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Bioinformatical Analysis of PHB Depolymerases from the Phototrophic Bacterium *Rhodopseudomonas Palustris* Using Computational Tools

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Abstract

In this study, PHB depolymerases from seven bacterial *Rhodopseudomonas palustris* were analyzed and presented in this communication. The composition of alanine, leucine and valine were the highest while lowest concentrations of asparagine and lysine residues were seen when compared to other aminoacids. pI value of Rp3 was 10.47 while the lowest pI of 5.67 was seen in Rp1. The instability index of all the depolymerases varied while for most of them it was less than 40 showing that some of them are stable while others are unstable. Aliphatic index was found to span within a range of 104 to 121. Secondary structural analysis of the depolymerases showed the pre-dominance of α-helices followed by random coils for all the depolymerases except Rp1 depolymerase. Significance of the above results are discussed in the light of existing literature.

Keywords: PHB, Depolymerases, *Rhodopseudomonas palustris*, Secondary structure.

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1. Introduction

Polyhydroxyalkanoates (PHA) are energy storage compounds and get accumulated as granules in the cytoplasm of cell. They are generally produced under nutrient limitation condition such as nitrogen and phosphorus but in excess carbon source. It has potential applications in different fields of biomedical, chemical and environment. The major commercial drawback is their high production cost which makes them more expensive than synthetic plastics. Polyhydroxyalkanoates are storage polymers [1] produced by bacteria to survive under unfavourable conditions. PHB is the most commonly found homo polymer used as carbon source when other sources of carbon get depleted [2]. PHB is released after bacterial lysis and is degraded by depolymerases in the environment [3]. It can be used as bioplastic as it is degradable by the depolymerases [4]. Some bacterial and fungal degradation of secreted PHB has been fully understood [5] while some are being investigated. The presence of intracellular PHB depolymerases has been reported [6-11]. PHB depolymerase system in phototrophic bacteria was first identified in *Rhodospirillum rubrum* in 1964 [6-8]. It contained a thermo stable activator and a thermolabile esterase. Intracellular soluble PHB depolymerase in *Rhodospirillum rubrum* was found to be similar with extracellular PHB depolymerases of *Acidovorax* sp. [12] . Gene cloning studies on PHB depolymerases have been reported in *Ralstonia eutropha* [13-17]. In the present study, bioinformatic analysis of PHB depolymerases from the most versatile phototrophic bacterium *Rhodopseudomonas palustris* is communicated.

2. Material and Methods

UniProtKB/Swiss-Prot, a protein sequence database, was used to retrieve the complete sequences of all the PHB depolymerases [18]. These sequences were used for further analysis. The computation of various physical and chemical parameters was done using ExPASy's ProtParam tool [19]. SOPMA tool (Self-Optimized Prediction Method with Alignment) server was used to characterize the secondary structural features [20]. The SOSUI server was used to predict the transmembrane regions which were further classified as membrane bound and soluble proteins [21].

3. Results and Discussion

PHA (iPHA) depolymerases (PhaZs) are enzymes that catalyze the depolymerization of accumulated PHAs [2, 22]. PHB depolymerase of *R.rubrum* was found to be located in the periplasm [6, 7, 23, 24]. Pretreatment of PHB granules with trypsin or an activator increases the activity of the enzyme [23]. *Rhodobacter sphaeroides* produced PHB as the major component (97%) and a small amount of PHV(3%) under anaerobic light conditions [24]. Nutrient limitation of nitrogen, sulphate and phosphate is necessary to initiate PHB accumulation. In continuation of our earlier studies in this group of bacteria [25-39], the PHB depolymerases from *Rhodopseudomonas* genus family were analyzed and the results are presented. Comparative analysis of the PHB depolymerases may give new inputs as to which groups of the depolymerases are vital in the degradation process.

Table 1 shows that the amino acid composition of eleven different PHB depolymerases of *Rhodopseudomonas palustris* species found in biological databases. The composition of alanine, leucine and valine was the highest while lowest concentrations of asparagines and lysine residues were seen when compared to other aminoacids. The number of negative charged residues are more than the positively charged residues (Table 2). Molecular weight of Rp8 depolymerase was the highest while Rp1 had the lowest molecular weight. pI value of Rp3 was 10.47 while the lowest pI 5.67 was seen in Rp1. The instability index of all the depolymerases varied while for most of them it was less than 40 showing that some of them are stable while others are unstable. Aliphatic index showing the relative volume of protein occupied by aliphatic side chains was found to span within a range of 104 to 121. From Table 3, Secondary structural analysis of the depolymerases showed the pre-dominance of α-helices followed by random coils for all the depolymerases except Rp1 depolymerase. SOSUI server analysis (Table 4) has shown that all the depolymerases were membrane bound proteins. These *in silico* findings can be used for working on properties of depolymerases in solution.

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Table-1. Amino acid composition of different PHB polymerases from *Rhodopseudomonas palustris* species

Species	ala	arg	asn	asp	cys	glu	glu	gly	his	ile	leu	lys	met	phe	pro	ser	thr	trp	tyr	val
Rp1	13.9	2.8	0.0	2.8	1.4	2.8	4.2	9.7	5.6	9.7	11.1	0.0	2.8	6.9	8.3	2.8	1.4	1.4	4.2	8.3
Rp2	17.6	7.1	0.8	3.9	1.2	2.0	3.1	10.6	1.2	5.5	14.5	0.8	1.6	2.0	6.7	5.1	4.7	2.4	0.4	9.0
Rp3	19.3	8.8	0.0	4.0	1.6	2.8	2.4	8.8	0.4	6.8	12.4	1.6	2.0	1.6	5.6	4.8	5.2	2.4	0.8	8.4
Rp4	15.6	3.2	1.9	5.7	0.6	1.0	1.9	9.8	6.3	7.0	9.8	1.0	1.9	3.8	4.8	5.1	5.1	2.5	1.6	11.4
Rp5	15.9	3.2	2.2	4.8	0.6	1.3	2.2	9.2	6.1	6.4	9.9	0.6	2.2	3.8	4.8	6.1	4.8	2.5	1.6	11.8
Rp6	19.1	3.6	0.9	1.3	0.0	3.6	3.1	9.3	3.1	3.6	15.1	1.8	1.3	7.4	4.4	4.9	4.6	1.3	4.0	8.4
Rp7	14.9	3.7	2.1	5.5	1.2	2.7	2.4	9.5	7.0	6.1	10.1	0.9	2.4	3.4	4.9	4.6	5.2	2.1	1.2	10.1
Rp8	14.2	4.0	3.0	4.6	0.3	1.9	3.2	9.4	5.9	6.7	9.9	1.1	2.4	4.0	5.9	6.5	4.8	2.4	0.8	8.9
Rp9	15.5	4.0	2.1	5.8	0.9	1.5	2.4	9.1	7.0	6.1	11.6	1.2	2.4	2.7	4.6	6.1	4.6	2.4	1.8	8.2
Rp10	15.5	3.6	2.1	6.1	0.9	0.9	2.7	10.0	6.4	6.7	10.3	0.6	2.4	4.3	4.9	5.5	4.3	2.7	0.6	9.4
Rp 11	15.9	3.2	2.2	4.8	0.6	1.3	2.2	9.6	6.1	6.4	9.6	0.6	2.2	3.8	4.8	6.1	4.8	2.5	1.6	11.8

Table-2. Physico chemical characteristics of PHB depolymerases

Name of species	No of amino acids	Molecular weight	pI	-ve charged residues	+ve charged residues	Instability index	Aliphatic index	gravy
Rp 1	72	7790.1	5.62	5	2	45.12	119.31	0.828
Rp2	255	26186.7	8.63	18	20	32.42	121.80	0.631
Rp3	249	25926.6	10.47	16	26	47	118.92	0.576
Rp4	315	32973.0	5.92	24	13	25.47	114.32	0.622
Rp5	314	32854.9	5.96	22	12	28.50	113.44	0.640
Rp6	225	23747.8	8.93	10	12	36.70	116.40	0.778
Rp7	328	34623.8	6.04	26	15	30.20	107.13	0.422
Rp8	372	39303.1	6.08	29	19	42.15	104.97	0.370
Rp9	329	34817.9	6.11	27	17	31.81	108.05	0.382
Rp10	329	34551.7	5.67	29	14	30.15	109.21	0.526
Rp11	314	32798.8	5.96	22	12	28.28	112.20	0.627

Table-3. Secondary structure of PHB depolymerases

Species	Alpha helix	310 helix	Pi helix	Beta bridge	Extended strand	Beta turn	Bend region	Random coil	Ambiguous state	Other states
Rp1	33.3	0	0	0	16.67	9.72	0	40.28	0	0
Rp2	58.82	0	0	0	7.84	4.31	0	29.02	0	0
Rp3	61.85	0	0	0	10.84	4.42	0	22.89	0	0
Rp4	50.48	0	0	0	16.83	5.40	0	27.30	0	0
Rp5	53.82	0	0	0	14.01	4.14	0	28.03	0	0
Rp6	59.56	0	0	0	12.89	8.44	0	19.11	0	0
Rp7	51.52	0	0	0	10.98	2.13	0	35.37	0	0
Rp8	51.34	0	0	0	10.75	2.96	0	34.95	0	0
Rp9	51.67	0	0	0	12.46	4.26	0	31.61	0	0
Rp10	52.28	0	0	0	13.07	3.04	0	31.61	0	0
Rp11	52.23	0	0	0	14.97	3.50	0	29.30	0	0

Table-4. Transmembrane regions of the depolymerases

No.	N terminal	transmembrane region	C terminal	type	length	Protein
Rp 1						
1	11	QPTIPAVIPVREILPWAIFG GLL	33	PRIMARY	23	Membrane
Rp2						
1	12	APLAGVPVGWKLTVLAA LGAGLA	34	PRIMARY	23	Membrane
2	39	PWLLAAAFGLSLCALLAT GLGPR	61	PRIMARY	23	
3	63	LWRGLKGPVVIVACIALL EGWQH	85	PRIMARY	23	
4	98	VTLICFAHAVTSSTSVLA MTAVI	120	SECONDARY	23	
5	140	LTLTLAIRFVPLIVDEIAAI REA	162	SECONDARY	23	
6	167	GLDRSIVALAVPLVVRIIL RAQD	189	SECONDARY	23	
Rp3						
1	12	APLVAVPVGWKLALLAL LGASVA	34	PRIMARY	23	Membrane
2	39	PWMLACGVASAACALML TGASPR	61	PRIMARY	23	
3	63	LWAGLKGTTIIVGCIGLFD YWSH	85	SECONDARY	23	
4	87	LASAAAVTTRLMILIGFA QAVTT	109	SECONDARY	23	
5	138	AGLTLTLAIRFVPLILDEIA AIR	160	SECONDARY	23	
6	167	GLDRSIVALAVPLVVKIIL RAQD	189	SECONDARY	23	
Rp4						
1	28	FGRAFAIGIALNVGFVIAE AAFG	50	PRIMARY	23	Membrane
2	99	ILAALFNAIFLLLAVGAIG WEAI	121	PRIMARY	23	
3	132	GTTVMIVAGIGIVINAATA WLF	153	PRIMARY	22	
4	181	VVAGVIILVTGWYWIDPA VSLLV	203	PRIMARY	23	
Rp5						
1	27	FGRAFAIGIALNMVFVVA EAAFG	49	PRIMARY	23	Membrane
2	97	SILAALFNAVFLLLAVGAI GWEA	119	PRIMARY	23	

Continue

3	131	GITVMVVAGIGIVINAVTAWLFA	153	PRIMARY	23	
4	180	VVAGVIILLTGWYWIDPAVSLIV	202	PRIMARY	23	
Rp6						
1	10	GAKITLSYVTAAGAGAYALGLAW	32	SECONDARY	23	Membrane
2	39	GIASLAARSVATTALVFSFFQLL	61	SECONDARY	23	
3	75	LGSTLFLLFGAAPAAFGLALGLL	97	PRIMARY	23	
4	108	LPQYGMNVTTLLVPLFALQALA	129	SECONDARY	22	
5	152	TAYQAGIVAWVAFWAFYGQGFGA	174	SECONDARY	23	
6	186	AYVAVIMFEPVFDLAVLAAAKSL	208	PRIMARY	23	
Rp7						
1	46	AIGIALNTGFVIAEATFGFLSNS	68	SECONDARY	23	Membrane
2	74	DAGHNLSDVLGLVVAWTAAVLS	95	SECONDARY	22	
3	109	GSSILAALFNAVFLLVAVGAIGW	131	PRIMARY	23	
4	145	EVTVMAVAAIGILINGVTAWLF	166	PRIMARY	22	
5	194	VAAGLVILLTGWNWIDAVTSLAI	216	PRIMARY	23	
Rp8						
1	85	FGRAFALGIGLNIAFVITEAAFG	107	SECONDARY	23	Membrane
2	115	LLADAGHNLSDVLGLAVAWIAAE	137	SECONDARY	23	
3	154	SSILAALFNAVFLLIAVGAIGWE	176	PRIMARY	23	
4	189	SVTMMVVAGIGILINGATAWLF	210	PRIMARY	22	
5	238	VVAGVVILFTGWNWIDPAVSLAV	260	PRIMARY	23	
Rp9						
1	42	FGTAFAIGILLNTGFVIGEAAFG	64	SECONDARY	23	Membrane
2	110	GSSILAALFNAAFLLVAVGAIGW	132	PRIMARY	23	
3	146	EITVMVVAGVGIVINGVTAWLF	167	PRIMARY	22	
4	184	MMADAAVSAGVVIAGLLILLTGW	206	PRIMARY	23	
5	208	WLDAVTSLLISAAIFWGTWGLLR	230	PRIMARY	23	
Rp10						
1	42	FGRAFAIGIGLNIGFVIVEAVFG	64	PRIMARY	23	Membrane
2	72	LLADAGHNLSDVLGLAVAWIAAE	94	SECONDARY	23	
3	110	GSSILAALFNAVFLLLAVGAIGW	132	PRIMARY	23	
4	146	GVTMMVVAGVGIGINAATAWLF	167	PRIMARY	22	
5	195	VVAGVVILFSGWTWIDPAVSLLV	217	PRIMARY	23	
Rp11						
1	27	FGRAFAIGIALNMVFVVAEAAFG	49	PRIMARY	23	Membrane
2	97	SILAALFNAVFLLLAVGAIGWEA	119	PRIMARY	23	
3	131	GITVMVVAGIGIVINAVTAWLFA	153	PRIMARY	23	
4	180	VVAGVIILSTGWYWIDPAVSLIV	202	PRIMARY	23	