Computational Analysis of Amino Acid Sequences in Relation to Thermostability of Interspecific Nitrile Degrading Enzyme (Amidase) from Various Thermophiles/Hyperthermophiles

Reena Kanwar, Nikhil Sharma and Tek Chand Bhalla*

Sub-Distributed Information Centre, Himachal Pradesh University, Summer-Hill, Shimla-171005, India

Abstract

Computational analysis of amino acid sequences of some thermophilic/hyper thermophilic microbial amidases for various physiochemical properties and amino acid has been done. Forty thermophilic and hyperthermophilic bacteria and archaea sequences were retrieved from NCBI database. These sequences were analyzed using ProtParam (ExPASy) tool for various physiochemical properties, whereas statistical significance was calculated using P value for the same, which indicates a clear distinction between thermophiles and hyperthermophiles. Physiochemical parameters, such as number of amino acid, molecular weight and negatively charged residues were found to be significantly higher in case of thermophiles. The number of amino acids, Leu, Arg and Ser were observed to be highly significant (1.15, 1.18 and 1.17 fold) for thermophiles, whereas in case of hyperthermophiles, Cys, Thr, His and Trp were found to be highly significant (2.29, 1.33, 1.56 and 1.93 fold), which makes a clear cut distinction between the thermostability of two groups (thermophiles/hyperthermophiles) of amidases.

Keywords: Amidase; Hyperthermophiles; Physiochemical properties; Thermophiles; Amino acid

Introduction

Various cyclic amide-metabolizing systems occur in nature and play significant roles in a variety of metabolisms, such as amino acid, antibiotic and pyrimidine/purine metabolisms [1]. The ability to degrade nitriles is quite common among micro-organisms. Amidase or amidohydrolase is an interesting member of the family hydrolase and superfamily nitrilase [2,3], used for the metabolism of amides. They act on the amide bonds (carbon-nitrogen (C-N) bond, other than peptide bonds), which are of considerable importance in biochemistry because many C-terminal amino acids act as hormones. Amidases participate in various metabolic pathways such as urea cycle, metabolism of amino groups, phenylalanine metabolism, tryptophan metabolism, cyano-amino acid metabolism, benzoate degradation and styrene degradation. Amidases have been used as biocatalyst for the production of ammonia, acrylic acid, and several other important compounds of industrial importance [4-6]. In industries, they are employed in combination with nitrile hydratase for the production of commercially important organic acids, such as acrylic acid, p-amino benzoic acid, pyrazinoic acid, nicotinic acid through biotransformation of nitriles [4,7].

There are special classes of enzymes which are stable at high temperature called thermostable enzymes (thermozymes), and explored as excellent biocatalyst in industrial processes [8-11]. There are certain micro-organisms which catalyses reaction even at the temperature above 100°C, such as *Pyrococcus furiosus*, *Methanopyrus kandleri* AV19 and *Thermatoga maritima* by hydrolyzing variety of substrates. The first hyperthermophilic microorganism *Sulfolobus* species was discovered in 1972 from hot acidic springs in Yellowstone Park [8,12]. During the past four decades, the molecular basis of thermal stability of protein has expanded as a vast research area. The importance of amidases in biotechnology is growing rapidly because of their potential applications in chemical and pharmaceutical industries, as well as in bioremediation [13]. Microbial amidases are a class of enzymes that have potential applications in the development of commercial bioprocesses [14,15]. They are used in the detoxification of industrial effluents containing toxic amides, such as acrylamide and formamide

[16,17], and their acyl transferase activity is exploited mainly for the synthesis of pharmaceutically active hydroxamic acids [18-20].

For efficient industrial applications, various parameters such as the number of amino acid residues, molecular mass, theoretical pI, amino acid composition, negatively charged residues, positively charged residues and extinction coefficients are needed to be studied carefully, which are considered to be important in exploring and comparing thermostability feature of enzymes, including amidases. These properties can be determined by *In silico* analysis of amino acid sequences available in the database, which provides meaningful information about the structure and function of proteins. In the present study, *In silico* analysis of some important, a physiochemical property of thermophilic/hyperthermophilic amidases, has been carried out, which has not been done so far.

Materials and Methods

Data collection and analysis

The information about micro-organisms (thermophilic/hyperthermophilic) producing amidases was searched from National Centre for Biotechnology Information (NCBI, http://www.ncbi.nlm. nih.gov/protein) and BRaunschweig ENzyme DAtabase (BRENDA, http://www.brenda-enzymes.info/). Various bacteria and archaea were searched from these databases, and their accession numbers were obtained. Amino acid sequences for both thermophilic and hyperthermophilic microorganisms which produce amidases were taken and these sequences are not fragmented, putative, pseudo and

*Corresponding author: Tek Chand Bhalla, Sub-Distributed Information Centre, Himachal Pradesh University, Summer-Hill, Shimla-171005, India, E-mail: bhallatc@rediffmail.com

hypothetical. Protein sequences of amidases (aliphatic and aromatic) of thermophiles and hyperthermophiles was searched from Expert Protein Analysis System (ExPASy) proteomics server of the Swiss Institute of Bioinformatics (SIB) and UniProt (http://www.uniprot.org/uniprot/). FASTA format of protein sequences were applied or saved for subsequent analysis of microorganisms, given in the tables. Different tools in the proteomic server (ProtParam, ProtScale, and Protein calculator) were applied to calculate various physiochemical properties of bacterial and archaeal amidases from their protein sequences. The physiochemical parameters computed by ProtParam included number of amino acid, molecular weight, theoretical pI, negatively charged residues (Asp & Lys), positive charge residues (Arg & Lys), extinction co-efficient (which depends upon the tyrosine residue, tryptophan residue and cysteine molecules), instability index and aliphatic index

Parameters		Microorganisms(MO's)										P-value
		1	2	3	4	5	6	7	8	9	10	
Number of amino acids	B	452.0	447.0	434.0	434.0	434.0	488.0	492.0	456.0	456.0	446.0	1.64ns
	A	504.0	504.0	403.0	417.0	401.0	401.0	431.0	412.0	472.0	473.0	
Molecular weight	B	49645.6	48998.0	46574.8	46649.7	46358.5	51605.3	53912.1	47981	47865.1	47530.4	0.53 ns
	A	55655.3	55735.3	42695.0	43772.1	43186.9	42406.7	44842.6	44303.0	51469.3	51941.1	
Theoretical pI	B	8.91	9.03	6.45	6.25	6.75	6.71	4.87	4.72	4.66	6.43	0.36 ns
	A	5.94	5.48	7.70	6.63	6.68	7.74	8.75	8.42	5.50	5.82	
Positively charged residues (Arg+Lys)	B	52.0	61.0	53.0	51.0	53.0	50.0	41.0	36.0	35.0	39.0	2.39 ns
	A	59.0	57.0	44.0	45.0	43.0	43.0	46.0	48.0	53.0	53.0	
Negatively charged residues (Asp+Glu)	B	58.0	53.0	55.0	54.0	54.0	52.0	62.0	55.0	55.0	43.0	1.51 ns
	A	64.0	64.0	43.0	46.0	44.0	42.0	41.0	46.0	58.0	64.0	
Extinction coefficients $(M^{-1}cm^{-1})$ at 280 nm	B	29910.0	30940.0	21430.0	21430.0	22920.0	37930.0	98890.0	55920.0	57410.0	40450.0	0.32 ns
	A	62800.0	67270.0	33350.0	33370.0	34840.0	31860.0	34380.0	38850.0	48250.0	85370.0	
Instability index	B	32.53.0	28.33	37.95	39.47	34.94	45.54	44.37	29.07	27.78	28.41	1.28 ns
	A	29.60	28.55	41.15	33.90	47.59	44.21	34.15	38.92	37.30	36.72	
Aliphatic index	B	99.89	97.36	105.48	103.02	102.79	84.51	85.08	87.35	86.91	95.81	2.42 ns
	A	90.14	90.52	97.15	100.74	98.30	98.15	100.44	102.35	97.99	85.58	
Grand average of hydropathicity (GRAVY)	B	-0.027	-0.077	-0.032	-0.068	-0.068	-0.097	-0.101	0.015	0.004	0.112	1.25 ns
	A	-0.197	-0.190	0.046	0.108	0.087	0.086	0.085	0.001	-0.062	-0.209	

(B) Bacteria: (1) *Thermotoga thermarum* DSM 5069; (2) *Thermotoga lettingae* TMO; (3) *Thermus thermophiles* SGO SOP17-16; (4) *Thermus sp* RL;(5) *Thermus aquaticus* Y51 MC23; (6) *Dietzia cinnamea* P4; (7) *Rhodococcus erythropolis* PR4; (8) *Rhodococcus jostii* RHA1; (9) *Rhodococcus opacus* B4; (10) *Pseudomonas Syringae py glycinea* str. racc14.

(A) Archaea: (1) *Sulfolobus solfataricus* 9812; (2) *Sulfolobus islandicum* M164; (3) *Thermoproteus neutrophilus* V24 Sta; (4) *Thermoproteus uzoniensis* 768-20; (5) *Pyrobaculum islandicum* DSM 4184; (6) *Pyrobaculum arsenatium* DSM 13514; (7) *Aeropyrum pernix* K1; (8) *Thermoproteus tenax* kra1; (9) *Acidianus hospitalis* W1; (10) *Thermofilium pendens* Hrk5.

Parameters		Microorganisms(MO's)										P-value
		11	12	13	14	15	16	17	18	19	20	
Number of amino acids	B	479.0	462.0	478.0	474.0	499.0	473.0	460.0	457.0	470.0	499.0	1.64ns
	A	501.0	489.0	434.0	455.0	454.0	451.0	455.0	463.0	501.0	433.0	
Molecular weight	B	50759.5	48227.4	53524.4	49614.4	52813.4	49559.6	51636.0	50542.2	51427.7	54181.1	0.53 ns
	A	54700.2	54916.2	48007.6	48829.3	48581.1	48833.3	49859.3	50522.2	54700.2	47501.2	
Theoretical pI	B	5.67	5.25	5.81	5.97	9.32	6.57	8.15	8.44	5.22	5.19	0.36 ns
	A	6.53	6.72	6.05	4.95	4.70	4.64	5.31	5.80	6.53	5.39	
Positively charged residues (Arg+Lys)	B	39.0	37.0	62.0	48.0	50.0	47.0	61.0	58.0	43.0	39.0	2.39 ns
	A	62.0	60.0	62.0	49.0	44.0	45.0	53.0	53.0	62.0	51.0	
Negatively charged residues (Asp+Glu)	B	49.0	51.0	66.0	51.0	46.0	48.0	59.0	55.0	60.0	56.0	1.51 ns
	A	63.0	60.0	64.0	68.0	69.0	71.0	61.0	56.0	63.0	57.0	
Extinction coefficients $(M^{-1}cm^{-1})$ at 280 nm	B	47900.0	52940.0	74260.0	59470.0	78950.0	57980.0	33810.0	34840.0	44350.0	51800.0	0.32 ns
	A	69330.0	58680.0	31290.0	33370.0	22350.0	20860.0	31290.0	45270.0	69330.0	31290.0	
Instability index	B	39.07	35.78	38.98	40.13	40.09	39.77	28.31	28.46	30.99	34.46	1.28 ns
	A	43.59	40.73	30.10	40.00	35.67	39.42	33.05	30.02	43.59	36.16	
Aliphatic index	B	87.72	82.86	89.35	91.56	86.97	94.42	90.15	92.60	92.74	93.13	2.42 ns
	A	102.57	99.57	91.64	92.42	93.92	92.37	96.88	88.06	102.57	87.39	
Grand average of hydropathicity (GRAVY)	B	-0.025	-0.031	-0.243	-0.057	-0.085	0.004	-0.255	-0.044	-0.110	-0.128	1.25 ns
	A	-0.002	-0.085	-0.192	-0.118	-0.079	-0.132	-0.109	-0.043	-0.002	-0.250	

(B) Bacteria: (11) *Pseudomonas fulva*12-x; (12) *Pseudonocardia dioxanivorans* CB1190; (13) *Aquifex aeolicus;* (14) *Frankia* sp. Elu IC; (15) *Frankia* sp. CCl$_3$; (16) Frankia sp. CN$_3$; (17) Thermosipho africanus TCF52B; (18) Thermosipho melanesiensis B1429; (19) *Geobacillus* sp. Y412MC52; (20) *Paenibacillus* sp. Y412 MC10.

(A) Archaea: (11) *Pyrolobus fumarii* 1A; (12) *Ignisphaera aggreganus* DSM 17230; (13) *Methanocaldococcus jannaschii* DSM 2661; (14) *Methanopyrus kandleri* AV19; (15) *Methanococcus vannielii* SB; (16) *Methanothermobacter marburgensis* str Marburg; (17) *Methanococcus maripaludis* C5; (18) *Archaeoglobus veneficus* SNP6; (19) *Archaeoglobus profundus* DSM 5631; (20) *Methanococcus voltae* A$_3$.

Table 1.1(a): Comparative analysis of physiochemical properties of amidases of bacteria and archaebacteria (hyperthermophiles).

Parameters		Microorganisms (MO's)										P- value
		1	2	3	4	5	6	7	8	9	10	
Number of amino acids	B	466.0	476.0	448.0	428.0	472.0	473.0	483.0	488.0	473.0	476.0	5.57 *
	A	475.0	471.0	470.0	471.0	472.0	445.0	454.0	417.0	457.0	455.0	
Molecular weight	B	48610.9	50195.8	46417.0	44976.3	50873.3	50860.3	51507.6	53669.8	51173.2	50458.0	4.97 *
	A	51211.4	51064.4	51136.3	51057.4	51469.3	48493.3	48581.1	45469.8	50211.5	49859.3	
Theoretical PI	B	4.76	5.23	6.65	8.19	5.40	5.29	6.04	5.56	5.37	5.92	1.63 ns
	A	4.86	5.92	7.61	6.16	5.50	5.41	4.70	6.06	5.93	5.31	
Positively charged residues (Arg+Lys)	B	37.0	43.0	38.0	39.0	40.0	39.0	46.0	54.0	49.0	44.0	0.54 ns
	A	40.0	50.0	51.0	53.0	53.0	57.0	44.0	40.0	58.0	53.0	
Negatively charged residues (Asp+Glu)	B	60.0	54.0	41.0	37.0	49.0	49.0	51.0	63.0	60.0	51.0	4.35 *
	A	58.0	55.0	50.0	54.0	58.0	62.0	69.0	48.0	61.0	61.0	
Extinction coefficients ($M^{-1}cm^{-1}$) at 280 nm	B	36440	77920	26470	28420	48360	43890	38850	53290	54430	37930	0.50 ns
	A	60740	55810	46760	43780	48520	50310	22350	30370	50770	31290	
Instability index	B	38.00	44.24	47.10	37.56	40.06	35.62	31.25	34.99	46.97	45.67	2.37 ns
	A	30.74	44.09	33.16	38.06	37.30	41.78	35.67	23.52	32.00	33.05	
Aliphatic index	B	100.24	92.12	96.07	87.34	96.36	97.00	89.05	91.99	87.12	91.97	2.81 ns
	A	79.24	90.59	90.64	93.38	97.99	87.24	93.92	88.44	86.65	96.80	
Grand average of hydropathicity (GRAVY)	B	0.088	-0.016	0.215	0.032	0.079	0.097	-0.140	-0.351	-0.104	0.037	0.58 ns
	A	-0.258	-0.034	-0.108	-0.058	-0.062	-0.240	-0.079	-0.210	-0.155	-0.109	

(B) Bacteria: (1) *Acidimicrobium ferrooxidans* DSM 10331; (2) *Acidothermus cellulolyticus* IIB; (3) *Alicycliphilus denitrificans* K601; (4) *Thermovibrio ammonificans* HB-1,(5) *Chloroflexus aggregans* DSM 9485; (6) *Chloroflexus* sp.Y.400.F1; (7) *Marinithermus hydrothermalis* DSM; (8) *Rhodococcus marinus* DSM 4252; (9) *Thermobifida fuscaxy*; (10) Thermobispora bispora.
(A) Archaea: (1) *Methanohalobium evestigatum* 2-7303; (2) *Methanosaeta thermophila* PT; (3) *Metallosphaera cuprina* Ar-4; (4) *Metallosphaera yellowstonesis* MKI; (5) *Methanosaeta concilli* GP6; (6) *Ignicoccus hospitalis* KIN4/T; (7) *Methanothermobacter thermautotrophicus* Str.Delta H; (8) *Methanobacterium* sp. AL-21; (9) *Archaeoglobus fulgidus* DSM 4304; (10) *Methanothermus fervidus* DSM 2088.

Parameters		Microorganisms(MO's)										P-value
		11	12	13	14	15	16	17	18	19	20	
Number of amino acids	B	466.0	475.0	477.0	472.0	453.0	484.0	490.0	422.0	486.0	431.0	5.57*
	A	433.0	434.0	425.0	476.0	471.0	463.0	453.0	425.0	451.0	425.0	
Molecular weight	B	49837.3	52257.6	52221.1	51943.9	50124.3	52603.2	53388.4	49367.0	52062.6	48324.5	4.97*
	A	46428.7	45482.9	44132.5	51334.7	51275.5	50522.2	50506.2	44204.6	48833.3	45109.7	
Theoretical PI	B	5.28	5.58	5.07	4.86	6.87	5.80	5.75	10.05	9.15	4.59	1.63 ns
	A	5.67	4.10	4.08	5.94	8.28	5.80	6.25	4.06	4.64	4.69	
Positively charged residues (Arg+Lys)	B	41.0	55.0	53.0	52.0	58.0	58.0	57.0	81.0	57.0	30.0	0.54 ns
	A	47.0	26.0	25.0	52.0	54.0	53.0	59.0	25.0	45.0	34.0	
Negatively charged residues (Asp+Glu)	B	54.0	61.0	67.0	69.0	58.0	63.0	63.0	25.0	52.0	62.0	4.35 *
	A	51.0	76.0	70.0	58.0	52.0	56.0	61.0	72.0	71.0	74.0	
Extinction coefficients ($M^{-1}cm^{-1}$) at 280 nm	B	56380	42290	41260	36790	34270	39310	62800	56160	27960	57300	0.50 ns
	A	35300	44810	43320	55810	46760	45270	45270	43320	20860	23950	
Instability index	B	43.65	33.80	41.45	34.21	28.30	39.06	40.55	35.39	37.08	34.11	2.37 ns
	A	37.98	38.11	35.05	36.57	38.49	30.02	35.67	36.77	39.42	42.79	
Aliphatic index	B	80.67	88.65	82.81	86.44	86.93	87.91	87.00	94.72	94.12	89.74	2.81 ns
	A	80.72	77.24	78.16	85.46	91.25	88.06	89.14	78.14	92.37	82.49	
Grand average of hydropathicity (GRAVY)	B	-0.084	-0.109	-0.295	-0.220	-0.269	-0.157	-0.122	-0.462	-0.177	-0.406	0.58 ns
	A	-0.135	-0.256	-0.188	-0.135	-0.118	-0.043	-0.163	-0.192	-0.132	-0.281	

(B) Bacteria: (11) *Natranaerobius thermophiles*; (12) *Fervidobacterium nodosum* Rt 17-b1; (13) *Halothermothrix* orenii H168; (14) *Hippea maritime* DSM 10411; (15) *Petrotoga mobilis* SU95, (16) *Thermodesulfatator indicus* DSM 15286; (17) *Thermovirga lienii* DSM 17291; (18) *Caminibacter mediatlanticus* B-2;(19) *Heliobacterium modesticaldum* Ice-1; (20) Thermovirga lienii DSM 17291.
(A) Archaea: (11) *Methanoculleus marisnigri* JR1; (12) *Halorhabdus utahensis* DSM 12940; (13) *Methanoculleus marismortui* ATCC 43049; (14) *Methanosarcina mazei* G01; (15) *Metallosphaera sedula* DSM 5348; (16) *Methanosarcina acetivorans* C2A; (17) *Methnosaeta harundinacea* 6AC; (18) *Haloarcula hispanica* ATCC 33960; (19) *Methanosarcina barkeri str.fusaro*; (20) Halorhabdus tiamaea SARL 4B.
** Significant at a level of 1% of probability (p<0.01),* Significant at a level of 5% of probability (0.01=< p< 0.05), ns= Non Significant (p>=0.05),

Table 1.1(b): Comparative analysis of physiochemical properties of bacteria and archaebacteria (thermophiles).

for thermostability, and grand average of hydropathicity (GRAVY). The extinction co-efficient of various amidases was calculated using the equation [21].

E (prot)=Numb (Tyr)*Ext (Tyr)+Numb (Trp)*Ext (Trp)+Numb (cysteine)*Ext (cysteine)

The values of the aliphatic index of various amidase sequences were obtained using the ProtParam (ExPASy) tool [22]. The instability index and grand average of hydropathicity (GRAVY) were assessed by following the method [23]. The molecular weight in kilodaltons (kda) and the pI of amidases were deduced using pK values of amino acid.

Atomic composition and amino acid percent count were also estimated for all protein sequences.

Results

In the present studies, using ProtParam (http://web.expasy.org/protparam/) tool, various physicochemical properties of amino acid sequences of amidases in thermophiles/hyperthermophiles of bacteria and archaea were determined. The significant differences in the physiochemical properties of the two groups (thermophiles and hyperthermophiles) have been observed (Table 1.1 a and b). The total number of amino acid residues were found to be significantly higher (1.02 fold) in thermophilic bacteria and archaea, as when compared to hyperthermophilic bacteria and archaea, as shown in table 1.1 a and b. Theoretical pI varied between 4.66-9.32; 4.64-8.75; 4.59-10.05 and 4.06-8.28, respectively for hyperthermophilic and thermophilic bacteria and archea. Negative charge residues (Glu and Asp) were found to be significantly higher, i.e 1.16 fold in hyperthermophiles, in comparison

Amino Acid		Micro-organisms (MO's)																				P-value
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Ala (A)	B	9.5	9.2	12.2	12.0	12.0	15.2	11.0	12.5	12.3	10.1	13.2	14.1	7.5	16.5	15.2	16.1	7.0	7.4	8.3	10.0	1.62 ns
	A	6.0	5.8	14.9	16.8	12.2	15.7	13.5	13.3	8.5	11.4	10.2	8.2	6.9	11.6	9.7	8.6	7.5	8.6	10.2	6.7	
Arg (R)	B	5.3	4.7	8.3	8.5	7.6	9.6	6.1	5.3	5.0	5.6	6.9	6.7	4.4	8.6	9.2	8.7	3.9	3.3	3.8	4.8	0.27 ns
	A	4.2	4.8	6.7	7.0	5.2	6.5	8.6	7.3	3.6	7.0	8.6	7.6	3.7	8.6	4.8	6.2	3.1	4.3	8.6	3.5	
Asn (N)	B	4.2	2.9	1.6	1.4	1.4	1.4	3.0	1.3	1.5	3.4	1.5	1.1	2.1	2.3	2.4	2.3	7.2	5.7	3.6	4.6	0.75 ns
	A	3.8	4.2	3.2	2.6	3.2	3.2	1.4	2.9	4.7	1.9	2.4	3.7	3.7	2.9	2.9	2.4	4.6	3.2	2.4	5.3	
Asp (D)	B	4.2	5.1	3.0	3.0	3.2	7.0	6.3	7.5	8.1	5.6	4.8	6.3	4.0	5.5	4.6	5.1	6.3	4.8	4.9	6.4	0.27 ns
	A	6.3	5.8	3.2	4.8	4.2	3.7	3.0	3.6	5.5	3.8	5.0	5.3	6.0	5.1	7.0	7.8	5.9	4.8	5.0	5.5	
Cys (C)	B	0.9	1.1	0.0	0.0	0.0	0.2	0.6	0.9	0.9	2.0	0.8	0.9	0.4	0.2	0.4	0.2	0.4	0.4	0.6	0.6	5.25*
	A	0.8	0.8	0.7	0.2	0.7	0.7	1.9	0.2	0.2	0.4	1.2	6.6	2.3	2.0	0.9	0.9	1.5	1.3	1.2	2.1	
Gln (Q)	B	2.2	2.2	1.6	2.1	1.4	1.0	2.8	2.0	2.0	2.2	2.7	1.9	2.1	2.3	1.8	2.1	1.3	1.1	2.3	3.4	17.65**
	A	1.4	1.4	1.0	0.7	1.0	0.7	0.7	0.7	0.6	1.9	0.8	1.2	0.9	1.1	1.5	2.4	2.4	1.5	0.8	2.3	
Glu (E)	B	7.3	6.7	9.7	9.4	9.2	3.7	6.3	4.6	3.9	4.0	5.4	4.8	9.8	5.3	4.6	5.1	6.5	7.2	7.9	4.8	5.58*
	A	6.3	6.9	7.4	6.2	6.7	6.7	6.5	7.5	6.8	9.7	7.6	7.0	8.8	9.9	8.1	8.0	7.5	7.3	7.6	7.6	
Gly (G)	B	7.3	8.3	10.1	9.9	10.6	9.0	8.3	9.9	10.3	10.8	10.0	10.4	7.5	9.9	9.8	9.5	7.4	7.7	8.1	7.8	0.35 ns
	A	9.3	9.7	9.4	9.4	8.5	9.7	12.1	8.0	8.9	9.9	9.0	7.0	8.3	10.1	10.6	10.4	8.6	10.2	9.0	8.8	
His (H)	B	1.8	2.2	1.6	1.6	1.8	2.9	1.8	1.3	1.3	3.4	2.5	2.4	0.6	0.8	1.6	1.1	0.9	0.0	2.6	2.6	6.43*
	A	1.4	0.8	1.5	1.4	1.7	2.2	0.5	1.5	0.4	3.2	0.8	0.2	0.5	1.3	1.3	1.1	0.7	0.4	0.8	0.5	
Ile (I)	B	6.6	8.7	2.8	2.8	2.3	2.3	3.7	4.4	4.4	5.8	3.3	2.2	6.3	3.0	4.2	3.2	8.7	9.8	7.4	7.2	3.62 ns
	A	6.5	6.5	3.2	3.4	6.2	4.7	2.6	4.1	9.7	3.0	5.6	11.0	8.8	6.2	7.7	8.4	11.4	6.3	5.6	8.3	
Leu (L)	B	10.0	9.4	15.7	15.4	15.7	10.0	9.1	8.8	8.6	10.3	11.9	8.9	9.4	11.0	9.4	11.4	9.6	6.8	8.5	10.2	1.12 ns
	A	10.3	10.3	10.7	11.8	9.0	9.7	16.5	12.4	9.1	8.7	11.0	8.6	7.1	7.7	8.8	8.4	6.6	8.6	11.0	8.1	
Lys (K)	B	7.5	8.9	3.9	3.2	4.6	0.6	2.2	2.6	2.6	3.1	1.3	1.3	8.6	1.5	0.8	1.3	9.3	9.4	5.3	3.0	2.48 ns
	A	7.5	6.5	4.2	3.8	5.5	4.2	2.1	4.4	7.6	4.2	3.8	4.7	10.6	2.2	4.8	3.8	8.6	7.1	3.8	8.3	
Met (M)	B	2.2	2.0	0.9	0.9	0.9	1.6	3.3	1.5	1.5	1.3	2.1	1.7	1.0	1.3	2.2	1.3	1.3	1.8	1.7	2.0	12.70 **
	A	4.4	4.4	1.7	1.2	2.7	1.7	1.2	1.0	3.2	2.5	2.2	2.7	3.2	1.5	3.1	3.3	2.2	3.9	2.2	2.8	
Phe (F)	B	4.2	4.7	3.0	3.0	2.8	3.9	3.9	3.7	3.5	4.7	3.5	3.0	4.6	2.5	2.2	2.7	7.0	7.0	4.0	2.8	13.69**
	A	2.8	2.6	2.2	2.9	3.0	2.5	1.4	2.4	2.5	3.2	1.8	3.1	2.8	2.2	2.6	3.1	3.1	4.1	1.8	2.8	
Pro (P)	B	4.4	4.9	7.4	8.1	7.8	8.6	6.3	8.8	9.0	5.4	7.1	8.0	5.9	9.3	10.2	9.5	3.3	3.9	7.0	5.0	9.15**
	A	6.5	6.7	5.7	6.0	5.5	5.2	6.7	7.3	4.7	5.9	6.4	5.1	4.1	6.4	4.2	4.2	4.0	4.1	6.4	3.5	
Ser (S)	B	4.9	5.6	3.7	3.7	3.9	6.4	6.5	5.7	5.3	6.7	7.5	5.6	7.3	5.1	4.6	5.7	6.3	7.4	5.1	8.2	1.59 ns
	A	6.5	6.3	5.2	5.8	5.5	4.2	7.9	5.8	7.4	5.3	6.2	7.4	5.5	5.1	7.0	6.7	5.9	7.1	6.2	7.2	
Thr (T)	B	6.0	4.9	5.1	5.3	5.3	6.8	5.1	7.7	7.7	5.2	6.9	8.9	4.8	5.1	6.6	4.9	5.2	5.3	7.0	6.4	20.30**
	A	4.4	4.2	5.0	3.8	5.2	5.2	5.8	4.6	5.3	2.7	3.0	4.7	4.1	3.7	4.6	4.9	5.3	4.3	3.0	6.2	
Trp (W)	B	0.7	0.9	0.5	0.5	0.5	1.0	3.0	1.8	1.8	1.3	1.3	1.7	1.5	2.1	2.6	2.1	0.2	0.4	0.9	0.8	7.49**
	A	1.2	1.2	0.5	0.5	0.5	0.5	0.7	0.7	0.4	2.5	1.6	0.4	0.0	0.4	0.0	0.0	0.0	0.4	1.6	0.0	
Tyr (Y)	B	2.0	1.3	1.6	1.6	1.8	1.4	2.2	1.8	2.0	1.1	2.1	1.3	5.0	0.6	1.0	0.4	4.1	3.5	3.2	4.0	27.48**
	A	4.0	4.6	3.7	3.1	4.0	3.5	2.8	3.6	5.3	2.7	3.4	6.5	4.8	2.9	3.3	3.1	4.6	5.0	3.4	4.8	
Val (V)	B	8.8	6.0	7.4	6.9	7.1	7.8	8.3	8.1	8.3	7.8	5.2	8.9	7.1	7.2	6.4	7.4	4.1	7.0	7.7	5.2	0.87 ns
	A	6.3	6.5	9.7	8.6	9.2	9.0	4.4	8.5	5.5	9.9	9.6	5.1	7.8	9.2	6.8	6.2	6.6	7.3	9.6	5.8	

(B) Bacteria: (1) *Thermotoga thermarum* DSM 5069; (2) *Thermotoga lettingae* TMO; (3) *Thermus thermophiles* SGO SOP17-16; (4) *Thermus sp* RL; (5) *Thermus aquaticus* Y51 MC23; (6) *Dietzia cinnamea* P4; (7) *Rhodococcus erythropolis* PR4; (8) *Rhodococcus jostii* RHA1; (9) *Rhodococcus opacus* B4, (10) *Pseudomonas Syringae* py glycinea str. racc14; (11) *Pseudomonas fulva12-x*; (12) *Pseudonocardia dioxanivorans* CB1190; (13) *Aquifex aeolicus*; (14) Frankia sp. Elu IC; (15) *Frankia* sp. CCl3; (16) *Frankia* sp. CN3; (17) *Thermosipho africanus* TCF52B; (18) *Thermosipho melanesiensis* B1429; (19) *Geobacillus* sp. Y412MC52; (20) *Paenibacillus* sp. Y412 MC10.
(A) Archaea: (1) *Sulfolobus solfataricus* 9812; (2) *Sulfolobus islandicum* M164; (3) *Thermoproteus neutrophilus* V24 Sta; (4) *Thermoproteus uzoniensis* 768-20; (5) *Pyrobaculum islandicum* DSM 4184; (6) Pyrobaculum arsenatium DSM 13514; (7) Aeropyrum pernix K1; (8) *Thermoproteus tenax* kra 1; (9) *Acidianus hospitalis* W1; (10) *Thermofilium pendens* Hrk5; (11) Pyrolobus fumarii 1A; (12) *Ignisphaera aggregans* DSM 17230; (13) *Methanocaldococcus jannaschii* DSM 2661; 14) *Methanopyrus kandleri* AV19; (15) *Methanococcus vannielii* SB; (16) *Methanothermobacter marburgensis str Marburg*; (17) *Methanococcus maripaludis* C5; (18) *Archaeoglobus veneficus* SNP6; (19) *Archaeoglobus profundus* DSM 5631; (20) *Methanococcus voltae* A_3.
** Significant at a level of 1% of probability (p<0.01), * Significant at a level of 5% of probability (0.01=< p< 0.05), ns= Non significant (p>=0.05),

Table 1.2(a): Comparison between amino acids percent count of amidases of bacteria and archaebacteria (hyperthermophiles).

Amino Acid		Micro-organisms (MO's)																			P-value
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Ala (A)	B	15.7	15.5	20.1	18.5	12.1	12.5	14.9	8.2	12.9	15.8	13.1	8.2	7.8	8.9	6.4	9.7	9.0	4.3	9.5	6.3	2.04 ns
	A	8.0	10.6	6.8	8.1	8.5	9.4	9.7	9.4	9.4	7.5	12.2	14.1	14.4	9.9	6.8	8.6	8.8	13.9	8.6	13.2	
Arg (R)	B	7.1	8.2	8.0	8.2	7.0	7.0	6.4	4.1	8.9	8.4	7.1	4.4	4.4	3.4	4.0	3.9	4.5	4.0	7.0	4.4	2.94 ns
	A	3.2	7.0	5.1	6.4	3.6	7.2	4.8	2.6	5.9	3.1	7.9	3.7	3.3	3.2	6.4	4.3	6.2	3.3	6.2	7.5	
Asn (N)	B	1.7	1.5	1.8	1.4	2.5	2.3	3.1	5.9	1.7	1.5	2.4	4.6	3.8	4.0	5.7	1.9	2.0	9.5	3.5	7.9	0.03 ns
	A	5.5	2.1	5.1	4.0	4.7	2.5	2.9	5.0	3.3	4.6	1.8	3.0	2.8	3.2	4.2	3.2	3.8	2.8	2.4	1.2	
Asp (D)	B	6.9	6.9	4.7	4.0	5.3	5.9	5.0	6.1	6.3	5.3	6.7	5.5	6.9	8.7	6.8	5.4	5.3	2.6	5.8	6.7	0.65 ns
	A	5.7	4.9	5.5	5.5	5.5	4.3	7.0	6.0	5.3	5.9	6.0	8.1	8.9	5.3	5.7	4.8	4.6	8.9	7.8	8.0	
Cys (C)	B	0.9	0.8	1.6	1.6	1.7	1.5	0.2	0.4	1.3	1.1	1.1	0.6	0.8	1.3	0.4	1.0	1.6	0.7	0.2	0.0	0.06 ns
	A	1.3	1.7	0.2	0.2	0.5	0.7	0.9	1.2	1.1	1.5	1.4	0.2	0.7	1.3	0.2	1.3	1.5	0.7	0.9	0.5	
Gln (Q)	B	0.9	2.3	1.8	5.8	3.8	4.2	3.7	2.9	2.3	1.9	2.1	1.7	1.5	2.8	1.3	2.5	1.6	2.6	3.9	5.3	11.48**
	A	1.9	0.8	1.1	0.8	0.6	0.7	1.5	3.8	1.1	2.4	2.1	1.2	2.1	1.3	0.8	1.5	1.1	1.6	2.4	2.6	
Glu (E)	B	6.0	4.4	4.5	4.7	5.1	4.4	5.6	6.8	6.3	5.5	4.9	7.4	7.1	5.9	1.0	7.6	7.6	3.3	4.9	7.7	13.55**
	A	6.5	6.8	5.1	5.9	6.8	9.7	8.1	5.5	8.1	7.5	5.8	9.4	7.5	6.9	5.3	7.3	8.8	8.0	8.0	9.4	
Gly (G)	B	8.2	8.8	8.7	9.6	8.5	8.2	8.7	8.0	9.3	8.4	9.7	8.4	8.4	7.6	8.2	8.7	9.6	4.3	8.2	7.0	19.27**
	A	9.7	9.8	9.1	10.4	8.9	9.4	10.6	8.2	9.8	8.6	9.9	10.6	11.5	9.2	9.3	10.2	8.2	11.3	10.4	12.0	
His (H)	B	1.9	1.5	4.0	1.9	1.5	1.5	1.9	1.6	1.7	2.3	2.1	0.8	1.0	0.8	0.7	1.0	1.2	1.4	0.6	2.8	2.08 ns
	A	1.3	1.5	0.2	0.2	0.4	0.0	1.3	3.8	0.7	0.7	0.7	1.2	0.9	1.9	0.2	0.4	0.9	0.9	1.1	4.7	
Ile (I)	B	4.9	3.2	4.0	3.3	6.6	6.6	3.9	7.2	3.0	2.7	3.2	8.0	5.5	8.1	7.5	6.4	6.5	10.7	4.5	4.9	1.24 ns
	A	6.1	7.2	7.0	7.4	9.7	4.0	7.7	5.5	5.5	11.4	4.8	4.1	3.5	5.9	6.6	6.3	5.1	3.3	8.4	5.9	
Leu (L)	B	11.8	9.5	9.8	10.0	8.9	9.3	10.8	10.7	10.6	11.3	7.7	5.9	8.8	8.1	7.7	8.9	8.2	9.0	10.9	10.0	8.50**
	A	6.5	9.3	8.9	8.9	9.1	9.9	8.8	9.6	8.3	6.6	6.7	6.9	7.1	7.6	8.9	8.6	9.9	7.1	8.4	6.1	
Lys (K)	B	0.9	0.8	0.4	0.9	1.5	1.3	3.1	7.8	1.5	0.8	1.7	7.2	6.7	7.6	8.8	8.1	7.1	15.2	4.7	2.6	0.40 ns
	A	5.3	3.6	5.7	4.9	7.6	5.6	4.8	7.0	6.8	8.6	3.0	2.3	2.1	7.8	5.1	7.1	6.8	2.6	3.8	0.5	
Met (M)	B	0.9	0.8	2.0	2.3	1.7	1.9	2.1	1.8	1.3	1.3	2.6	2.1	2.5	2.1	2.6	1.9	3.3	0.5	1.6	1.6	17.15**
	A	2.7	1.9	2.8	2.8	3.2	2.0	3.1	2.9	3.3	2.2	2.8	2.1	2.1	2.5	3.0	3.9	3.3	2.1	3.3	1.6	
Phe (F)	B	1.9	1.9	2.9	3.5	3.6	3.8	2.9	2.0	4.9	3.8	4.1	5.3	3.4	4.9	4.4	4.1	3.1	4.5	2.9	2.8	4.29*
	A	2.1	3.6	3.4	3.4	2.5	2.7	2.6	3.4	3.7	3.1	3.0	2.1	2.1	3.2	2.8	4.1	4.0	2.1	3.1	2.8	
Pro (P)	B	7.3	8.8	5.8	5.8	8.5	8.5	8.1	4.9	8.9	9.0	7.5	3.8	5.0	3.8	4.6	5.4	5.5	3.8	8.2	3.2	8.25**
	A	4.8	5.7	4.5	4.7	4.7	6.5	4.2	4.8	3.7	4.0	6.2	6.2	5.6	5.5	4.7	4.1	4.0	5.9	4.2	4.5	
Ser (S)	B	6.7	5.3	5.1	5.1	4.4	4.2	4.8	6.8	3.8	3.6	5.4	7.4	8.0	7.0	7.3	7.2	7.8	4.5	7.2	6.3	5.18*
	A	8.8	7.4	10.0	8.1	7.4	7.2	7.0	5.5	6.6	5.9	5.8	6.0	5.9	7.6	9.8	7.1	5.5	6.1	6.7	4.2	
Thr (T)	B	7.5	6.1	5.6	4.9	5.1	5.7	5.4	5.5	4.9	7.6	6.0	4.8	6.5	5.3	5.1	5.6	3.7	6.2	6.2	6.0	0.42 ns
	A	6.7	5.5	6.4	5.7	5.3	4.9	4.6	5.3	3.7	5.3	6.9	6.9	6.1	5.0	6.4	4.3	4.4	6.1	4.9	5.6	
Trp (W)	B	1.1	2.5	0.9	0.7	1.1	1.1	0.6	0.8	1.7	1.1	1.5	0.4	0.2	0.2	0.0	0.4	1.2	0.2	0.8	1.2	5.54*
	A	0.8	1.1	0.4	0.4	0.4	0.9	0.0	0.5	0.7	0.0	0.2	0.7	0.7	1.1	0.4	0.4	0.4	0.7	0.0	0.7	
Tyr (Y)	B	1.3	1.7	0.7	1.9	3.0	2.3	3.1	4.3	1.5	1.5	2.6	4.4	5.0	4.4	5.1	3.9	4.1	8.1	0.8	4.6	5.22*
	A	5.5	4.0	5.1	4.7	5.3	4.3	3.3	3.1	5.0	4.6	4.6	4.4	4.2	4.0	5.1	5.0	5.1	4.2	3.1	1.2	
Val (V)	B	6.7	9.5	7.6	5.8	8.3	7.8	5.8	4.9	7.4	7.4	8.6	9.1	6.7	5.1	7.3	6.4	7.1	4.7	8.4	8.8	0.11 ns
	A	7.6	5.3	7.4	7.4	5.5	8.1	6.8	7.0	8.1	6.6	8.1	6.9	7.8	8.0	8.3	7.3	7.5	8.2	6.2	7.8	

(B) Bacteria: (1) *Acidimicrobium ferrooxidans* DSM 10331; (2) *Acidothermus cellulolyticus* IIB; (3) *Alicycliphilus denitrificans* K601; (4) *Thermovibrio ammonificans* HB-1; (5) Chloroflexus aggregans DSM 9485; (6) Chloroflexus sp.Y.400.F1; (7) Marinithermus hydrothermalis DSM; (8) *Rhodococcus marinus* DSM 4252; (9) *Thermobifida fuscayx*; (10) *Thermobispora bispora*; (11) *Natranaerobius thermophiles*, (12) *Fervidobacterium nodosum* Rt 17-b1; (13) *Halothermothrix orenii* H168; (14) *Hippea maritime* DSM 10411;(15) *Petrotoga mobilis* SU95; (16) *Thermodesulfatator indicus* DSM 15286; (17) *Thermovirga lienii* DSM 17291; (18) *Caminibacter mediatlanticus* B-2; (19) *Heliobacterium modesticaldum* Ice-1; (20) *Thermovirga lienii* DSM 17291.

(A) Archaea: (1) *Methanohalobium evestigatum* 2-7303; (2) *Methanosaeta thermophila* PT; (3) *Metallosphaera cuprina* Ar-4; (4) *Metallosphaera yellowstonesis* MKI; (5) *Methanosaeta concilli* GP6; (6) *Ignicoccus hospitalis* KIN4/T; (7) *Methanothermobacter thermautotrophicus* Str.Delta H, (8) *Methanobacterium* sp. AL-21; (9) *Archaeoglobus fulgidus* DSM 4304; (10) *Methanothermus fervidus* DSM 2088; (11) *Methanoculleus marisnigri* JR1, (12) *Halorhabdus utahensis* DSM 12940; (13) *Methanoculleus marismortui* ATCC 43049; (14) *Methanosarcina mazei* G01; (15) *Metallosphaera sedula* DSM 5348; (16) *Methanosarcina acetivorans* C2A; (17) *Methnosaeta harundinacea* 6AC; (18) Haloarcula hispanica ATCC 33960; (19) *Methanosarcina barkeri* str.fusaro; (20) *Halorhabdus tiamaea* SARL 4B.

** Significant at a level of 1% of probability (p<0.01), * Significant at a level of 5% of probability (0.01=< p< 0.05), ns= Non significant (p>=0.05)

Table 1.2(b): Comparison between amino acids percent count of bacteria and archaebacteria (thermophiles).

to thermophilic archaeal and bacterial amidases. Positively charged (Arg and Lys) residues were found higher in hyperthermophilic amidases, in comparison to thermophiles. Instability index was also found to be higher for amidases of thermophiles, in comparison to hyperthermophiles.

Aliphatic index is defined also regarded to increase the thermostability of proteins, and was found to be higher in case of hyperthermophiles, when compared to thermophilic bacteria and archea. The values for grand average of hydropathicity (GRAVY) were substantially higher for amidase sequences of hyperthermophiles than that of thermophiles (1.07 fold). The results of amino acid analysis hyperthermophilic and thermophilic (bacteria and archea) amidase are shown in table 1.2 a and b. The comparison of amino acid composition for both groups of amidases (thermophiles/hyperthermophiles) has

revealed that glycine (Gly), one of the simplest amino acid was found to be the predominant residue in hyperthermophiles; its percentage was more 1.01 folds higher in case of hyperthermophiles. The amino acid cysteine (Cys) was considered to be an important parameter in the calculation of extinction co-effieient of proteins, and its content was 1.03 fold higher in hyperthermophiles. The amino acid Ala, Cys, Glu, Gly, Pro, His and Trp (1.02, 1.07, 1.07, 1.10, 1.03 and 1.36 fold) were found to be significantly higher for hyperthermophilic amidases, whereas thermophilic amidases contained Asn, Asp, Gln, Lys, Met, Phe and Ser (1.14, 1.15, 1.31, 1.0, 1.08, 1.06 and 1.07 fold). The comparison of atomic composition revealed that the sulphur content was observed significantly higher in amidases of hyperthermophilic origin.

Discussion

The present study aims to compare the parameters responsible for thermostability of amidases present in thermophiles and hyperthermophiles (bacteria and archaea), on the basis of their amino acid sequences and physiochemical properties. The variation in total number of amino acid residues and molecular weight might be playing some role in thermostability of proteins, in both groups of amidases producing thermophiles/hyperthermophiles (bacteria and archaea). Significant difference for extinction co-efficient and aliphatic index was found between these two groups of microorganisms. Investigation of aliphatic index is important as the hydrophobicity of aliphatic amino acids is a potentially attractive measure of the stability of proteins at high temperature, as well as against denaturants, such as urea and initial scanning of amino acid. Composition of several thermostable proteins invariably showed a high content of Gly, Ala, Glu, and Leu [24]. The distribution of pI of proteins exhibited a clear relationship with subcellular localization, ecology, length of proteins and taxonomy of organisms [25]. The proteins with instability index less than 40 were considered stable, and greater than 40 are considered to be unstable as shown in table 1.1a and b. The present study revealed that greater the number of cystine and more will be the formation of disulphide bonds (S-S bonds), which imparts stability for hyperthermophilic amidases. Thus, the majority of the thermophilic archaea are sulphur dependent [26]. In contrast to sulphur reducing microorganisms, mainly *Sulfolobus, Acidianus*, *Metallosphaera* and *Sulfurisphaera*, undergo highly exothermic reaction of bio-oxidation, which significantly improve the leaching kinetics, accelerate the reaction rate and shorten the leaching cycle because of the inherent advantages of tolerating the high temperature [1,27-29]. Disulfide bonds are an important factor concerning the structural stabilization of intracellular proteins, which are found in oxidizing environment, while in chemically reducing conditions; it favours thiol form of cysteine, which is not thermodynamically responsible for stability of proteins in hyperthermophilic archaea and bacteria [30].

In the present investigations, the structural basis for thermal stability in thermophilic and hyperthermophilic archaeal and bacterial amidases was deduced. Glu and Asp participate in the formation of salt bridges, which provide extra stability to thermal proteins [31,32]. Methionine is sulphur containing amino acids form salt bridges, which are strong bonds, and thus provide stability to proteins. Methionine is an aliphatic amino acid, which shows that by increasing the length of the aliphatic side chain of amino acid in the protein, thermostability increases [33]. Comparative analysis of complete proteomes showed extremely strong bias toward arginine-to-lysine replacement in hyperthermophilic organisms, and overall much greater content of lysine than arginine in hyperthermophiles [34,35].

Proline being one of the responsible residues for the stability,

maintaining a common fold conformation in the polypeptide chain had less conformational freedom, when compared with other amino acids, as the pyrrolidine ring of proline imposes N-C rotation. Glutamine was significantly higher in hyperthermophilic bacteria and archaea,which are responsible for stability of amidases at higher temperature. Some researchers found that the decreased Gln content may minimize deamidation, which results in increased thermostability of proteins. Alanine is the best helix-forming residue [36,37], and is found to be more in case of hyperthermophilic bacteria. Cysteine (Cys) on other hand was found to be more in case of hyperthermophiles, when compared to thermophiles.

In the present studies, aromatic amino acids (tryptophan, tyrosine and phenylalanine) were found to be significantly higher in thermophilic amidases, as thermophilic amidases are found to be more stable than hyperthermophilic amidases. Aromatic amino acids are bulkier and hydrophobic in nature, and form complex with a histidine residue, which provided extra stability to heat-resistant proteins [38,39], and is found to increase in thermophilic amidases. Hydrophobic effect plays a crucial role in protein folding and considered to be a major factor responsible for protein stability [40,41]. In both heat–resistant archaeal and bacterial proteins, mostly Glu and Asp (negatively charged residues) are higher in amount, which participates in the formation of salt bridges, responsible for thermostability of proteins. However, the present investigation revealed that higher amount of Glu was found in hyperthermophilic amidases, in comparison to thermophilic amidases (1.07 fold). Investigations in recent years have indicated that there are disparities in the heat-resistant mechanism between archaeal proteins and bacterial proteins [42,43,31]. Archaeal organisms resist high temperature by substituting non-charged polar amino acids, with Glu, Lys and non-polar amino acids with Ile on protein surfaces [44].

The ratio of salt bridge network is higher in hyperthermophilic archaea than thermophilic archaea. Accordingly, salt bridges significantly contribute to the ability of the organism to withstand high temperatures. These results demonstrate that salt bridges are the most important factor determining the heat resistance in archaeal or bacterial proteins. Previous studies have shown that salt bridge networks are major factors that affect the thermostability of protein [2,45-47]. In addition, there was high Ser and Thr content in hyperthermophilic amidases, in comparison to thermophilic or mesophilic amidases [48]. Ser and Thr are more flexible, so increasing flexibility of proteins to withstand the extreme heat. Due to higher number of sulphur atoms in hyperthermophiles, more salt bridges can be formed, which increase the thermostability of proteins, in comparison to thermophiles [49].

Conclusion

A number of amino acid sequences of thermophilic/hyperthermophilic amidases were analyzed *In silico*, for some of the physiochemical properties. The hyperthermophilic amidases are found to be more stable than thermophilic amidases. The hyperthermophilic amidases and thermophilic amidases show significant difference in amino acid residues, molecular weight, and percent count of some amino acids. Amino acids which are responsible for thermostability of these amidases such as Cys, Glu, Ala, Arg, Pro, Tyr, Trp, His and Val, which have been found to be significantly higher in case of hyperthermophilic amidases. The results of present investigation will be quite useful in the prediction of extent of thermostability among amidases of thermophiles and hyperthermophiles of various forms of bacteria.

References

1. Soong CL, Ogawa J, Shimizu S (2000) A novel amidase (half-amidase) for half-

half-amide hydrolysis involved in the bacterial metabolism of cyclic imides. Appl Environ Microbiol 66: 1947-1952.

2. Pace CN, Fu H, Fryar KL, Landua J, Trevino SR, et al. (2011) Contribution of hydrophobic interactions to protein stability. J Mol Biol 408: 514-528.

3. Banerjee A, Sharma R, Banerjee UC (2002) The nitrile-degrading enzymes: current status and future prospects. Appl Microbiol Biotechnol 60: 33-44.

4. Madhavan NK, Roopesh K, Chacko S, Pandey A (2005) Comparative study of amidase production by free and immobilized *Escherichia coli* cells. Appl Biochem Biotechnol 120: 97-108.

5. Bjellqvist B, Hughes GJ, Pasquali C, Paquet N, Ravier F, et al. (1993) The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences. Electrophoresis 14: 1023-1031.

6. Berezovsky IN, Chen WW, Choi PJ, Shakhnovich EI (2005) Entropic stabilization of proteins and its proteomic consequences. PLoS Comput Biol 1: e47.

7. Fournand D, Arnaud A (2001) Aliphatic and enantioselective amidases: from hydrolysis to acyl transfer activity. J Appl Microbiol 91: 381-393.

8. Ding Y, Cai Y, Han Y, Zhao B (2012) Comparison of the structural basis for thermal stability between archaeal and bacterial proteins. Extremophiles 16: 67-78.

9. Kyte J, Doolittle RF (1982) A simple method for displaying the hydropathic character of a protein. J Mol Biol 157: 105-132.

10. Nagasawa T, Yamada H (1989) Microbial transformations of nitriles. Trends Biotechnol 7: 153-158.

11. Orell A, Navarro CA, Arancibia R, Mobarec JC, Jerez CA (2010) Life in blue: copper resistance mechanisms of bacteria and archaea used in industrial biomining of minerals. Biotechnol Adv 28: 839-848.

12. Berezovsky IN, Shakhnovich EI (2005) Physics and evolution of thermophilic adaptation. Proc Natl Acad Sci USA 102: 12742-12747.

13. Hakamada Y, Hatada Y, Ozawa T, Ozaki K, Kobayashi T, et al. (2001) Identification of thermostabilizing residues in a Bacillus alkaline cellulase by construction of chimeras from mesophilic and thermostable enzymes and site-directed mutagenesis. FEMS Microbial Lett 195: 67-72.

14. Wu S, Fallon RD, Payne MS (1998) Cloning and nucleotide sequences of amidase gene from *Pseudomonas putida*. DNA Cell Biol 17: 915-920.

15. Andrade J, Karmali A, Carrondo MA, Frazão C (2007) Structure of amidase from Pseudomonas aeruginosa showing a trapped acyl transfer reaction intermediate state. J Bio Chem 282: 19598-19605.

16. Mizuguchi K, Sele M, Cubellis MV (2007) Environment specific substitution tables for thermophilic proteins. BMC Bioinformatics 8: S15.

17. Kumar S, Bansal M (1998) Geometrical and sequence characteristics of alpha-helices in globular proteins. Biophys J 75: 1935-1944.

18. Dill KA (1990) Dominant forces in protein folding. Biochemistry 29: 7133-7155.

19. Gupta V, Gaind S, Verma PK, Sood N, Srivastava AK (2010) Purification and characterization of intracellular nitrilases from *Rhodococcus* sp.- Potential role of periplasmic nitrilase. Afr J Microbiol Res 4: 1148-1153.

20. Holmes LB (1996) Hydroxamic acid: a potential human teratogen that could be recommended to treat ureaplasma. Teratology 53: 227-229.

21. Gill SC, von Hippel PH (1989) Calculation of protein extinction coefficients from amino acid sequence data. Anal Biochem 182: 319-326.

22. Kiraga J, Mackiewicz P, Mackiewicz D, Kowalczuk M, Biecek P, et al. (2007) The relationships between the isoelectric point and: length of proteins, taxonomy and ecology of organisms. BMC Genomics 8:163.

23. Fournand D, Bigey F, Arnaud A (1998) Acyl transfer activity of an amidase from *Rhodococcus* sp. strain R312: formation of a wide range of hydroxamic acids. Appl Environ Microbiol 64: 2844-2852.

24. Zhao J, Huang GR, Zhang MN, Chen WW, Jiang JX (2011) Amino acid composition, molecular weight distribution and antioxidant stability of shrimp processing byproduct hydrolysate. Am J Food Technol 6: 904-913.

25. Jorda J, Yeates TO (2011) Widespread disulfide bonding in proteins from thermophilic archaea. Archaea 409156.

26. Zhu W, Xia JI, Yang Y, Nie ZY, Zheng L, et al. (2011) Sulphur oxidation activities of pure and mixed thermophiles and sulphur speciation in bioleaching of chalcopyrite. Bioresour Technol 102: 3877-3882.

27. Buranasilp K, Charoenpanich J (2011) Biodegradation of acrylamide by *Enterobacter aerogenes* isolated from wastewater in Thailand. J Environ Sci (China) 23: 396-403.

28. Rathi PC, Radestock S, Gohlke H (2012) Thermostabilizing mutations preferentially occur at structural weak spots with a high mutation ratio. J Biotechnol 159: 135-144.

29. Niehaus F, Bertoldo C, Kahler M, Antranikian G (1999) Extremophiles as a source of novel enzymes for industrial application. Appl Microbial Biotechnol 51: 711-729.

30. Guruprasad K, Reddy BVB, Pandit MW (1990) Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting *in vivo* stability of a protein from its primary sequence. Protein Eng 4: 155-161.

31. D'Hugues P, Foucher S, Galle-Cavalloni P, Morin D (2002) Continuous bioleaching of chalcopyrite using a novel extremely thermophilic mixed culture. International Journal of Mineral Processing 66: 107-119.

32. Das R, Gerstein M (2000) The stability of thermophilic proteins: a study based on comprehensive genome comparison. Funct Integr Genomics 1: 76-88.

33. Soundrarajan N, Edwardraja S, Lee SG, Yun H, Aygadurai N (2012) Enhancing the productivity of soluble green fluorescent protein through methionine-residue specific consensus approach. Afr J Biotechnol 11: 1059-1064.

34. Best RB, De Sancho D, Mittal J (2012) Residue-specific α-helix propensities from molecular simulation. Biophys J 102: 1462-1467.

35. Acevedo F, Gentina JC (2007) Bioreactor design fundamentals and their application to gold mining. In: Microbial processing of metal sulfides, Donati ER, Sand W (Eds) Springer, Dordrecht, 151-168.

36. Kar K, Kishore N (2007) Enhancement of thermal stability and inhibition of protein aggregation by osmolytic effect of hydroxyproline. Biopolymers 87: 339-351.

37. Alsop E, Silver M, Livesay DR (2003) Optimized electrostatic surfaces parallel increased thermostability: a structural bioinformatic analysis. Protein Eng 16: 871-874.

38. Kumar N, Bhalla TC (2011) *In silico* analysis of amino acid sequences in relation to specificity and physiochemical properties of some aliphatic amidases and kynurenine formamidases. Journal of Bioinformatics and Sequence Analysis 3: 116-123.

39. Pace HC, Brenner C (2002) The nitrilase superfamily: classification, structure and function. Genome Biol 2.

40. Chan CH, Yu TH, Wong KB (2011) Stabilizing salt-bridge enhances protein thermostability by reducing the heat capacity change of unfolding. PLoS ONE 6: e21624.

41. Nikookar E, Badie K, Sadeghi M (2012) A novel statistical method for thermostable proteins discrimination. International Journal of Research in Computer Science 2: 1-5.

42. Trivedi S, Gehlot HS, Rao SR (2006) Protein thermostability in Archaea and Eubacteria. Genet Mol Res 5: 816-827.

43. Zaparty M, Esser D, Gertig S, Haferkamp P, Kouril T, et al. (2010) "Hot standards" for the thermoacidophilic archaeon *Sulfolobus solfataricus*. Extremophiles 14: 119-142.

44. Loewenthal R, Sancho J, Fersht AR (1992) Histidine-aromatic interaction in barnase. Elevation of histidine pKa and contribution to protein stability. J Mol Biol 224: 759-770.

45. Chacko S, Ramteke PW, John SA (2009) Amidase from plant growth promoting rhizobium. J Bacteriol 1: 46-50.

46. Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, et al. (2002) Alpha-synuclien is phosphorylated in synucleinopathy lesions. Nat Cell Biol 4: 160-164.

47. Brock TD, Brock KM, Belly RT, Weiss RL (1972) *Sulfolobus*: A new genus of sulfur-oxidizing bacteria living at low pH and high temperature. Arch Mikrobiol 84: 54-68.

48. Nawaz MS, Khan AA, Bhattacharayya D, Siitonen PH, Cerniglia CE (1996) Physical, biochemical, and immunological characterization of a thermostable amidase from *Klebsiella pneumoniae* NCTR 1. J Bacteriol 178: 2397- 2401.

49. Posner M, Kiss AJ, Skiba J, Drossman A, Dolinska MB, et al. (2012) Functional validation of hydrophobic adaptation to physiological temperature in the small heat shock protein αA- crystallin. PLoS One 7: e34438.