by bacteria for example, it has been repored for *Rhodococcus* sp. [4.] Many microbial amidases have been purified and characterized. Many of the *Geobacillus* spp. are reported for the production of many organic acid using thermostable amidases [5, 6].

Online server like Expert Protein Analysis System (ExPASy) create ease to use platform to reveal the hidden characteristics of the protein. Physico-chemical properties like quantification of amino acids with composition and its molecular mass along with theoretical pI, negatively and positively charged residues can be calculated presciely using online tool. Atomic composition, extinction coefficients ($M^{-1}.cm^{-1}$) at 280 nm, instability index/aliphatic index, grand average hydropathicity (GRAVY), etc. can disclose the stability and specificity of a particular protein [7]. These properties can be either determined experimentally or deduced from the *in silico* analysis of amino acid sequences of enzyme available in the databases. Amidases are still not adequately investigated and their classification is not definitely formulated. Number of amino acids involved in binding, nature of the amino acid and its interactions with thermosolutes as well as mutation study expands the view of thermal stability [8,9,10].

Molecular structure and the mechanism of protein ligand binding are very critical approach to study the integrity and stability of the compound under various condition. For unknown protein, 3D structure construction and its minimum energy is necessary for the docking study, which yield the optimum binding position with amino acid residues. Stability and conformation change at higher temperature are most commonly described using molecular docking [11]. Enzyme-substrate binding and its stability study gives modern approach to maximize the yield. In spite of significant progress in expanding the knowledge of amidases, the spatial organization of these proteins remains unknown and systematic comparative analysis of amidases are far from complete. Apart from catalytic activity, amidases were also classified based on amino acid sequences and phylogentic relationship [12, 13]. *In silico* statistical optimization of the amidase production are carefully studied and has provided the information for further characterization [14, 15].

The novel approach taken is reported here with the aim to study the general physico-chemical properties that are true for the sequence analysis and structural analysis; these properties of the sequences then can be used to predict there substrate specificity, thermal stability for the thermostable aliphatic amidases. Furthermore, the structure and sequences based difference and evolutionary changes in particular species can be revealed.

2. MATERIAL AND METHODS

2.1. Dataset

To investigate the plausible mechanism of substrate binding specificity, physico-chemical nature and thermal stability, 14 amidases from thermophiles were selected from UniProtKB database. This dataset was used to evaluate the structural insights of theomorphilic bacterial aliphatic amidases that is as shown in Table 1. The ligands were retrieved from Pub Chem database in 2D format and then converted in to 3D format using Marvin suite (Fig. 1). Further, addition of hydrogen were followed by cleaning process. For molecular docking experiments, energy minimization was performed using the steepest descent technique of Amber 03 force field [16].

2.2. Sequence based analysis

Information about all the selected aliphatic amidases from thermophilic microorganisms was obtained from the Swiss-Prot database (UniProt). Amino acid sequences of all retrieved amidases were checked for having experi-mentally proved substrate specificity as well as complete nucleotide sequences in terms of non-fragmented, pseudo, or hypothetical sequences. The complete nucleotide sequences for its amidase gene as well as experimentally proved substrate specificity were examined using various tools available in the Proteomic server namely Prot Param, Protein calculator, Compute pI/Mw, Prot Scale. These tools were applied to calculate different physico-chemical properties of cyclic amidases from the retrived protein sequences. Physico-chemical data were generated from the Swiss Prot and Expert Protein Analysis System (ExPASy) that is the proteomic server of Swiss Institute of Bioinformatics (SIB) which is ProtParam. Blastp (Protein BLAST) was performed to study the homology among the various amidase sequences under study and were used for further characterization [17].

Table 1: Selected thermophilic bacteria with the UniProt accession number for aliphatic amidases

Sr. No.	Entry	Microorganisms
1	Q5L060	*Geobacillus kaustophilus* (strain HTA426)
2	D3E8W1	*Geobacillus* sp. (strain Y412MC10)
3	G8N388	*Geobacillu sthermoleovorans* CCB_US3_UF5
4	D3ELZ1	*Geobacillus* sp.(strain Y412MC10)
5	U2WW35	*Geobacillus kaustophilus* GBlys
6	A0A023DJT5	*Geobacillus caldoxylosilyticus* NBRC 107762
7	A0A023CKZ7	*Geobacillus stearothermophilus* NUB3621
8	A0A0E0TBA6	*Geobacillus* sp. (strain Y412MC52)
9	Q9L543	*Bacillus* sp.
10	I3DTI3	*Bacillus methanolicus* MGA3
11	R4G0S3	*Anoxybacillus flavithermus* NBRC 109594
12	W9EAC9	*Thermoanaerobacterium aotearoense* SCUT27
13	A0A085L3H5	*Schleiferia thermophile* str. Yellowstone
14	A0A0B3BFJ9	*Thermoanaerobacter* sp. YS13

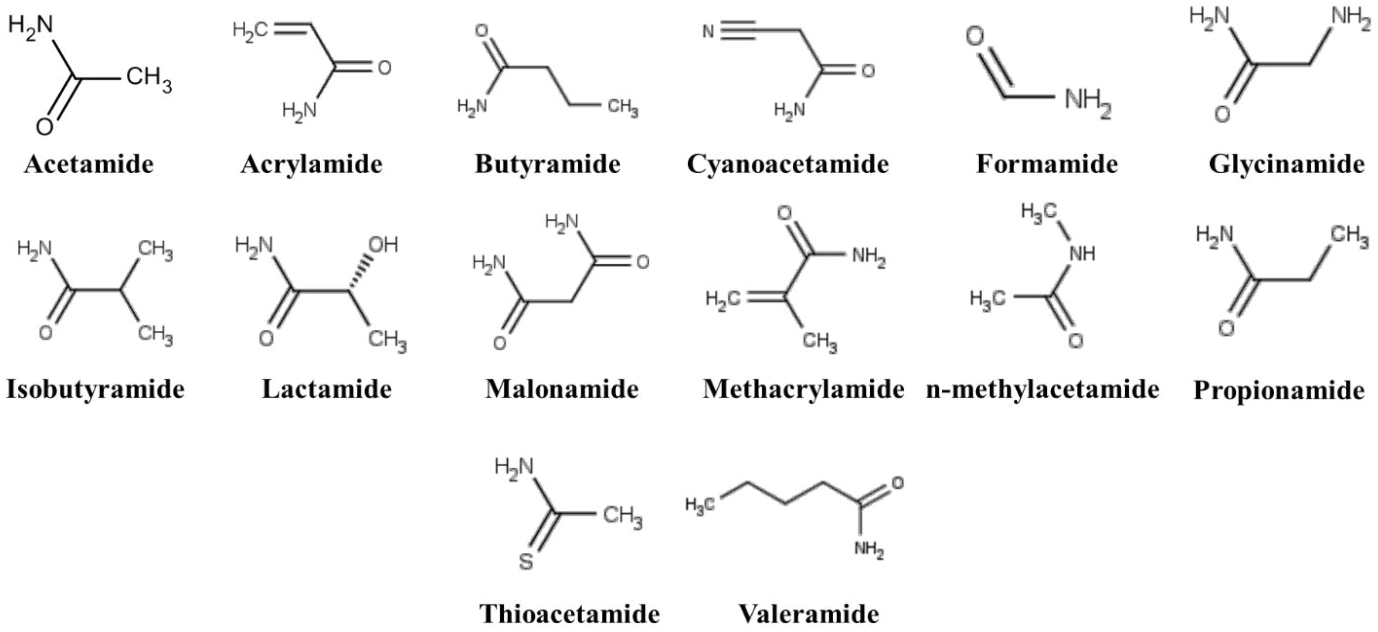


Fig. 1: 2D representation of selected substrates (Ligands)

The molecular weight (kDa) of thermophilic aliphatic amidases were calculated by the addition of average isotopic masses of amino acid in the protein and deducting the average isotopic mass of one water molecule. The pI of amidases was calculated using pK values of amino acid [18, 19]. The atomic composition of amidases was derived using the ProtParam tool, available at Ex PASy.

Molecular weight (kDa) is very important to study and was calculated by taking average isotopic masses. Apart from this, pK values were used for the calculation of theoretical pI values of the selected thermostable amidases. Determination of extinction coefficient of various amidases was calculated using Equation 1 by Edelhoch method [20].

E (Prot) = Numb (Tyr)*Ext (Tyr) + Numb (Trp)*Ext (Trp) + Numb (Cystine)*Ext (Cystine) (1)

where, E= Extinction coefficients; Numb= Number of amino acid residues; Ext= molar extinction coefficient

Aliphatic index, instability index and grand average of hydropathicity (GRAVY) were estimated by ProtParam tool [21]. Clustal W was used for multiple sequence alignment and evaluation of phylogenetic relationships along with importance of catalytic traid [22, 23].

2.3. Structure based analysis

2.3.1. Homology modelling

As 3D structure of the selected proteins were not available, FASTA sequences were used for the construction of 3D structure of the protein using SWISS-Model [24-26]. Out of all the predicted interactive structures, one structure was selected based on optimum sequence identity and coverage [27].

2.3.2. Protein preparation

For the exploration of protein-ligand binding mechanism, 14 proteins were prepared based on the UniprotKB database and was considered for further analysis. Three-dimensional structure of the proteins were prepared through the YASARA energy parameters including addition of H ions, removal of water, removal of unwanted cofactors, ligands and metal ions. Energy minimization was performed for the structural evaluation with compared to natural state [28].

2.3.3. Molecular docking

Molecular docking study involves binding of two unknown or known molecules with each other at specific binding energy and bonds. The 14 generated proteins were optimized using energy minimization with chemical all-atom force field using YASARA software and docked with selected ligands, which demonstrated docking positions, docking energy ($kcal.mol^{-1}$) and root mean squared deviation (RMSD). The best docked pose was identified with respect to the lowest binding energy of the bound ligand [29]. The docking energy was calculated using Equation 2 [15].

$$\Delta G = \Delta G_{vdW} + \Delta G_{Hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta G_{desolv}$$

Where, ΔG_{vdW} = van der Waals term for docking energy; ΔG_{Hbond} = H bonding term for docking energy; ΔG_{elec} = electrostatic term for docking energy; ΔG_{tor} = torsional free energy term for ligand when the ligand transits from unbounded to bounded state; ΔG_{desolv} = desolvation term for docking energy.

2.3.4. Molecular dynamics simulations

To understand the reasonable mechanism of conformation as well as stabilization, molecular dynamics (MD) simulations was carried out on all the 14 docked receptor-ligand complexes, which require removal of water molecules. Also, the optimization was performed using (Y) AMBER force field, acid dissociation constant (pKa), and density 0.997 g L^{-1} set as per the YASARA Structure software to neutralize the system and was subjected to energy minimization using the steepest gradient approach (100 cycles). As per the software parameters, force constant was kept at 1000 $kJmol^{-1}nm^{-2}$, while number of atoms N, pressure P, and temperature T were stored to standard level including temperature of 298 K (physiological condition, pH=7.4) and pressure of 1 bar using Berendsen thermostat and [10, 26] and barostat [30] respectively. One nano second (1 ns) time interval was chosen for the MD simulation evaluation including root mean squared deviation (RMSD) and root mean squared fluctuation (RMSF) [28, 31]. The protein ligand interaction patterns obtained from the averaged conformations were graphically illustrated using Discovery Studio Visualizer 2016.

3. RESULTS AND DISCUSSION

3.1. Sequence based analysis of selected thermostable aliphatic amidases from thermophiles

Physico-chemical characterization of all the selected thermostable aliphatic amidases showed difference amongst *Geobacillus* sp. (301-523) and other thermophilic species (296-323) in terms of number of amino acids. Isoelectic point of proint plays very important role in stability and substrate specificity so the theoretical pI using online tool was calculated, which showed 9.18 for *Anoxybacillus flavithermus* NBRC 109594 and 9.07 for *Schleiferia thermophile* str. Yellowstone posses that was higher in comparison to the rest.

Instability index of aliphatic amidases from *Geobacillus* sp. (strain Y412MC10), *Anoxybacillus flavithermus* NBRC 109594, and *Schleiferia thermophile* str. Yellowstone is higher than 40, which proved less stability in *in vitro* condition. Apart from this, *Thermoanaerobacter* sp. YS13 and *Bacillus* sp. showed maximum and minimum volume occupied by site chains. Based on these results *Thermoanaerobacter* sp. YS13 is having more thermal stability than the other amidases studied (Table 2).

Results of amino acid analysis of selected thermostable aliphatic amidases are shown in Table 3.

The comparison of the amino acid composition of the aliphatic amidases revealed that in most of the *Geobacillus* sp. alanine (Ala), glycine (Gly), proline (Pro) were found to be higher than the rest of the thermophiles. Moreover, Lysine (Lys) and Tyrosine (Tyr) in the studied selected thermophiles except *Geobacillus* sp. were two fold higher. *Anoxybacillus flavithermus* NBRC 109594 showed significant difference that is three fold

less Alanine (Aln) and two fold higher Lysine (Lys). Isolucine (Ile), Lucine (Leu), Lysine (Lys), Aspartate (Asp) and Tyrosine (Tyr) were significantly higher in *Thermoanaerobacter* sp. YS13 as compared to the rest.

Table 2: Physico-chemical characterization of selected thermostable aliphatic amidases

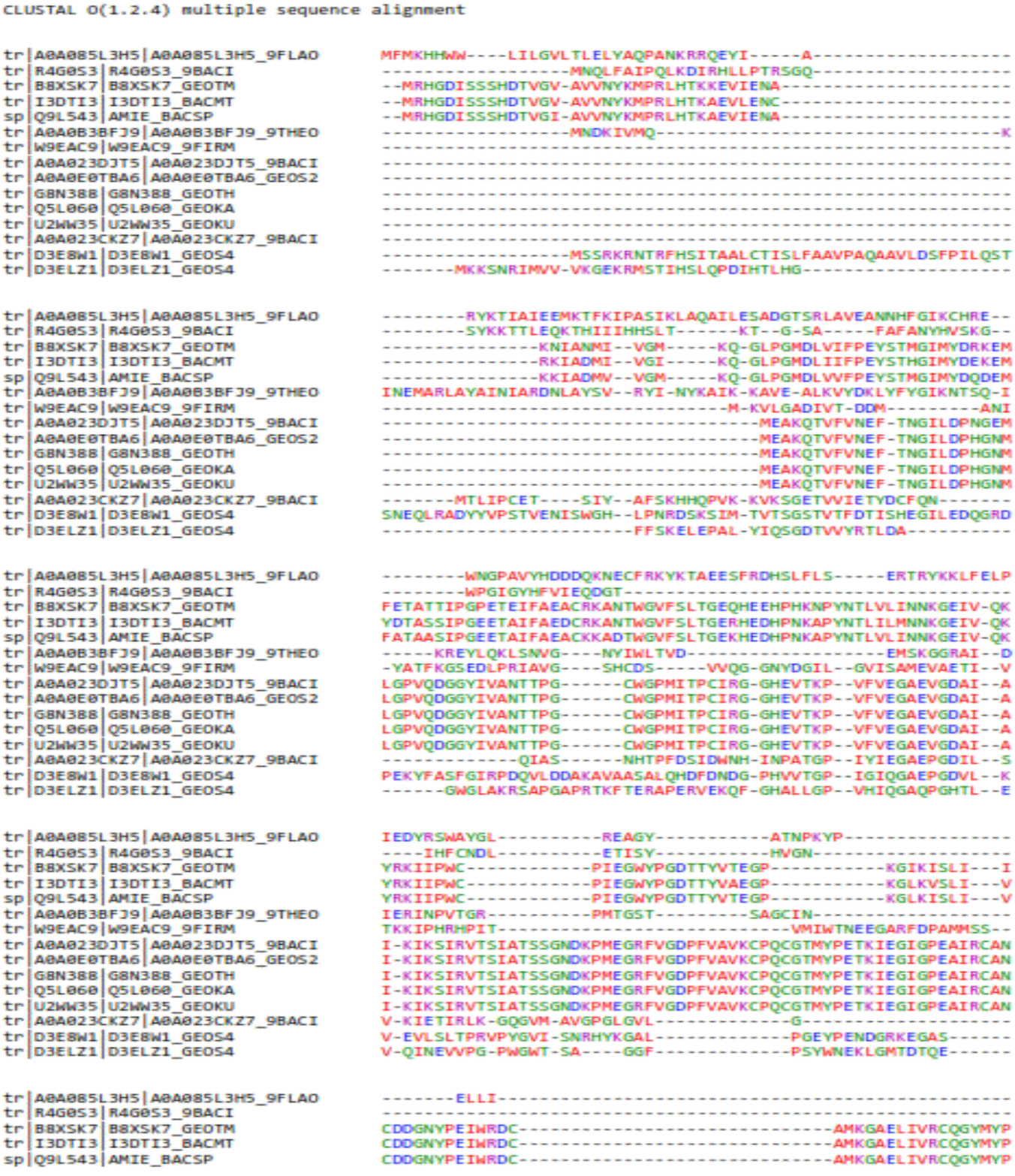
Properties	1	2	3	4	5	6	7	8	9	10	11	12	13	14
No. of amino acids	434	523	434	334	434	434	301	434	348	349	323	360	296	369
MW	46671.4	56375.8	46671.4	36882.3	46671.4	46525.3	32779.3	46686.5	38597	39127.4	37017.4	39890.9	34985.8	40747.3
Theoritical pI	5.6	5.16	5.6	5.78	5.6	5.27	5.06	5.51	5.48	5.54	9.18	5.7	9.07	7.53
Total - residue (Asp+Glu)	48	64	48	39	48	50	33	49	45	47	34	51	39	45
Total + residue (Arg+Lys)	38	45	38	31	38	36	18	38	36	37	45	42	46	46
Extinction co-eff. Cys	27890	48820	27890	46535	27890	24910	18575	27890	57340	58830	36245	34755	60405	33935
Extinction co-eff. Cys reduced	27390	48820	27390	46410	27390	24410	18450	27390	56840	58830	35870	34380	60280	33810
Instability index	27.78	34.44	27.78	41.62	27.78	25.84	31.6	26.25	30.2	30.37	44.54	32.28	45.67	21.01
Aliphatic Index	89.15	88.8	89.15	75.33	89.15	90.28	102.29	89.38	74.57	75.16	83.56	87.44	77.53	108.56
GRAVY	-0.078	-0.161	-0.078	-0.24	-0.078	-0.027	0.163	-0.077	-0.324	-0.398	-0.662	-0.205	-0.72	-0.012

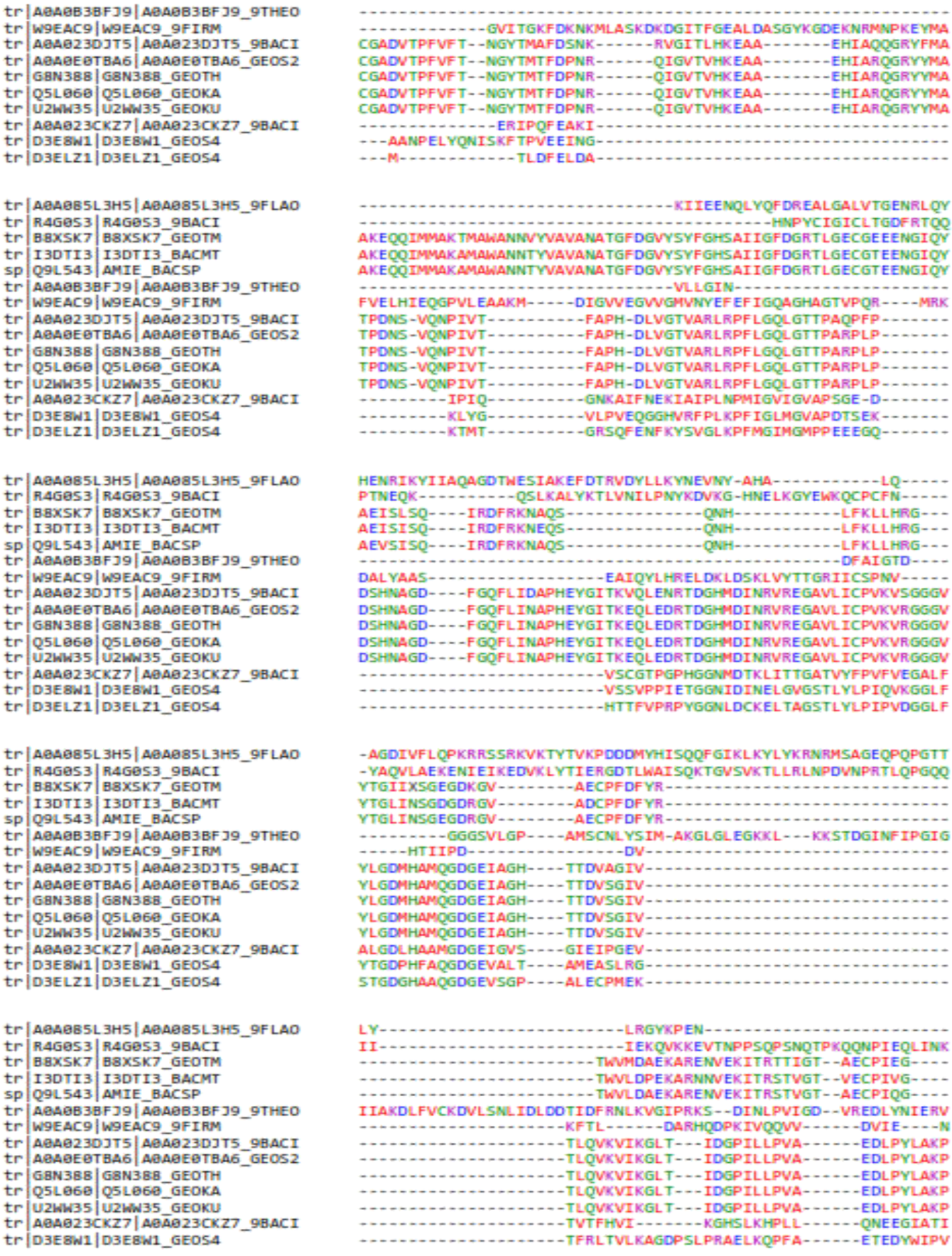
Table 3: Comparative investigation of amino acid composition of thermostable aliphatic amidases

	Microorganism													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Ala (A)	7.4	9.6	7.4	6.6	7.4	7.8	6.6	7.4	8.3	6.6	3.1	7.5	8.1	5.7
Arg (R)	4.4	3.6	4.4	4.2	4.4	3.9	1.3	4.4	3.7	4.6	3.1	3.3	7.1	3.3
Asn (N)	4.1	3.8	4.1	1.8	4.1	3.9	3.3	3.9	4.6	4.9	7.1	3.6	4.4	7
Asp (D)	5.1	5.9	5.1	4.8	5.1	5.3	4	5.3	5.2	5.7	4.3	7.8	4.7	7.3
Cys (C)	1.8	0.2	1.8	0.6	1.8	1.8	1	1.8	2.3	2.6	1.9	1.7	0.7	0.8
Gln (Q)	3.2	2.7	3.2	3.3	3.2	3.5	4.3	3.2	3.2	2.9	7.1	2.5	4.7	1.1
Glu (E)	6	6.3	6	6.9	6	6.2	7	6.2	7.8	7.7	6.2	6.4	8.4	4.9
Gly (G)	10.8	8.8	10.8	10.5	10.8	11.1	9.6	10.8	9.8	9.5	6.5	7.8	4.7	9.2
His (H)	2.5	2.5	2.5	3.3	2.5	2.5	3.7	2.5	2.3	2.9	3.4	3.1	3	0.3
Ile (I)	7.4	6.5	7.4	3.9	7.4	7.6	9.6	7.6	7.8	8.3	8	7.8	6.4	11.9
Leu (L)	6.9	8.4	6.9	9.3	6.9	6.7	8	6.9	4.3	4.6	8.7	6.1	9.1	9.2
Lys (K)	4.4	5	4.4	5.1	4.4	4.4	4.7	4.4	6.6	6	10.8	8.3	8.4	9.2
Met (M)	2.5	1.9	2.5	5.1	2.5	2.5	2.7	2.5	3.7	3.4	0.9	4.2	1.7	2.7
Phe (F)	3.2	3.4	3.2	4.5	3.2	3.7	4	3.2	3.2	2.9	3.1	3.1	3.7	2.7
Pro (P)	6.9	6.9	6.9	6.6	6.9	6.7	6.6	6.9	4.3	4.6	5	3.9	3.7	2.4
Ser (S)	3.2	7.1	3.2	5.4	3.2	2.5	5	3.2	4	4..3	4	5	4.1	5.4
Thr (T)	8.1	5.9	8.1	7.8	8.1	8.1	7	8.1	6	5.7	6.5	5	4.7	4.3
Trp (W)	0.5	0.8	0.5	1.8	0.5	0.5	0.7	0.5	1.7	1.7	0.9	0.8	1.7	0.3
Tyr (Y)	2.5	3.4	2.5	2.7	2.5	2.1	1.7	2.5	4.6	4.9.	4	3.3	7.4	5.1
Val (V)	9	7.3	9	6	9	9.2	9.3	8.8	6.6	6.3	5.3	8.9	3	7

As shown in Fig. 2, the multiple sequence alignment of the aliphatic amidases under study showed some unique differences for the position specific presence of some amino acids. The present work has contributed to conclude that there is a clear difference between selected aliphatic amidases in terms of position specific presence of certain amino acids. Position of amino acid glycine (57, 63, 95, 96, 158, 360, 405, 410, 442, 455), proline (62, 312), glutamate (66, 102, 131, 337, 412), histidine (68, 70, 203, 260), lysine (163, 404) and arginine (314) are conserved in the selected aliphatic amidases. Amino acid histidine and tyrosine at position

227 are conserved in all the amidases under study. Aspartate (Asp), valaine (Val), glutamine (Glu) and isolucine (Ilu) were position specific and most conserved amino acid in all selected thermostable amidases. No conserved regions were found at C and N terminal of the sequences. Catalytic triad (KAP) was observed amongst selcected thermophiles and many position specific amino acid substitution were noted which leads to the evolutionary relationship between them.

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tr|A0A0E0TBA6|A0A0E0TBA6_GEOS2
tr|G8N388|G8N388_GEOTH
tr|Q5L060|Q5L060_GEOKA
tr|U2WW35|U2WW35_GEOKU
tr|A0A023CKZ7|A0A023CKZ7_9BACI
tr|D3E8W1|D3E8W1_GEOS4
tr|D3ELZ1|D3ELZ1_GEOS4
tr|A0A085L3H5|A0A085L3H5_9FLAO
tr|R4G0S3|R4G0S3_9BACI
tr|B8XSK7|B8XSK7_GEOTM
tr|I3DTI3|I3DTI3_BACMT
sp|Q9L543|AMIE_BACSP

Fig. 2: Multiple sequence alignment of the amino acid sequences of the selected thermostable aliphatic amidases

3.2. Homology modeling

Homology modelling using SWISS-MODEL predicts and suggest various 3D model for the selected sequences based on template structure, identification and alignment. Evaluation and selection of the optimum model for the docking purpose were carried out using Global Model Quality Estimation (GMQE) score, coverage and sequence identity. The high value of the GMQE score proved a more reliable model among other proposed models. Based on which the single best model was chosen for each protein and selected models are presented in Fig. 3.

3.3. Molecular docking

The binding modes of receptor-ligand complexes were implemented to illuminate molecular docking. The 14 receptors and 14 thermostable amidases were docked with each other. Data presented in Table 4 shows the best binding of all the docked ligands with proteins, in which malanomide ligand remained in top three docked ligand. The binding energy, hydrogen bonding and contacting residues are listed in Table 5. Malanomide docked with *Geobacillus stearothermophilus* NUB3621 at 5.098 kcal.mol^{-1} binding energy, which was the best in all the docked proteins. In addition, 5 hydrogen bonds were formed between malanomide and *Geobacillus stearothermophilus* NUB3621 protein, and Cys 36, Phe 37, Asn 57, Asn 154, Asp 156, Asp 181, His 183, Glu 190, Val 193, Ser 194, Gly 195, Glu 197, Val 276, Asp 277 were found as contacting amino acids residues.

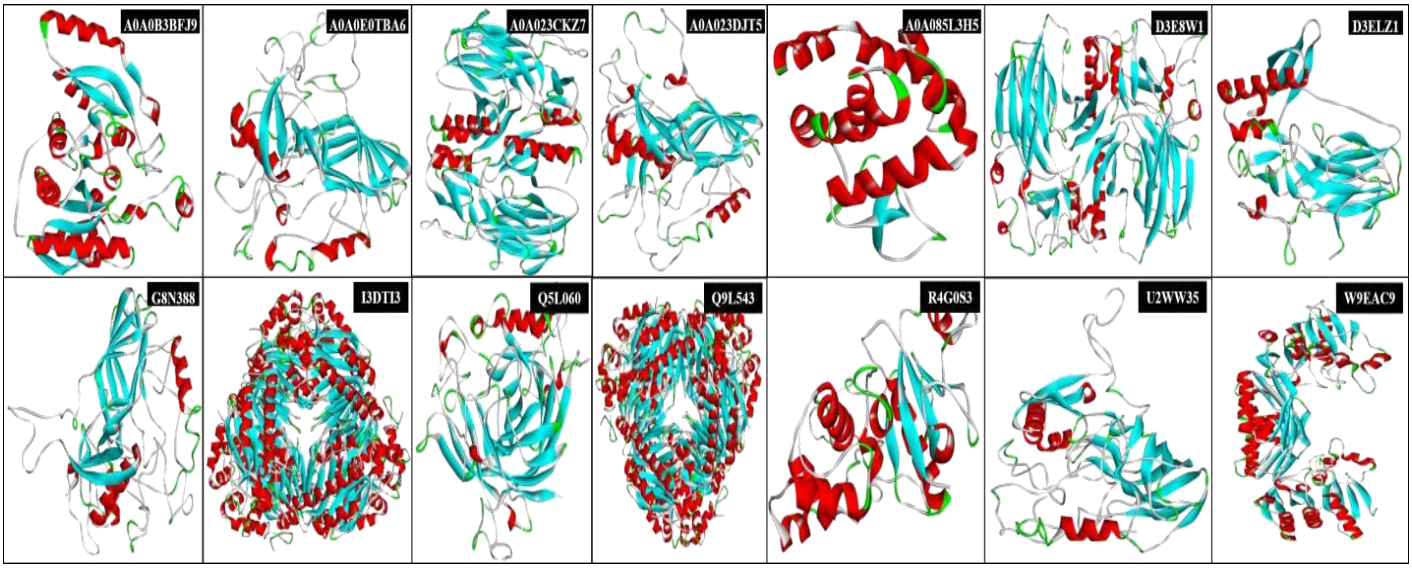


Fig. 3: 3D structure of selected thermostable aliphatic amidases by Homology Modelling

Table 4: Docked ligands with proteins analyzed through molecular docking

Proteins	A0A023CKZ7	A0A023DJT5	A0A085L3H5	A0A0B3BFJ9	A0A0E0TBA6	D3E8W1	D3ELZ1	G8N388	I3DTI3	Q5L060	Q9L543	R4G0S3	U2WW35
Ligands	**9**	9	14	8	9	9	9	9	9	9	9	9	9
	8	14	10	14	14	14	14	8	14	14	14	10	8
	6	7	9	9	8	4	4	10	10	8	8	3	10
	14	8	3	3	3	8	8	7	8	10	4	8	4
	4	10	7	7	4	7	7	14	4	7	10	7	7
	7	3	8	10	12	10	10	12	7	3	2	14	14
	3	4	12	2	10	3	3	4	11	4	7	4	3
	10	6	2	4	7	6	6	3	12	6	3	12	12
	2	2	4	12	2	12	12	2	2	2	6	2	2
	12	12	11	6	6	2	2	6	3	12	12	6	6
	1	11	6	1	1	1	1	1	6	1	11	11	11
	13	1	1	11	11	11	11	11	5	11	1	1	1
	11	13	5	13	13	13	13	13	1	13	5	13	13
	5	5	13	5	5	5	5	5	13	5	13	5	5

Table 5: Docking score, binding energy, hydrogen bonds, dissociation constant, and contacting receptor of Malanomide ligand with all selected proteins

Protein	Binding Energy [kcal mol]	Hydrogen bonds	Contacting receptor residues
A0A0B3BFJ9	4.325	Gly 89, Gly 107	Gly 89, Thr 106, Gly 107, Gly 131, Ser 132, Gly 135, Pro 136, Met 138, Pro 269, Ile 272, Phe 273, Gly 295, Lys 296, Ile 299
A0A0E0TBA6	4.801	Cys 41, Ile 83, Thr 85, Ser 86	Pro 39, Gly 40, Cys 41, Trp 42, Gly 43, Pro 44, Ile 83, Ala 84, Thr 85, Ser 86, Asp 293, Val 294, Ser 295
A0A023CKZ7	5.098	Asn 57, Asn 154, Asp 156, Asp 181, His 183	Cys 36, Phe 37, Asn 57, Asn 154, Asp 156, Asp 181, His 183, Glu 190, Val 193, Ser 194, Gly 195, Glu 197, Val 276, Asp 277
A0A023DJT5	4.437	Cys 41, Met 45, Ala 84, Asp 293	Gly 40, Cys 41, Trp 42, Gly 43, Pro 44, Met 45, Ala 84, Thr 85, Ser 86, Asp 293, Val 294, Ala 295
A0A085L3H5	3.859	Glu 40, Phe 109, Ser 113	Ile 37, Glu 40, Glu 41, Thr 44, Phe 109, Arg 110, Ser 113
D3E8W1	4.342	Glu 326, Lys 328, Gln 329, Arg 356, Lys 365	Glu 326, Leu 327, Lys 328, Gln 329, Phe 331, Arg 356, Glu 357, Gly 360, Phe 361, Lys 365
D3ELZ1	4.342	Gly 58, Arg 71, Lys 84, Gly 87	Glu 326, Leu 327, Lys 328, Gln 329, Phe 331, Arg 356, Glu 357, Gly 360, Phe 361, Lys 365
G8N388	4.824	Cys 41, Thr 85, Ser 86, Ile 83, Asp 293	Pro 39, Gly 40, Cys 41, Trp 42, Gly 43, Pro 44, Ile 83, Ala 84, Thr 85, Ser 86, Asp 293, Val 294, Ser 295
I3DTI3	4.124	Leu 25, Met 66, Asp 68, Glu 71	Pro 23, Arg 24, Leu 25, His 26, Gly 64, Ile 65, Met 66, Tyr 67, Asp 68, Glu 71, Asp 224
Q5L060	4.439	Arg 214, Gln 242, Asp 245, Leu 349, Leu 353	Pro 212, Arg 214, Pro 215, Pro 217, Gln 242, Asp 245, Arg 246, Lys 267, Leu 349, Glu 350, Leu 353, Pro 354, Ile 355
Q9L543	4.574	His 232	Arg 188, Gln 190, Gln 200, Ile 201, Ala 204, Lys 205, Val 217, Phe 230, His 232, Ser 233, Ala 234, Ile 236, Glu 245
R4G0S3	4.165	His 39, His 40, Tyr 65, Gln 147	Ile 38, His 39, His 40, Leu 42, Ile 63, Tyr 65, His 137, Asn 138, Lys 146, Gln 147, Cys 148, Pro 149
U2WW35	4.807	Cys 41, Ile 83, Thr 85, Ser 86, Asp 293	Pro 39, Gly 40, Cys 41, Trp 42, Gly 43, Pro 44, Ile 83, Ala 84, Thr 85, Ser 86, Asp 293, Val 294, Ser 295
W9EAC9	4.668	Asp 45, Glu 79, Glu 80, Gly 307, His 308, His 332	His 34, Asp 45, Glu 79, Glu 80, Ala 87, Met 88, His 143, Gln 146, Pro 306, Gly 307, His 308, His 332

The docked positions of malaonomide and *Geobacillus stearothermophilus* NUB3621 protein with 2D interaction profiles is shown in Fig. 4. On the basis of the results, the generation of hydrogen bonds fluctuated in protein binding while some van der Waals and unfavorable donor-donor as well as unfavorable acceptor-acceptor were also detected. So, these may be the responsible factors for the possible binding.

3.4. Molecular dynamics simulations

The binding affinity and changes occurred in conformation of receptor-ligand complexes were seen in the molecular dynamics simulations. For the MD simulations analysis, standard physiological conditions were maintained according to the Yasara software. All the 14 receptor-ligand complexes were kept for 1 ns time period to decipher the molecular dynamics simulation evaluation. To understand this study, Time vs. Energy and Time vs. RMSD graph plots were taken, which revealed the exact mechanism of these selected ligands against proteins. A0A085L3H5 and W9EAC9 proteins remained separated with respect to other proteins that usually ranged from -340000 kcal.mol^{-1} to -190000 kcal.mol^{-1} based on the software algorithm, while RMSD of all complexes showed higher fluctuations at simultaneous MD simulations time interval (Fig. 5). On the basis of these results, it is easily recognized that all ligands possessed similar stability and

changed in their conformation except A0A085L3H5 and W9EAC9 protein. Moreover, to understand this in detail, protein-ligand interaction maps were generated using Accelrys Discovery studio visualizer 2016 that showed that dominance of hydrogen bonds were the major anchoring sites for ligand binding and these hydrogen bonds had diverse pattern in their combination with protein complexes. A0A0B3BFJ9, D3E8W1, D3ELZ1, Q5L060, Q9L543, R4G0S3, and U2WW35 protein-ligand complexes described massive change in their conformation (Fig. 6), which clarified the changes occurred in their structural conformation. The selected protein–ligand complex was used in receptor-based superposition to study the collaboration of dock poses, which showed the potential binding against native structured receptors.

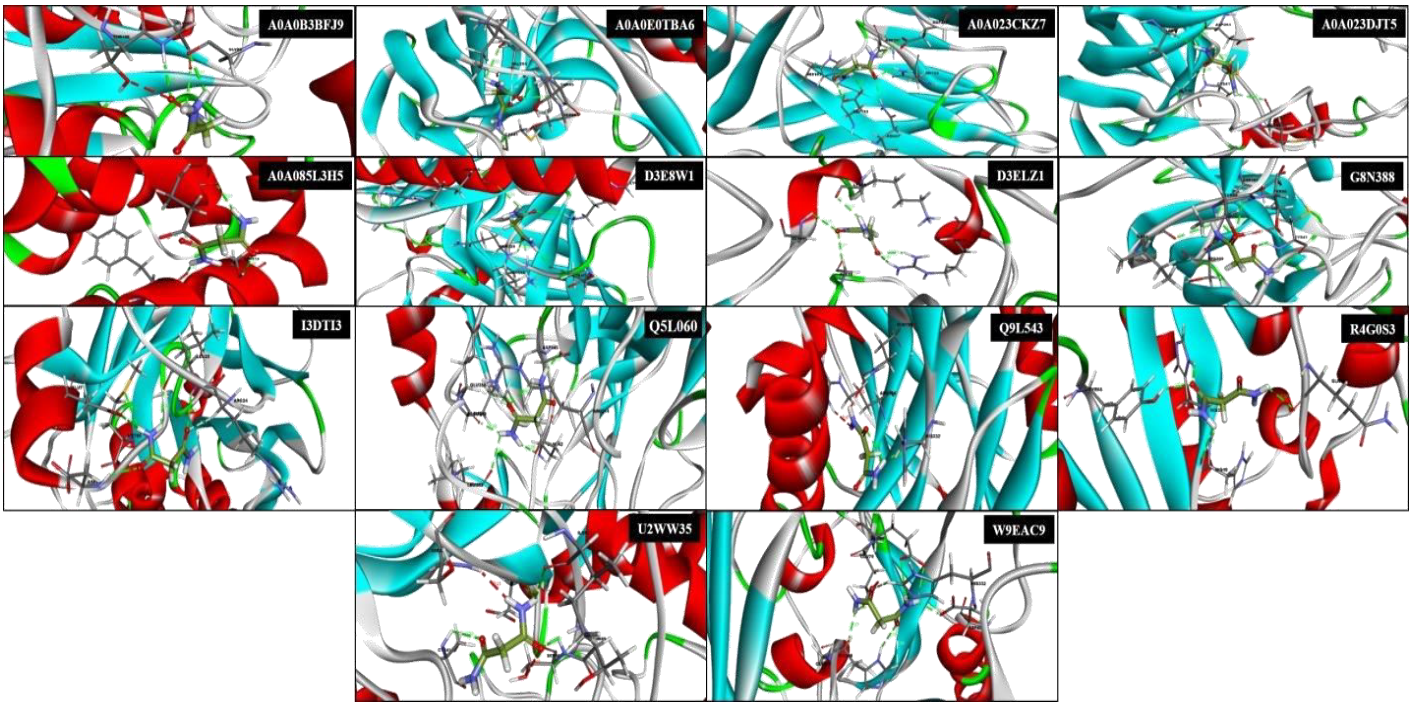


Fig. 4: Dock pose of docked ligands with selected proteins

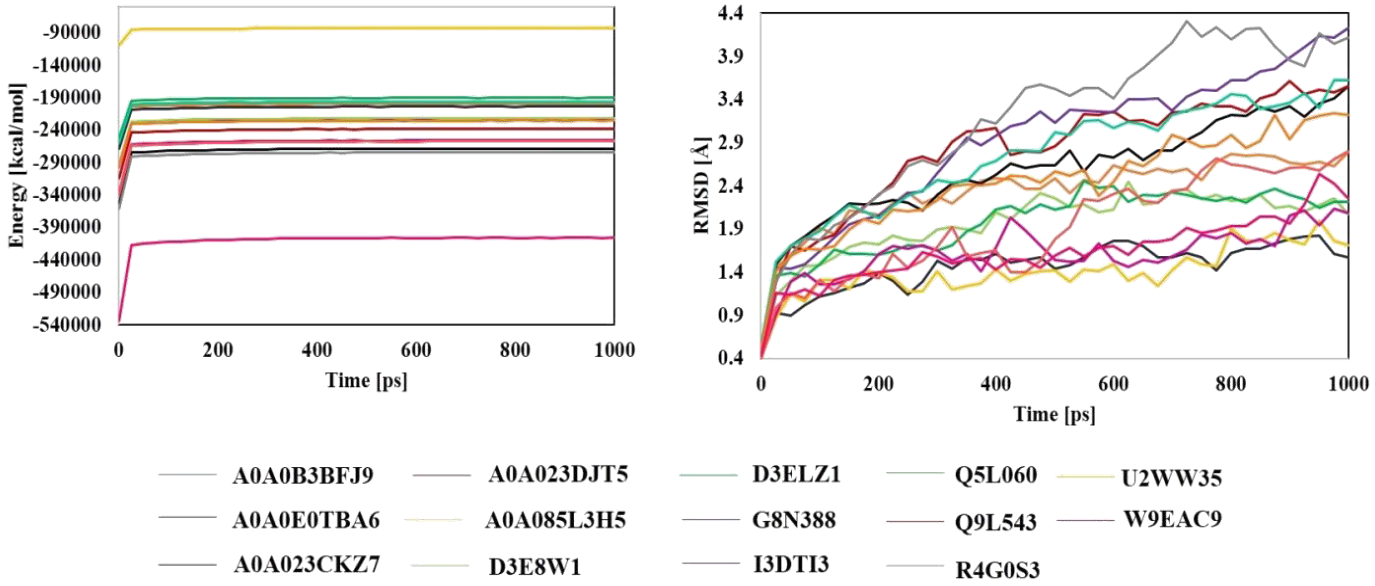
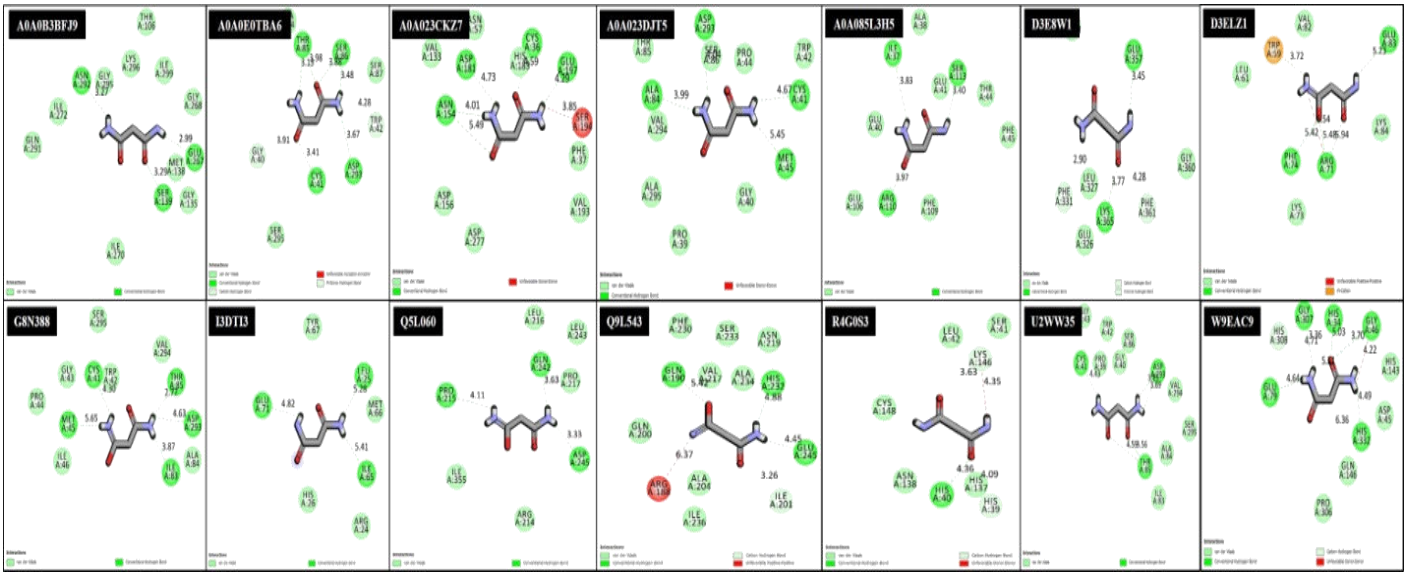


Fig. 5: Energy and RMSD plots produced from MD trajectories of prioritized targets



(Hydrogen: blue color, Hydrophobic interactions: Gray colour with dotted spots, π-stacking: Green colour with dashed line, van der Waals interactions: light green color, Unfavorable positive-positive interaction: red color) developed from Accelrys Discovery Studio visualize

Fig. 6: The protein-ligand interaction maps developed from molecular dynamics (MD) conformations

4. CONCLUSION

Aliphatic amidase plays very crucial role in the metabolism in all the living creature especially in the microorganisms. Thermophiles show the best amidase activity at higher temperature, which prompt scientific community to explore the nature of aliphatic amidases, with respect to structural and sequence based correlation with substrate specificity and thermo stability.

A number of physico-chemical characteristics and sequence analysis of thermostable aliphatic amidases have been calculated for total number of amino acid, molecular weights and composition of amino acids, which clearly stated the presence of Glu, Leu, Ile and Ala amino acid and its location that is responsible for thermo stability. This study has found difference between various thermophiles in conserved amino acid residues at several positions. Glutamine is significantly different and plays important role in enantioselectivity. The results of the present work will be quite useful in prediction and selection of thermostable aliphatic amidases or from the large number of sequenced microbial genomes. The docking studies conducted revealed that among all the selected substrates, malaonomide showed the best binding affinity towards all the chosen proteins. Molecular dynamics simulation study showed the thermal stability of amidase reported from *Geobacillus stearothermophilus* NUB3621 that was confirmed by RMSD, which in turn depended on structural arrangements and interactions of all atoms. In addition, more fluctuation were observed at 1 ns time trajectory so more time interval will be the next step to perceive the higher level structural integrity.

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Conflict of interest

There is no conflict of interest of the study.

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