IN SILICO CHARACTERIZATION OF SINORHIZOBIUM AND MESORHIZOBIUM AMYLASES.

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ABSTRACT

Microbial amylases are the dominant enzymes in industrial markets. These enzymes hydrolyze the starch molecule to the glucose unit polymers. A large numbers of amylases were obtained from diverse species of microbes. Starch as the main substrate for these enzymes which plays the important role in human diet obtains from many economically important crops such as wheat, rice, tapioca and potato. The final products of maltodextrin, modified starches, or glucose and fructose syrups were obtained by this enzymatic reaction. As there are large numbers of amylases from different microbial sources for different applications, the molecular characterization of the enzyme is vital for the researchers and producers. This research paper provided the comprehensive *in silico* information on amylases from two distinct rhizobia genera *Sinorhizobium and Mesorhizobium*. Thermostability and pH stability of amylases from *Sinorhizobium* and *Mesorhizobium* genera as well as the genomic distribution, physicochemical and structural features were analyzed. The physicochemical characterizations such as grand average hydropathy (GRAVY), aliphatic index (AI), extinction coefficient (EC), isolelectric point (pI), and instability index (II) were evaluated. S-S bridges, secondary structures and their homology modeling of these enzymes were presented with PyMol model visualization. This research finding could provide the analytical information for isolating and producing these enzymes for industrial purposes.

Keywords: Amylases, Rhizoba, *Sinorhizobium*, *Mesorhizobium*

INTRODUCTION

One of the six distinct categories of the enzyme is hydrolases. Generally, enzymes can obtain from plants, animals and microbes. Microbial enzymes mostly preferred for their easier isolation, low cost production and stability compared to the other sources [1]. Furthermore, their modification to adapt the new environment is also easier. Vast application of microbial enzyme in industry mostly referred to their capability to produce and secret them to the media which made the purification and other process convenient. Generally three kinds of microbial enzymes, proteases, lipases and amylases are dominant enzymes in the industry. Several microbes were isolated from different sources for production of extracellular hydrolysis [2]. Amylases are classified in three categories α, β, and γ . α Amylases which called α-1-4 glycoside hydrolases can act on α-1-4 glyosidic bonds [3]. It is noteworthy to mention that the first isolated microbe was from Anselme Payen in 1833. Amylases substrates are cheap plant sources and can be divided in two categories, endohydrolases and exohydrolases. Endohydrolases catalyze hydrolysis the starch molecule in random manner that provide linear and branched oligosaccharides of various lengths however the exohydrolases hydrolyze the substrate from the non-reducing end [4]. Starch the main substrate of alpha amylases are the polymer of glycose that connected by glyosidic bonds. Amylose and amylopectin the polymers in the starch, have different structures and properties.

It showed that alpha amylase is the metalloenzyme which requires the calcium ion to maintain its stability. In the structure of this enzyme four conserved arrangements reported. These regions are β-strands 3, 4, and 5 in the loop connecting β-strand 7 to α-helix 7[5]. Isolation of the better strain is the first and main step in better production of this enzyme. Sinorhizobium and Mesorhizobium are among the most important genera in microbial agriculture. Ubiquitous and accessibility of isolation for these genera from the soil rhizosphere made them better choices for research and study about amylases. However there was no enough information about amylases from these genera, therefore this paper provided the *in silico* characterization of amylase from them.

MATERIALS AND METHODS

Proteins sequences were retrieved from the UniProt and NCBI Databases [http://www.ncbi.nlm.nih.gov/]. Secondary structure of each protein was built based on the PSIPRED [6] and SOPMA server secondary structure prediction servers. With help of this information the best template was found for tridimensional (3D) structure modeling of each protein and the structural models were obtained by using SWISS MODEL [http://swissmodel.expasy.org/] [7] server. The resulting 3D-models were analyzed by RAMPAGE servers [http://www.ebi.ac.uk/ thornton-srv/databases/profunc/] [8] for functional and structural motifs predication and stereo-chemically evaluation, respectively. Subcellular localization of each protein was predicted from PSORTb v2.0.4 [9]. PRED-TMBB [http://biophysics.biol.uoa.gr/PRED-TMBB/input.jsp] using a 2.965 threshold value was used to analysis the β-barrel structures [10]. The structure analysis of amylases has done by the SOSUI server. ProtParam prediction server was used for sequence analysis.

RESULTS AND DISCUSSIONS

The proteins for this study were retrieved from UniProt and NCBI database. Physiological feature of Amylases were presented in figure 1. The total numbers of residue were ranged from 550 to 857. Molecular weights of different amylases were 61053.35 to 94599.86. The results of structure analysis of amylases showed that all of them are hydrophilic (SOSUI server). The results indicated that this protein is cytoplasmic by PSORTb server. Isoelectric point (pI) is the pH that the net charge of the protein is zero thus protein is stable and compact. The pI of the amylases were in the range of 4.72 to 6.03 which showed that they are acidic. Average pI was around 5 which was indicated the acidic condition. It is noteworthy to mention that for the purification of protein the pI value could be very useful for developing the buffer system.

Extinction coefficient (EC) of proteins which were calculated with ProtParam prediction server had ranging from 96620 to172355 $M^{-1}.cm^{-1}$ with respect to the concentration of Cys, Trp and Tyr. EC can help in predicting the protein –protein or protein-ligand interaction. The high EC value showed high concentration of Cys Trp, Tyr. If there isno EC it indicates that protein cannot analyzed using UV spectral methods (Table 1). The instability index which is an estimation of the enzyme in vitro was calculated by the same server. These values were in the range of 26.15 to 41.7. It showed that Sinorhizobium and Mesorhizobium amylases were stable in vitro but the enzyme No.9 showed instability. Aliphatic index which is the thermal stability of enzyme factor calculated based on the aliphatic side chains of A, V and L amino acids. This index ranged from 72.46 To89.03 which showed the stability of the enzymes from these sequences. The Grand Average Hydropathy (GRAVY) which is the value index for hydrophilic activity of protein was

provided by ProtParam prediction server. The value indexes showed that A0A2Z3G5P6 sequence is the most hydrophilic among the retrieved sequences. Overall the index showed that low GRAVY sequences can interact better with water. The secondary structure of amylase sequence was predicated by SOPMA server. This information illustrates the helix, strand and coil in the structure of protein. It is noteworthy to mention that results provided by default parameters of the server. The results showed that the secondary structure that alpha helix -strand -beta sheet are dominated the structure. The sys-Rec provided the information about the s-s Bonds and possible bonding pairs. There was not S-S bridge observed in the structure of amylases (Figure 1). Three-dimensional structure of proteins and enzymes provide important information about the proteins. This information can lead to design the Lab experiments. This information can predict from sequences with the help of many homology modeling websites. In this study the structure of these eight enzymes were provided by Swiss-model server. The templates of PDB were which is obtained by BLASTp search. After model predicted the model was checked with different website servers for the calculation of Ramachandran Map. The stereo chemical quality of the model proteins was provided with comparison of the model with well refined high resolution structure (Figure 2).

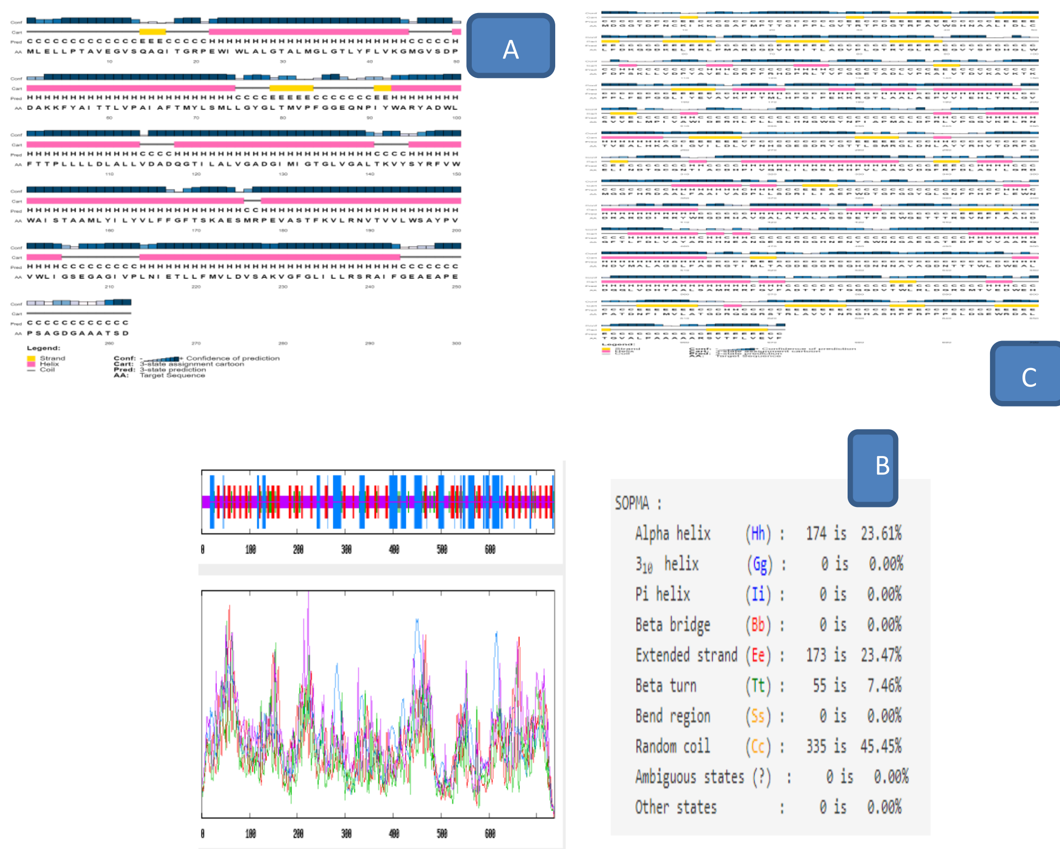

Figure 1. A) The secondary structure of the sequence X5R0H1predicted by PSIPRED server, B) The secondary structure of the sequence X5R0H1 predicted by SOUPA, C) The secondary structure of the sequence A0A2Z3FYG4 predicted by PSIPRED server.

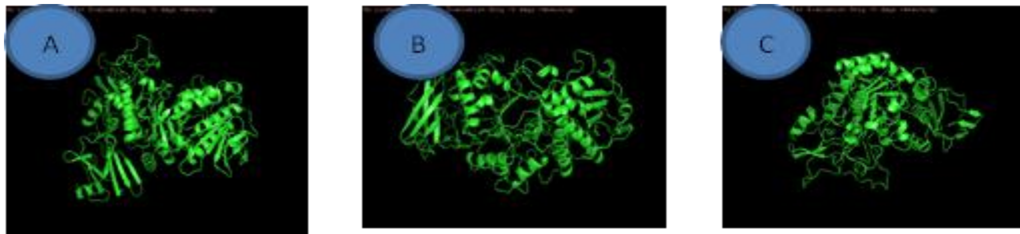

Figure 2. A: Mesorhizobium sp.(X5R0H1) B: Mesorhizobium sp.(X5R013) C: Sinorhizobium fredii (A0A2Z3F

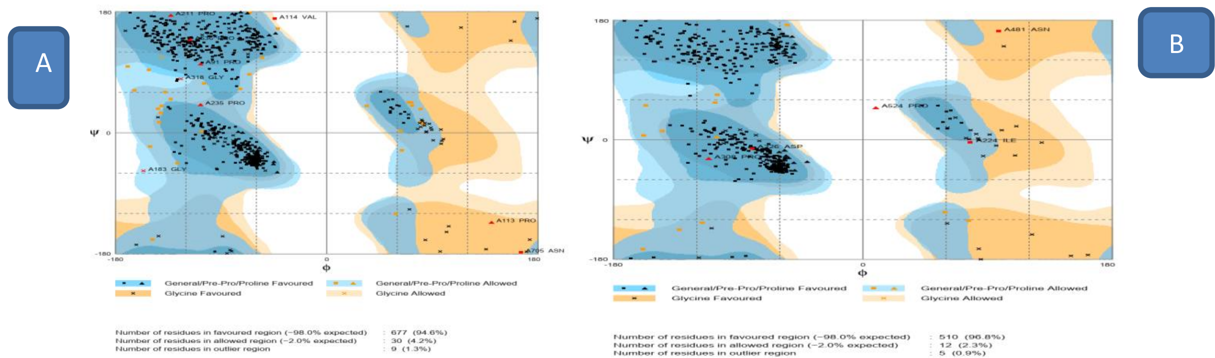

Figure 3. A) Ramachandran Map of the X5R0H1 predicted by RAMPAGE Server. B) Ramachandran Map of the A0A2Z3FYG4 predicted by RAMPAGE Server.

Table 1. Physiological feature of Sinorhizobium and Mesorhizobium amylases Amino Acids=AA, Molecular weight=MW, Theoretical pI=pI, Total number of negatively charged residues (Asp + Glu) = (Asp+Glu), Total number of positively charged residues (Arg + Lys)= (Arg+Lys), Total number of atoms=No. Atoms, Grand average of hydropathicity =(GRAVY)

No	Entry	No. AA	MW	pI	(Asp + Glu)	(Arg + Lys)	Formula	No. of atoms	GRAVY)	Ext. coefficient	Ext. coefficient	half-life (hours)	Instability index:	SOSUI server	Aliphatic index
1	X5R0H1	737	82329.53	6.03	93	76	C3742H5567N1029O1055S15	11408	-0.365	172355	172230	30	26.15 Stable	soluble	73.26
2	X5R013	666	72658.76	5.8	85	73	C3220H5023N951O953S11	10158	-0.315	107495	107370	31	31.36 Stable	soluble	84.19
3	X5R0H9	549	61053.35	4.72	79	48	C2742H4119N747O812S16	8436	-0.263	127350	126850	32	33.04 Stable	soluble	79.98
4	X5Q168	550	61596.95	5.2	72	52	C2777H4152N758O812S14	8513	-0.379	125165	124790	33	35.65 Stable	soluble	74.91
5	A0A2Z3G096	736	83221.52	5.59	98	72	C3765H5608N1038O1071S21	11503	-0.406	176950	176700	34	37.08 Stable	soluble	73.56
6	A0A2Z3GB95	598	65987.13	5.35	83	58	C2953H4479N841O857S16	9146	-0.254	96620	96370	35	37.74 Stable	soluble	78.58
7	A0A2Z3G5P6	696	78001.08	4.83	110	65	C3477H5230N960O1045S25	10737	-0.437	120000	119750	36	39.29 Stable	soluble	72.46
8	A0A2Z3G0N2	671	73592.09	5.68	85	66	C3279H5075N937O964S17	10272	-0.225	96620	96370	37	30.72 Stable	soluble	85.08 Cytoplasmic Membrane
9	A0A2Z3GKS5	857	94599.86	5.35	119	91	C4206H6563N1191O1257S21	13238	-0.198	112800	112300	38	41.7 Unstable	soluble	89.03
10	A0A2Z3FV96	551	61912.62	5.12	76	51	C2806H4197N757O808S14	8582	-0.314	119080	118830	39	36.72 Stable	soluble	78.17
11	A0A2Z3FYG4	557	62599.2	5.34	76	56	C2787H4227N787O825S20	8646	-0.359	111185	110810	40	30.97 Stable	soluble	76.54

The results showed that averaged maximum residues in favored region were 677 more than 98%, allowed region 30 (2%) For the sequences X5R0H1 and 510 residues in favored region and 12 (2%) in allowed region for A0A2Z3FYG4 sequence predicted by RAMPAGE Server (Figure 3).

CONCLUSION

Bacterial amylases as considerable group of enzyme offer great potentials for various applications and therefore identifying and developing of new amylases are necessary. This enzyme is one of the most produced enzymes in the industry. Therefore, Isolation and screening and characterization of new bacterial amylases need many efforts from different fields of study are justified. In silico characterization of bacterial soil amylases can provide great information about microbial amylases and can introduce better enzyme candidate for industrial process. This information is vital for engineering and classification of bacterial amylases.

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