

Computational Analysis of Amino Acid Sequences of Hydantoinase and Allantoinase from Selected Bacteria

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ABSTRACT: Development of computational tools for the analysis of biological data plays a significant role especially in the field of proteomics. Present work showed computational analysis of amino acid sequences of hydantoinase and allantoinase for their physicochemical properties and substrate specificity. Composition and total number of amino acid clearly indicated the difference between these two subclass of cyclic amino hydrolases. Negatively charged residues were less in allantoinase as well as its instability index was found to be lower, which showed stability. Multiple sequence alignment and statistical analysis proved clear distinction at several positions and shows evolutionary relationship among them. Catalytic triad was the conserved triad in both the hydrolases. Result obtained from this computational study have significance as it may play role for further selection of hydantoinase and allantoinase from reported or from genome.

KEYWORDS: Hydantoinase, allantoinase, amino acids, substrate specificity, multiple alignments

I. INTRODUCTION

Amidases (EC 3.5.1 and 3.5.2) a subclass of acylamideamidohydrolases are mainly involved in nitrogen exchange, which leads to the production of respective acid and release ammonia. These complex enzyme, are of primary interest for large scale production of acrylamide, nicotinamide, nicotinic acid, benzoic acid and acrylic acid in industry [1]. Various applications in pharmaceutical product and bioremediation of hazardous amide leads to the in-depth computation study of this cyclic amidases. Diversity in this class of cyclic amide-metabolizing systems plays an important role in various pyrimidine and purine; amino acid and antibiotic metabolism [2, 3]. Amidases (EC 3.5.1.4) are ubiquitous enzymes in the living world and can be divided into two types. They include hydantoinase (hydrolyzing short and mid chain aliphatic amides) and broad range amidases (hydrolyzing various substrates, some acrylamides, α -aminoamides, α -hydroxyamides and allantoinase). Amidases can be assigned to phylogenetically

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

unrelated families on the basis of amino acid sequence. One family comprises the allantoinase group (3.5.1.9), and other may be termed as hydantoinase on the basis of substrate specificity. Amidases turned out to be efficient tools for the synthesis of various compounds. Many microbial amidases have been purified and characterized. Many microbial genera for eg. *Rhodococcus*, *Mycobacterium*, *Pseudomonas*, *Bacillus*, *Micrococcus*, *Blastobacter*, *Arthrobacter*, *Alcaligenes*, *Geobacillus*, and *Methylophilus* are reported for these enzyme production [4, 5]. Physiochemical properties like. number of amino acid residues, molecular mass, theoretical pI, amino acid composition, negatively charged residues (Asp+Glu), positively charged residues (Arg+Lys), atomic composition, total number of atoms, extinction coefficients ($M^{-1}.cm^{-1}$) at 280 nm, instability index/aliphatic index, grand average hydropathicity (GRAVY) plays critical role in sequence base analysis [6]. Amidases are still not sufficiently investigated and their classification is not definitely formulated. Rare integrity is seen in these enzymes with different structural organization, mechanism, and catalytic properties based on the substrate specific classification [7]. The spatial organization of these proteins remains unknown and systematic comparative analysis of allantoinase and hydantoinase is far from complete. The unique approach taken is to study the sequence analysis and these properties of the sequence can then be used to predict their substrate specificity. Many of the amidase are discovered till date but few of the amidases are characterized in detailed, which play very important role in the development of the computational tool. Aliphatic amidase were screened and characterized by *in vitro* as well as *in silico* method and the results of this revealed the nature of the amidase [8]. Purification of thermophilic amidases were carried out with respect to the physico-chemical characteristics and stability. Hydrophathy values were also studied in detailed to describe the nature of the enzyme [9, 10]. Cyclic amidases were checked for the substrate specificity and also checked for the amidase activity and acyl transferase activity. Few of the researches focus on the substrate specificity of the enzyme with respect to physico-chemical characterization using statistical tools [11].

II. METHOS AND MATERIALS

Data collection and analysis: All selected sequences of allantoinase and hydantoinase of few microorganisms were obtained from the SwissProt (ExPASy proteomic server) database (Table 1a and b), which were having experimentally proved substrate specificity as well as complete nucleotide sequences. Physico-chemical data were generated from the ProtParam proteomic server of Swiss Institute of Bioinformatics (SIB).

Table 1a. Name of microorganism with the SwissProt accession number for Hydantoinase.

No.	Accession No.	Abbreviation	Name of Microorganism	Amino Acid
1	Q9I676	HYDA_PSEAE	<i>Pseudomonas aeruginosa</i>	479
2	Q45515	HYDA_GEOSE	<i>Geobacillus stearothermophilus</i>	471
3	Q59699	HYDA_PSEPU	<i>Pseudomonas putida</i>	495
4	P81006	HYDL_ARTAU	<i>Arthrobacter aureus</i>	458
5	O69809	HYDA_STRCO	<i>Streptomyces coelicolor</i>	467
6	Q8VTT5	HYDA_RALPI	<i>Ralstonia pickettii</i>	457
7	B7LF59	PHYDA_ECO55	<i>Escherichia coli</i> (strain 55989 / EAEC)	461
8	Q1R7F8	PHYDA_ECOUT	<i>Escherichia coli</i> (strain UTI89 / UPEC)	461
9	Q31WE3	PHYDA_SHIBS	<i>Shigella boydii</i> serotype 4	461
10	Q44184	HYDA_RHIRD	<i>Rhizobium radiobacter</i>	457

Sequences were used for analysis like Blastp (Protein BLAST), which was performed to study the homology among the various amidase sequences and 20 sequences belonging to same E.C. group (E.C.3.5.2.) of microbial cyclic amidases were selected (Table 1a and b).

International Journal of Innovative Research in Science, Engineering and Technology

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Table 1b.Name of microorganism with the SwissProt accession number for allantoinase.

No.	Accession No.	Abbreviation	Name of Microorganism	Amino Acid
1	P77671	ALLB_ECOLI	<i>Escherichia coli</i>	453
2	Q9KAH8	ALLB_BACHD	<i>Bacillus halodurans</i>	438
3	Q65LN0	ALLB_BACLD	<i>Bacillus licheniformis</i>	454
4	O32137	ALLB_BACSU	<i>Bacillus subtilis</i>	446
5	Q8XGS6	ALLB_SALTI	<i>Salmonella typhi</i>	453
6	Q5WBJ6	ALLB_BACSK	<i>Bacillus clausii</i>	448
7	A7Z8F5	ALLB_BACA2	<i>Bacillus amyloliquefaciens</i> subsp. <i>plantarum</i>	454
8	Q1J391	ALLB_DEIGD	<i>Deinococcus geothermalis</i>	449
9	Q9RV76	ALLB_DEIRA	<i>Deinococcus radiodurans</i>	448
10	Q24PT9	ALLB_DESHY	<i>Desulfitobacterium hafniense</i>	449

The extinction coefficient of various amidases was calculated using the following equation [12]

$$E(\text{Prot}) = \text{Numb}(\text{Tyr}) * \text{Ext}(\text{Tyr}) + \text{Numb}(\text{Trp}) * \text{Ext}(\text{Trp}) + \text{Numb}(\text{Cystine}) * \text{Ext}(\text{Cystine})$$

The values of aliphatic index of various amidase sequences were obtained using ProtParam (ExPASy) tool. The instability index and grand average of hydropathicity (GRAVY) were estimated following the method of respectively. Clustal W was used for multiple sequence alignment [13, 14].

Statistical analysis: An analysis of variance (ANOVA) was conducted on various physico-chemical parameter variables for each study with the statistical packages SPSS (v.20) and Excel. F-tests were used to determine the statistical significance.

III. RESULTS AND DISCUSSION

Results of the in-depth analysis was used to find difference and similarity between the physico-chemical properties of twenty amino acid sequences of hydantoinase and allantoinase (ten each) as illustrated in Table 2a. The total number of amino acid residues in these cyclic amidases differed substantially as allantoinase have lesser number of amino acid residues ranging between 438 to 454 amino acid; whereas, hydantoinase ranging from 457 to 495. The molecular weight of allantoinase ranged between 48331 to 49886.8 and that of hydantoinase ranged from 4959.6 to 53506.5 for the selected sequences. Theoretical pI varied from 5.08 to 6.04 in case of allantoinase and it was found to be 4.91 to 6.18 for hydantoinase. It was further found that the average pI value of hydantoinase was insignificantly higher than that of allantoinase (Table 2a).

Negative charge residues (Asp and Glu) were found to be substantially lower in hydantoinase that is 58.4 and 62 in allantoinase. Moreover, significant difference was not found between allantoinase (47.2) and hydantoinase (45.4) for positively charged amino acid residues. Instability indices of these two groups of cyclic amidases indicated that hydantoinase (34.208) were more stable as compared to allantoinase (38.684) as shown in Table 2a. The aliphatic index and values for grand average hydropathicity (GRAVY) were found to be 1.01 and 1.11 fold higher respectively in allantoinase than that of hydantoinase.

International Journal of Innovative Research in Science, Engineering and Technology

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Table 2a: Comparative analysis of physico-chemical properties of allantoinase and hydantoinase. Note: SS= Substrate specificity;HYD =Hydantoinase; ALL= Allantoinase; ** = Significant at a level of 1% probability

Parameters	SS	Microorganisms										Mean	Stdev	F value	P value
		1	2	3	4	5	6	7	8	9	10				
No. of amino acids	HYD	479	471	495	458	467	457	461	461	461	457	466.7	12.16598	17.831**	0.0005
	ALL	453	438	454	446	453	448	454	449	448	449	449.2	4.871687		
MW	HYD	52212	51725	53506.5	49596.6	50748.1	49931.9	51057.2	50971.1	51043.1	49939.7	51073.1	1179.815	23.76**	0.00012
	ALL	49601.6	48542.3	49488.2	48331	49886.8	48710	49454.3	48998.3	48558.7	49228	49079.9	528.8431		
Theoretical pI	HYD	5.78	5.13	6.09	4.91	5.29	5.68	6.18	6.1	6.18	5.46	5.68	0.465522	1.518	0.233
	ALL	5.22	5.9	5.16	5.22	5.22	6.04	5.63	5.35	5.08	5.73	5.455	0.341703		
Total - residue (Asp+Glu)	HYD	55	67	53	66	63	63	51	52	51	63	58.4	6.552353	1.9234	0.183
	ALL	65	55	68	62	67	55	63	67	61	57	62	4.944132		
Total + residue (Arg+Lys)	HYD	41	49	44	44	42	52	44	44	44	50	45.4	3.627059	1.215	0.284
	ALL	48	45	52	44	48	49	51	50	40	45	47.2	3.675746		
Extinction Coeff. Cys	HYD	67755	50225	64775	28670	44725	44600	43485	421120	43485	44600	85344	118508.2	1.452	0.244
	ALL	36160	41870	37860	40505	37650	40630	40505	43555	41035	41995	40176.5	2276.188		
Instability index	HYD	29.02	23.64	36.9	38.32	29.86	26.09	44.28	42.23	43.33	28.41	34.208	7.675254	2.874	0.107
	ALL	40.91	41.33	35.66	39.06	38.07	41.67	42.77	37.44	38.3	31.7	38.684	3.28784		
Aliphatic index	HYD	83.38	88.79	81.45	90.9	80.06	85.8	89.52	91	89.31	83.89	86.41	4.024953	0.214	0.648
	ALL	86.09	87.26	88.92	84.26	89.89	87.81	78.99	88.91	90.42	89.29	87.184	3.423904		
Gravy	HYD	0.171	0.152	0.178	0.123	0.196	0.163	0.166	0.128	0.167	0.19	0.1634	0.02375	0.863	0.365
	ALL	0.081	0.224	0.217	0.091	0.243	0.183	0.194	0.236	0.201	0.147	0.1817	0.057579		

Results of amino acid analysis of two types of amidases are shown in Table 2b. These enzymes contained all the common amino acids. The comparison of the amino acid composition of the allantoinase and hydantoinase has shown that alanine (Ala), glycine (Gly) and leucine (Leu) is higher in both the cyclic amidase. The amino acid cysteine (Cys) is considered to be an important parameter in the calculation of extinction coefficient of proteins and its content was 2.12 fold higher in hydantoinase as compared to allantoinase. The amino acid Glu, Ile, Lys, Phe, and Ser were (1.20, 1.00, 1.32, 1.02 and 1.04 respectively) non-significantly higher in allantoinase while in case of hydantoinase enzyme

International Journal of Innovative Research in Science, Engineering and Technology

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the amino acid, Arg, Asn, Asp, Gln, His, Met, Pro, Thr, Tyr and Val were (1.09, 1.20, 1.10, 1.13, 1.12, 1.19, 1.04, 1.12, 1.13, and 1.07 respectively) non-significantly higher. The amino acid glycine (G) was found to be significantly higher in allantoinase as oppose to amino acid Trp (W) which was very low in both the cyclic amidases. Glutamate and Histidine were found to be higher and conserved at specific position to 205-283 in both the cyclic amidases under study. The multiple sequence alignment of hydantoinase and allantoinase showed some unique differences for the position specific presence of some amino acids (Fig. 1). The present work has contributed to conclude that there is a clear difference between allantoinase and hydantoinase in terms of position specific presence of certain amino acid.

Table 2b: Comparison between amino acids presents in allantoinase and hydantoinase. Note: SS = Substrate specificity; HYD = Hydantoinase; ALL = Allantoinase; * = Significant at a level of 5% probability

Amino acid composition	SS	Microorganism										Mean	F value	P value
		1	2	3	4	5	6	7	8	9	10			
Ala (A)	HYD	11.9	7.9	11.1	8.7	12	10.5	8.2	8.2	8.2	10.3	9.7	0.05	0.825
	ALL	8.4	6.6	8.1	11.2	8.2	10.6	12.1	10.9	10.5	8.7	9.53		
Arg (R)	HYD	6.5	3	6.7	3.7	6.6	5.3	6.9	6.9	6.9	5.5	5.8	0.523	0.478
	ALL	4.9	4.8	3.5	4.3	4.9	5.6	4.6	9.6	6.5	4.2	5.29		
Asn (N)	HYD	2.5	2.8	2.2	2.8	2.1	2.4	3.7	3.3	3.7	2.4	2.79	0.33	0.572
	ALL	2.9	2.7	3.7	1.3	3.3	3.3	1.5	0.9	1.8	4.2	2.56		
Asp (D)	HYD	5	7	5.5	7	5.6	7.2	6.1	6.3	6.1	7.7	6.35	0.862	0.365
	ALL	7.3	5.5	1	5.8	7.1	5.4	5.1	6.5	6.5	7.3	5.75		
Cys (C)	HYD	1.3	1.5	1.4	1.1	1.5	1.1	2.4	2.6	2.4	1.1	1.64	1.314	0.267
	ALL	2.6	1.8	1.8	2.2	2.6	2.9	2.2	0.7	0.7	2.4	1.99		
Gln (Q)	HYD	3.8	3.4	3.8	4.4	3	2	4.6	4.6	4.6	2.4	3.66	1.121	0.284
	ALL	4.2	3.7	2.6	2.7	4	3.3	2.6	2.2	4.2	2.9	3.24		
Glu (E)	HYD	6.5	7.2	5.3	7.4	7.9	6.6	5	5	5	6.1	6.2	7.165*	0.016
	ALL	7.1	7.1	7.9	8.1	7.7	6.9	8.8	8.5	7.1	5.3	7.45		
Gly (G)	HYD	9.4	9.6	9.9	9.6	9.9	8.8	8.9	8.9	8.9	9	9.29	0.084	0.775
	ALL	9.7	8.2	9.9	8.5	9.5	8.9	9.3	10.5	10.3	8.9	9.37		
His (H)	HYD	4.2	2.3	3.8	2.2	3.9	2.8	3.7	3.7	3.7	2.6	3.29	1.458	0.243
	ALL	2.6	3.7	2	2.9	3.1	2.5	2.9	3.1	3.1	3.6	2.95		
Ile (I)	HYD	4.2	6.4	4.4	6.1	4.9	5.9	6.7	6.9	6.5	6.1	5.81	0.001	0.965
	ALL	6.4	6.6	9.3	6.3	6.8	5.4	5.5	3.1	2.5	6.5	5.84		
Leu (L)	HYD	9.2	8.1	8.5	8.3	6.6	6.6	9.8	9.8	9.8	6.6	8.33	0.308	0.585
	ALL	7.3	9.1	5.7	7	7.5	9.8	7.7	11.6	13.4	8.9	8.8		
Lys (K)	HYD	2.1	7.4	2.2	5.9	2.4	6.1	2.6	2.6	2.6	5.5	3.94	2.2114	0.154
	ALL	5.7	5.5	7.9	5.6	5.7	5.4	6.6	1.6	2.5	5.8	5.23		
Met (M)	HYD	2.3	1.9	2.4	3.5	2.8	2.8	2.2	2.2	2.2	2.8	2.51	2.475	0.133
	ALL	2.9	2.3	1.5	2.9	2.9	1.8	2.4	1.3	1.3	1.8	2.11		
Phe (F)	HYD	3.8	4.5	3.4	3.5	3.4	3.5	4.1	4.1	4.1	3.7	3.81	0.156	0.697
	ALL	4	3.9	3.7	3.8	3.5	3.1	5.1	3.8	3.8	4.2	3.89		
Pro (P)	HYD	5.2	4	5.7	4.6	4.3	3.9	5.2	5	5.2	4.4	4.75	0.547	0.468
	ALL	4.2	4.6	3.7	4.3	4.2	5.4	4.4	5.3	5.4	4	4.55		
Ser (S)	HYD	5.6	3.4	6.5	5.2	3.9	4.2	5	5.4	5	4.2	4.84	0.288	0.598
	ALL	4.2	6.8	5.3	6.7	3.3	5.1	5.5	4.5	4.7	4.7	5.08		
Thr (T)	HYD	5.6	7	6.5	4.6	7.3	7	5.2	4.8	5.2	7.2	6.04	2.438	0.136
	ALL	4.4	6.6	4.6	5.4	5.1	4.9	4.8	6	6.5	5.6	5.39		
Trp (W)	HYD	1.9	1.1	1.8	0.7	0.9	0.9	0.9	0.9	0.9	0.9	1.09	1.155	0.296
	ALL	0.9	0.9	0.7	0.9	0.9	0.9	0.9	1.3	1.1	0.9	0.94		
Tyr (Y)	HYD	2.5	3.2	2	1.7	3.2	3.3	3	2.8	3	3.3	2.8	1.808	0.195
	ALL	2	3	3.1	2.7	2.2	2.7	2.6	1.6	2	2.9	2.48		
Val (V)	HYD	6.7	8.5	6.9	9	7.9	9.2	5.9	6.1	6.1	8.3	7.46	1.084	0.311
	ALL	8.4	6.6	7.7	7.4	7.5	6.2	5.3	7.1	6.2	7.1	6.95		

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

Position of amino acid glycine (57, 63, 95, 96, 158, 360, 405, 410, 442, and 455), proline (62, 312), glutamate (66, 102, 131, 337, 412), histidine (68, 70, 203, 260), lysine (163, 404) and arginine (314) is conserved in both the cyclic amidase. Amino acid histidine and tyrosine at position 227 is conserved in hydantoinase and allantoinase respectively. In hydantoinase asparagine is conserved at position 365 whereas allantoinase contains glutamine at the same position. WNA and GGI triad were found to be conserved for both the cyclic amides.

Q91676 HYDA_PSEAE	1	---MSLLRGATVTHEESYRADVLCAAGLTQATGE---NLETP---SGCDVLDGGQYL	51
Q45515 HYDA_GEOSE	1	---MTKLKNGITVATDIYEADLLIQDGLAVIGR---NLDES---GAEDVATSCYV	50
Q59699 HYDA_PSEPU	1	---MSLLRGATVTHEESYPADVLCDGLRAATGP---NLEPP---TDCEILDGSGQYL	51
P81006 HYDL_ARTAU	1	---MFDVIVKNCRLVSSDGITEADILVKDGLVAASDQT---SOLVE---ASRTIDAGKQV	52
Q69809 HYDA_STRCO	1	---MSRTVIRGGLVTSASDEIHADVLIEDGRVAALAA---TGTAAEAFTAEVTDASQYV	57
Q8VTT5 HYDA_RALPI	1	---MDIILKNGITVADGISRADLGKDGKLTQGG---ALGPA---ERTIDAGRYV	49
B7LF59 PHYDA_ECOS5	1	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	51
Q1R7F8 PHYDA_ECOUT	1	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	51
Q31NE3 PHYDA_SHIBS	1	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	51
Q44184 HYDA_RHIRD	1	---MDIILKNGITVADGISPADLGKDGKLTQGG---TFGPA---GRTIDAGRYV	49
P77671 ALLB_ECOLI	1	---MSFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	51
Q9KAH8 ALLB_BACHD	1	---MKRFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	51
Q651N0 ALLB_BACLD	1	---MNFDSILKNGITVLENGEVEVGIKDGKLAAGQ---DL-GT---SAKIDAKSTV	51
Q32137 ALLB_BACSU	1	---MAYDVRKGAALTPDGVIEADIVVQGVVAETGS---DTEAS---GTETIDAGRYV	52
Q8XG56 ALLB_SALTI	1	---MSFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	51
A7Z8F5 ALLB_BACA2	1	---MAYDVRKGAALTPDGVIEADIVVQGVVAETGS---DTEAS---GTETIDAGRYV	52
Q1J391 ALLB_DEIGD	1	---MYDVLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	51
Q9RV76 ALLB_DEIRA	1	---MSLDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	51
Q24PT9 ALLB_DESHY	1	---MSHYDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	51
Q5WB36 ALLB_BACSK	1	---MSLDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	51
Q91676 HYDA_PSEAE	52	---MSLLRGATVTHEESYRADVLCAAGLTQATGE---NLETP---SGCDVLDGGQYL	51
Q45515 HYDA_GEOSE	51	---MTKLKNGITVATDIYEADLLIQDGLAVIGR---NLDES---GAEDVATSCYV	50
Q59699 HYDA_PSEPU	52	---MSLLRGATVTHEESYPADVLCDGLRAATGP---NLEPP---TDCEILDGSGQYL	51
P81006 HYDL_ARTAU	51	---MFDVIVKNCRLVSSDGITEADILVKDGLVAASDQT---SOLVE---ASRTIDAGKQV	52
Q69809 HYDA_STRCO	57	---MSRTVIRGGLVTSASDEIHADVLIEDGRVAALAA---TGTAAEAFTAEVTDASQYV	57
Q8VTT5 HYDA_RALPI	49	---MDIILKNGITVADGISRADLGKDGKLTQGG---ALGPA---ERTIDAGRYV	49
B7LF59 PHYDA_ECOS5	51	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	51
Q1R7F8 PHYDA_ECOUT	51	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	51
Q31NE3 PHYDA_SHIBS	51	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	51
Q44184 HYDA_RHIRD	49	---MDIILKNGITVADGISPADLGKDGKLTQGG---TFGPA---GRTIDAGRYV	49
P77671 ALLB_ECOLI	51	---MSFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	51
Q9KAH8 ALLB_BACHD	51	---MKRFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	51
Q651N0 ALLB_BACLD	51	---MNFDSILKNGITVLENGEVEVGIKDGKLAAGQ---DL-GT---SAKIDAKSTV	51
Q32137 ALLB_BACSU	52	---MAYDVRKGAALTPDGVIEADIVVQGVVAETGS---DTEAS---GTETIDAGRYV	52
Q8XG56 ALLB_SALTI	51	---MSFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	51
A7Z8F5 ALLB_BACA2	52	---MAYDVRKGAALTPDGVIEADIVVQGVVAETGS---DTEAS---GTETIDAGRYV	52
Q1J391 ALLB_DEIGD	51	---MYDVLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	51
Q9RV76 ALLB_DEIRA	51	---MSLDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	51
Q24PT9 ALLB_DESHY	51	---MSHYDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	51
Q5WB36 ALLB_BACSK	51	---MSLDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	51
Q91676 HYDA_PSEAE	109	---MSLLRGATVTHEESYRADVLCAAGLTQATGE---NLETP---SGCDVLDGGQYL	109
Q45515 HYDA_GEOSE	108	---MTKLKNGITVATDIYEADLLIQDGLAVIGR---NLDES---GAEDVATSCYV	108
Q59699 HYDA_PSEPU	109	---MSLLRGATVTHEESYPADVLCDGLRAATGP---NLEPP---TDCEILDGSGQYL	109
P81006 HYDL_ARTAU	109	---MFDVIVKNCRLVSSDGITEADILVKDGLVAASDQT---SOLVE---ASRTIDAGKQV	109
Q69809 HYDA_STRCO	115	---MSRTVIRGGLVTSASDEIHADVLIEDGRVAALAA---TGTAAEAFTAEVTDASQYV	115
Q8VTT5 HYDA_RALPI	107	---MDIILKNGITVADGISRADLGKDGKLTQGG---ALGPA---ERTIDAGRYV	107
B7LF59 PHYDA_ECOS5	110	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	110
Q1R7F8 PHYDA_ECOUT	110	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	110
Q31NE3 PHYDA_SHIBS	110	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	110
Q44184 HYDA_RHIRD	107	---MDIILKNGITVADGISPADLGKDGKLTQGG---TFGPA---GRTIDAGRYV	107
P77671 ALLB_ECOLI	108	---MSFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	108
Q9KAH8 ALLB_BACHD	112	---MKRFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	112
Q651N0 ALLB_BACLD	111	---MNFDSILKNGITVLENGEVEVGIKDGKLAAGQ---DL-GT---SAKIDAKSTV	111
Q32137 ALLB_BACSU	109	---MAYDVRKGAALTPDGVIEADIVVQGVVAETGS---DTEAS---GTETIDAGRYV	109
Q8XG56 ALLB_SALTI	108	---MSFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	108
A7Z8F5 ALLB_BACA2	109	---MAYDVRKGAALTPDGVIEADIVVQGVVAETGS---DTEAS---GTETIDAGRYV	109
Q1J391 ALLB_DEIGD	108	---MYDVLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	108
Q9RV76 ALLB_DEIRA	109	---MSLDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	109
Q24PT9 ALLB_DESHY	110	---MSHYDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	110
Q5WB36 ALLB_BACSK	108	---MSLDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	108
Q91676 HYDA_PSEAE	160	---MSLLRGATVTHEESYRADVLCAAGLTQATGE---NLETP---SGCDVLDGGQYL	160
Q45515 HYDA_GEOSE	160	---MTKLKNGITVATDIYEADLLIQDGLAVIGR---NLDES---GAEDVATSCYV	160
Q59699 HYDA_PSEPU	160	---MSLLRGATVTHEESYPADVLCDGLRAATGP---NLEPP---TDCEILDGSGQYL	160
P81006 HYDL_ARTAU	160	---MFDVIVKNCRLVSSDGITEADILVKDGLVAASDQT---SOLVE---ASRTIDAGKQV	160
Q69809 HYDA_STRCO	166	---MSRTVIRGGLVTSASDEIHADVLIEDGRVAALAA---TGTAAEAFTAEVTDASQYV	166
Q8VTT5 HYDA_RALPI	158	---MDIILKNGITVADGISRADLGKDGKLTQGG---ALGPA---ERTIDAGRYV	158
B7LF59 PHYDA_ECOS5	159	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	159
Q1R7F8 PHYDA_ECOUT	159	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	159
Q31NE3 PHYDA_SHIBS	159	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	159
Q44184 HYDA_RHIRD	158	---MDIILKNGITVADGISPADLGKDGKLTQGG---TFGPA---GRTIDAGRYV	158
P77671 ALLB_ECOLI	163	---MSFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	163
Q9KAH8 ALLB_BACHD	163	---MKRFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	163
Q651N0 ALLB_BACLD	163	---MNFDSILKNGITVLENGEVEVGIKDGKLAAGQ---DL-GT---SAKIDAKSTV	163
Q32137 ALLB_BACSU	167	---MAYDVRKGAALTPDGVIEADIVVQGVVAETGS---DTEAS---GTETIDAGRYV	167
Q8XG56 ALLB_SALTI	160	---MSFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	160
A7Z8F5 ALLB_BACA2	163	---MAYDVRKGAALTPDGVIEADIVVQGVVAETGS---DTEAS---GTETIDAGRYV	163
Q1J391 ALLB_DEIGD	159	---MYDVLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	159
Q9RV76 ALLB_DEIRA	160	---MSLDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	160
Q24PT9 ALLB_DESHY	161	---MSHYDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	161
Q5WB36 ALLB_BACSK	162	---MSLDLIRGGLVIREGVEQADLVGVEEIVETAP---ELMGD---AREELDARGLHV	162
Q91676 HYDA_PSEAE	220	---MSLLRGATVTHEESYRADVLCAAGLTQATGE---NLETP---SGCDVLDGGQYL	220
Q45515 HYDA_GEOSE	220	---MTKLKNGITVATDIYEADLLIQDGLAVIGR---NLDES---GAEDVATSCYV	220
Q59699 HYDA_PSEPU	220	---MSLLRGATVTHEESYPADVLCDGLRAATGP---NLEPP---TDCEILDGSGQYL	220
P81006 HYDL_ARTAU	220	---MFDVIVKNCRLVSSDGITEADILVKDGLVAASDQT---SOLVE---ASRTIDAGKQV	220
Q69809 HYDA_STRCO	226	---MSRTVIRGGLVTSASDEIHADVLIEDGRVAALAA---TGTAAEAFTAEVTDASQYV	226
Q8VTT5 HYDA_RALPI	218	---MDIILKNGITVADGISRADLGKDGKLTQGG---ALGPA---ERTIDAGRYV	218
B7LF59 PHYDA_ECOS5	219	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	219
Q1R7F8 PHYDA_ECOUT	219	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	219
Q31NE3 PHYDA_SHIBS	219	---MRVLKNGITVADGQAKQDLIESGIVRQGN---NISPQ---LPYEEIDATSCYV	219
Q44184 HYDA_RHIRD	218	---MDIILKNGITVADGISPADLGKDGKLTQGG---TFGPA---GRTIDAGRYV	218
P77671 ALLB_ECOLI	223	---MSFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	223
Q9KAH8 ALLB_BACHD	223	---MKRFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	223
Q651N0 ALLB_BACLD	227	---MNFDSILKNGITVLENGEVEVGIKDGKLAAGQ---DL-GT---SAKIDAKSTV	227
Q32137 ALLB_BACSU	220	---MAYDVRKGAALTPDGVIEADIVVQGVVAETGS---DTEAS---GTETIDAGRYV	220
Q8XG56 ALLB_SALTI	220	---MSFDLIRKNGITVLENEARVVDIAVGGKLAAGQ---DL-GD---AKEVHDASGLV	220

International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 6, Issue 7, July 2017

Amino acids like asparagine, glutamine and histidine along with cysteine in a hydrophobic core region provide more stability, which is considered higher in allantoinase. No conserved region was found at C and N terminal of the sequences for hydantoinase and allantoinase under study. Catalytic triad was found at various helix, which is present in both the cyclic amidases. The present study aims to differentiate two groups of amidases that is allantoinase and hydantoinase on the basis of their amino acid sequences and physico-chemical properties. The selected sequences in each group (i.e. hydantoinase and allantoinase) were homologous and more than 40% identical with respect to model sequence. Results of variation in total number of amino acid residue between hydantoinase and allantoinase shows that total number of amino acid and molecular weight might be playing some role in providing the substrate specificity to these two groups of amidases. No significant difference for theoretical pI value was found between the selected two groups of amidases. This indicates that theoretical pI value is not responsible with the substrate specificity of the amidase enzymes considered in the present study. The results of this work indicates that amino acid number and their percentage composition in cyclic amidases significantly affect the substrate specificity among the selected microbial sequences studied. An important amino acid that is found to play a significant role in amidases is Cys that generally provides stability to proteins due to the formation of disulphide bonds. The results in the present work also indicate that microbial allantoinase are more stable as is shown by the instability index values (Table 2a) of various amidases considered in the present work.

IV. CONCLUSION

A number of physico-chemical properties of hydantoinase and allantoinase have been derived from amino acid sequences of reported microorganisms. The allantoinase and hydantoinase mainly differs in the total number of amino acid, molecular weights and composition of amino acid. Evolutionary relationship among selected enzymes revealed many conserved threads. This study has found similarity between the two cyclic amidases in terms of conserved amino acid residues at several positions. Glutamate and histidine were found to be higher and conserved at specific position of 205-283 in both cyclic amidases, which plays important role in enantioselectivity. The results of the present work will be quite useful in prediction and selection of allantoinase / hydantoinase from reported cyclic amidases or from the large number of sequenced microbial genomes.

ACKNOWLEDGEMENT

We are thankful to Gujarat State Biotechnology Mission (GSBTM), Gandhinagar, Gujarat, for the financial support and research fellowship to one of the author (D.H. Bhagat). Chirag N. Patel, acknowledge financial assistance from University Grants Commission (UGC), Govt. of India as Rajiv Gandhi National Fellowship. The authors extend their sincere thanks to Department of Botany, Bioinformatics and Climate Change Impacts Management, Gujarat University for providing an opportunity to access the bioinformatics research facilities. We are also grateful to University Grants Commission (UGC), New Delhi, for the Emeritus Professor fellowship to S.R. Dave.

REFERENCES

- [1] Novo C., Fournand S., Tata R., Clemente A. and Brown P.R., "Support for a three-dimensional structure predicting a Cys-Glu-Lys catalytic triad for *Pseudomonas aeruginosa* amidase comes from site-directed mutagenesis and mutations altering substrate specificity", *Biochemistry*, vol. 365, 731-738, 2002.
- [2] Sharma M., Sharma N.N. and Bhalla, T.C., "Amidases: versatile enzymes in nature". *Environmental Science and Biotechnology*, vol. 8, no.4, 343-366, 2009.
- [3] Bhalla T.C., Computational analysis of amino acid sequences in relation to thermostability of interspecific nitrile degrading enzyme (amidase) from various thermophiles/hyperthermophiles, *Open Access Scientific Reports*, vol 1, no. 12, 2012.
- [4] Kumar N. and Bhalla T.C., "In silico analysis of amino acid sequences in relation to specificity and physicochemical properties of some aliphatic amidases and kynurenine formamidases", *Journal of Bioinformatics and Sequence Analysis*, vol. 3, no.6, 116-123, 2011.
- [5] Fournand D., Bigey F. and Arnaud A., "Acyl transfer activity of an amidase from *Rhodococcus* sp. strain R312: formation of a wide range of hydroxamic acids", *Applied and Environmental Microbiology*, vol. 64, no. 8, 2844-2852, 1998.
- [6] Bjellqvist B., Hughes G.J., Pasquali C., Paquet N., Ravier F., Sanchez J.C., Frutiger S. and Hochstrasser D., "The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences", *Electrophoresis*, vol. 14, 1023-1031, 1993.
- [7] Fournand D. and Arnaud A., "Aliphatic and enantioselective amidases: from hydrolysis to acyl transfer activity", *Journal of Applied Microbiology*, vol. 91, 381-393, 2001.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Website: www.ijirset.com

Vol. 6, Issue 7, July 2017

- [8] Gopal K., Sharma N. and Bhalla T.C., “*In silico* analysis of some microbial amidases for their amino acid and physicochemical parameters”, International Journal of Bioassays, vol. 2, no.4, 630-636, 2013.
- [9] Mehta P.K., Bhatia S.K., Bhatia R.K. and Bhalla T.C., “Purification and characterization of a novel thermo-active amidase from *Geobacillus subterraneus* RL-2a”, Extremophiles, vol. 17, no.4, 637–648, 2013.
- [10] Guruprasad K., Reddy B.V.B. and Pandit M.W., “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 Engineering, vol. 4, 155-161, 1990.
- [11] Makhongela H.S., Glowacka, A.E., Agarkar, V.B., Sewell, B.T., Weber, B., Cameron, R.A. and Burton, S.G., “A novel thermostable nitrilase superfamily amidase from *Geobacillus pallidus* showing acyl transfer activity”, Applied Microbiology and Biotechnology, vol. 75, no.4, 801–811, 2007.
- [12] Kyte J. and Doolittle R.A., “A simple method for displaying the hydropathic character of a protein”, Journal of Molecular Biology, vol. 157, 105-132, 1982.
- [13] Nawaz M.S., Khan A.A., Bhattacharya D., Sifton P.H. and Cerniglia C.E., “Physical, biochemical and immunological characterization of a thermostable amidase from *Klebsiella pneumoniae* NCTR1”. Journal of Biotechnology, vol. 178, 2397-2401, 1996.
- [14] Stanley C., Peter H. and Von-Hippel, “Calculation of protein extinction coefficients from amino acid sequence data”, Analytical Biochemistry, vol. 182, no. 2, 319-326, 1989.