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# Computational Analysis of Amino Acid Sequences of Hydantoinase and Allantoinase fromSelected 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 significanceas 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 includehydantoinase (hydrolyzingshort and mid chain aliphatic amides) and broad range amidases (hydrolyzing various substrates, some acrylamides, a-aminoamides, a-hydroxyamides and allantoinase). Amidases can be assigned to phylogenetically 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



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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<sup>-1</sup>.cm<sup>-1</sup>) 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 discover 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 be 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	Pseudomonas aeruginosa	479
2	Q45515	HYDA_GEOSE	Geobacillus stearothermophilus	471
3	Q59699	HYDA_PSEPU	Pseudomonas putida	495
4	P81006	HYDL_ARTAU	Arthrobacteraurescens	458
5	O69809	HYDA_STRCO	Streptomyces coelicolor	467
6	Q8VTT5	HYDA_RALPI	Ralstoniapickettii	457
7	B7LF59	PHYDA_ECO55	Escherichia coli (strain 55989 / EAEC)	461
8	Q1R7F8	PHYDA_ECOUT	Escherichia coli (strain UTI89 / UPEC)	461
9	Q31WE3	PHYDA_SHIBS	Shigellaboydiiserotype 4	461
10	Q44184	HYDA_RHIRD	Rhizobium radiobacter	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).



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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	Escherichia coli	453
2	Q9KAH8	ALLB_BACHD	Bacillus halodurans	438
3	Q65LN0	ALLB_BACLD	Bacillus licheniformis	454
4	O32137	ALLB_BACSU	Bacillus subtilis	446
5	Q8XGS6	ALLB_SALTI	Salmonella typhi	453
6	Q5WBJ6	ALLB_BACSK	Bacillus clausii	448
7	A7Z8F5	ALLB_BACA2	Bacillus amyloliquefacienssubsp.plantarum	454
8	Q1J391	ALLB_DEIGD	Deinococcusgeothermalis	449
9	Q9RV76	ALLB_DEIRA	Deinococcusradiodurans	448
10	Q24PT9	ALLB_DESHY	Desulfitobacteriumhafniense	449

The extinction coefficient of various amidases was calculated using the following equation [12] E (Prot) = Numb (Tyr)\*Ext (Tyr) + Numb (Trp)\*Ext (Trp) + Numb (Cystine)\*Ext (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 hydantoinasewas 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.



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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

Donomotoro	55	Microorganisms								Maar Stiller	Stelor	E volu-	Develop		
Parameters	55	1	2	3	4	5	6	7	8	9	10	Mean	Stdev	F value	P value
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		
	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
IVI W	ALL	49601.6	48542.3	49488.2	48331	49886.8	48710	49454.3	48998.3	48558.7	49228	49079.9	528.8431		
Theoriticalp I	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 -	HYD	55	67	53	66	63	63	51	52	51	63	58.4	6.552353	1.9234	0.183
(Asp+Glu)	ALL	65	55	68	62	67	55	63	67	61	57	62	4.944132		
Total +	HYD	41	49	44	44	42	52	44	44	44	50	45.4	3.627059	1.215	0.284
(Arg+Lys)	ALL	48	45	52	44	48	49	51	50	40	45	47.2	3.675746		
Extinction	HYD	67755	50225	64775	28670	44725	44600	43485	421120	43485	44600	85344	118508.2	1.452	0.244
Coeff. Cys	ALL	36160	41870	37860	40505	37650	40630	40505	43555	41035	41995	40176.5	2276.188		
Instability	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
index	ALL	40.91	41.33	35.66	39.06	38.07	41.6	42.77	37.44	38.3	31.7	38.684	3.28784		
Aliphatic	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
index	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		
C	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
Gravy	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, Lye. 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 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.



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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	gg				l	Micro	organis	m				Maria	E	Date
composition	22	1	2	3	4	5	6	7	8	9	10	Mean	F value	P value
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
Ala (A)	ALL	8.4	6.6	8.1	11.2	8.2	10.6	12.1	10.9	10.5	8.7	9.53	0.05	0.825
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
ng (R)	ALL	4.9	4.8	3.5	4.3	4.9	5.6	4.6	9.6	6.5	4.2	5.29	0.525	0.470
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	0.55	0.572
Asn (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
nop (D)	ALL	7.3	5.5	1	5.8	7.1	5.4	5.1	6.5	6.5	7.3	5.75	0.002	0.505
Cvs (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
0,5 (0)	ALL	2.6	1.8	1.8	2.2	2.6	2.9	2.2	0.7	0.7	2.4	1.99	1.511	0.207
Gln (O)	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		01201
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	0.001	01110
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
5 ( )	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	3.1	4.3	4.2	5.4	4.4	5.3	5.4	4	4.55		
Ser (S)		5.0	5.4	0.5	5.2	3.9	4.2	) = =	5.4 4 5	3	4.2	4.84	0.288	0.598
	ALL	4.2	0.8	5.5	6.7	3.3	5.1	5.5 5.0	4.5	4.7	4.7	5.08		
Thr (T)		5.0		0.5	4.0	7.3 5.1	10	5.2 4.9	4.8	5.2	1.2	0.04 5.20	2.438	0.136
	ALL	4.4	0.0	4.6	5.4	5.1	4.9	4.8	6	0.5	5.6	5.39		
Trp (W)		1.9	1.1	1.8	0./	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	1.5	1.1	0.9	0.94		
Tyr (Y)		2.5	3.2 2	2	1./	3.2 2.2	3.3 2.7	3 26	2.8 1.6	3	3.3	2.8 2.49	1.808	0.195
	ALL	2	5 0 =	3.I	2.1	2.2 7.0	2.7	2.0	1.0	2 6 1	2.9 0 2	2.48		
Val (V)		0./ 0./	8.3 6.6	0.9 77	9 74	1.9 75	9.2 6.2	5.9 5.2	0.1 7.1	0.1	8.5 7.1	/.40	1.084	0.311
	ALL	ð.4	0.0	1.1	/.4	1.5	0.2	5.5	/.1	0.2	/.1	0.95		

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 whereasallantoinase contains glutamine at the same position. WNA and GGI triad were found to be conserved for both the cyclic amides.



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Q91676 HYDA_PSEAE Q45515 HYDA_PSEAE Q55699 HYDA_PSEPU P81006 HYDA_PSEPU 069809 HYDA_STRC0 Q8VTT5 HYDA_STRC0 Q8VTT5 HYDA_STRC0 Q8VTT5 HYDA_STRC0 Q11WE3 PHYDA_STIBS Q44184 HYDA_STIBS Q44184 HYDA_STIBS Q44184 HYDA_STIBS Q44184 HYDA_STIBS Q44184 HYDA_STIBS Q44184 HILB_BACHD Q55140 ALLB_BACHD Q55140 ALLB_BACHD Q8X566 ALLB_DBLTRA Q24PT9 ALLB_DESHY Q5WB36 ALLB_BACSK	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	<ul> <li>MSLL RGATV THEESYRAD LCANGL QA GE - NLETP</li></ul>	51 50 51 57 49 51 51 51 51 55 55 55 52 51 52 52 51 52 52 51
Q91676 HYDA_PSEAE Q59699 HYDA_PSEPU PS1096 HYDA_PSEPU PS1096 HYDA_STRCO Q8VTTS HYDA_RALPI BYTFS HYDA_RALPI BYTFS HYDA_SHLBS Q44184 HYDA_SHLBS Q44184 HYDA_SHLBS Q44184 HYDA_SHLBS Q44184 HLB_BACLD Q9KAH8 ALLB_BACLD Q9KAH8 ALLB_BACLD Q3L37 ALLB_BACLD Q3137 ALLB_BACLD Q3137 ALLB_BCLD Q9KY6 ALLB_DETKA Q78F5 ALLB_DETKA Q24PT9 ALLB_DETKA Q54H936 ALLB_BACSK	52 51 52 58 50 52 52 52 52 52 52 52 52 52 52 53 53 53 53 53 53 53	M GI P T MULPFMGTV-ASEDFFSG AAGLA TTISIIFVIP-NPRQSLLEAFHTW GI P T MULPFMGTV-ASEDFFSG AAGLA TTISIIFVIP-NPRQSLLEAFHTW M T MULPFMGTV-ASEDFFSG AAGLA TTISIIFVIP-NPRQSLLEAFHTW W T EV T MULPFMGTV-ASEDFFSG AAGLA TTISIIFVIP-NPRQSLLEAFHTW W T EV T MULPFMGTV-ASEDFFSG AAGLA TTISIIFVIP-NPRQSLLEAFHTW M T MULPFMGTV-ASEDFFSG AAGLA TTISIIFVIP-NPRQSLLEAFHTW GI W T VETVSFMTQ-SADFFATA VAAGC TTISIIFVIP-SVPISLEAGLAWA GI W T VETVSFMTQ-SADFFATA VAAGC TTISIIFVIP-NPRQSLLEAFHTW M T HNIDWGIAK-SCDDFFTG RAAGC TTISIIFWIP-SPHIGCRLEHQLEVY F GV W T FNIDWGIAK-SCDDFFTG RAAGC TTISIIFWIP-SPHIGCRLEHQLEVY F GV W T FNIDWGIAK-SCDDFFTG RAAGC TTISIIFWIFOPHIGCRLEHQLEVY F GV W T FNIDWGIAK-SCDFFTG RAAGC TTISIIFWIFOPHIGCRUHGEND F W W W T FNIDWGIAK-SCDFFTG RAAGC TTISIIFWIFOPHIGCRUHGEND F W W W T FNIDWGIAK-SCDFFTG RAAGC TTISIIFWIFORUNGFTGUNGK F W W W W T FNIDWGIAK-SCHARAGC TISIIFWIFORUNGFTGUNGK F W W W W T FNIDWGIAK-SCHARAGC TISIIFWIFONUNGFTGUNGK F W W W W	109 108 109 109 115 107 100 110 100 110 108 112 111 109 108 109 108
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Q73895 ALL8_D&CAB Q9RV76 ALL8_DETRA Q24PT9 ALL8_DESHY Q5WBJ6 ALL8_BACSK	160 161 162 163	ADBRTLVEGNEGASOGEVYUALIAL DALTRALEARIXARGGETDVR0VGAS IPALAVO A ADDITLVEGNETARHELVVITIANEFTRETTETARAGGESOVRDVLESIPVITI LA NDDYTALVGNERLARLEUPINN C-JAETIKELTELSLANNKTGVRDVFAAIPPITI II N VDLNGLRAGNRLLAEKGULLCVIALUPSMISQLEAKLLSPVGADAVAS IPVEAIVA	220 220 221 220
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Q9I676 HYDA PSEAE	459	PSVYEVLGRRAERQRPVAVER	479
Q45515 HYDA GEOSE	459	GALMADQDVVKMS	471
059699 HYDA PSEPU	459	SVGVRGAGATRRTAAPDARSALRPLGLLRSPSPASOI	495
P81006 HYDL ARTAU	455	EASK	458
069809 HYDA STRCO	464	0YLT	467
OSVITS HYDA RALPT	456	κο	457
B71 E59 PHYDA ECOSS	457	VPPVI	451
018758 PHYDA ECOUT	457	VPPVI	401
O31WE3 DHVDA CHTRC	457	VDDVI	401
	437	VPFVL	401
Q44184 HTDA_KHIKD	450	NQ	457
P77671 ALLB_ECULI	454		453
Q9KAH8 ALLB_BACHD	439		438
Q65LN0 ALLB_BACLD	455		454
282658 ALLB_BACSU	454		456
A7Z8F5 ALLB BACA2	450	KEPIL	454
010391 ALLB DEIGD	449	S	449
98V76 ALLB DEIRA	449	-	448
24PT9 ALLB DESHY	450		449
SUBIC ALLE BACSK	449		449
CHILD HELD DHC3K	449		440

Fig. 1. Alignment of the amino acid sequences of hydantoinase and allantoinase.

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 theoreticalpI 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 Cysthat 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.

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