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In silico analysis for various proteomic parameters of nitrogenases from the phototrophic bacterium *Rhodobacter* species

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ABSTRACT

Nitrogenases from eight bacterial Rhodobacter species were analyzed and presented in this communication. The composition of alanine, glycine and leucine was high while low concentrations of asparagines, tyrosine, tryptophan, cysteine and lysine residues were seen when compared to other amino acids. The numbers of negative charged residues are more compared to positively charged residues. Molecular weight of AN2 was the highest while NIP3 nitrogenase had the lowest molecular weight. pI value of N2 was the highest with 6.1 while the lowest pI 4.98 was seen in NIP2. The instability index of all the nitrogenases varied while for most of them it was less than 40 except N3 showing that most of them are stable. The relative volume of protein occupied by aliphatic side chains was found to span within a range of 75 to 110. Secondary structural analysis of the nitrogenases showed the dominance of α-helices and random coils equally for all the nitrogenases. Beta turns were less in number for all the nitrogenases. SOSUI server analysis has shown that all the nitrogenases from Rhodobacter genus are soluble proteins except N3 which is a membrane protein

Key words: *Rhodobacter* species, nitrogenases, in silico analysis

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INTRODUCTION

Hydrogen production by phototrophic bacteria has an invaluable share in the total biohydrogen production. nitrogenase and hydrogenase enzymes play a key role in Hydrogen production from photosynthetic bacteria. Photobiological hydrogen production by photosynthetic bacteria has been investigated by many researchers [1-7]. Burris, (1991) [8] has proposed the presence of two component metalloproteins in nitrogenase system. *nifHDK* genes and *anfHDGK* genes are the genes which are involved in the expression of nitrogenases [9]. Glutamate should be limited in order to stimulate nitrogenase expression [10]. Nitrogenase requires electrons and ATP in order to produce hydrogen. Photosynthetic bacteria bacterial hydrogenase and nitrogenase are involved in hydrogen production under anaerobic conditions [11]. Nitrogenase is inhibited in the presence of ammonium [12]. Nitrogenase induction takes place in presence of light [13]. Molybdenum is required for the production of hydrogen and its limitation leads to reduced expression of *nifK* in *R. sphaeroides* [14]. In the present work, nitrogenases which play an important role in photobiological production have been analyzed for various proteomic parameters *in silico* and the results are discussed.

EXPERIMENTAL SECTION

Retrieval of nitrogenase sequences was done from UniProtKB/Swiss-Prot [15]. These sequences were used for further analysis. ExPASy's ProtParam tool was used for the computation of various physical and chemical

parameters [16]. SOPMA tool (Self-Optimized Prediction Method with Alignment) server was used to characterize the secondary structural features [17]. The transmembrane regions classified as membrane bound and soluble proteins were predicted by SOSUI server [18].

Table 1: Different nitrogenases from *Rhodobacter* species

Nitrogenases	*Rhodobacter* Species /strain
N1	Nitrogenase [Rhodobacter capsulatus] WP_023923925.1
N2	Nitrogenase [Rhodobacter capsulatus Y262] ETE53280.1
N3	Nitrogenase [Rhodobacter sphaeroides] WP_011907825.1
NIP1	Nitrogenase iron protein [Rhodobacter capsulatus] WP_013066316.1
NIP2	Nitrogenase iron protein [Rhodobacter capsulatus] WP_013067945.1
NIP3	Nitrogenase iron protein [Rhodobacter capsulatus] WP_013066329.1
AN1	Alternative nitrogenase [Rhodobacter capsulatus] CAA49627.1
AN2	Alternative nitrogenase [Rhodobacter capsulatus] CAA49625.1

Table 2*:* Amino acid composition of eight different nitrogenases from *Rhodobacter* species

Amino acids	N1	N2	N3	NIP1	NIP2	NIP3	AN1	AN2
Ala	20.3	20.7	17.7	13.4	9.8	8.7	7.8	8.6
Arg	7.3	7.5	7.1	7.6	4.4	4.7	3.5	4.8
Asn	0.9	0.9	1.1	1.7	4.1	4.0	3.0	3.8
Asp	3.6	3.6	3.8	7.9	4.7	5.8	7.4	6.1
Cys	1.1	1.1	0.9	1.4	2.4	2.2	1.5	2.1
Gln	1.1	1.1	1.8	2.1	3.7	3.3	2.4	2.5
Glu	5.5	5.5	5.8	4.5	10.2	9.1	6.5	7.8
Gly	13.0	13.0	13.3	11.0	10.2	10.2	7.6	8.4
His	2.1	2.1	1.6	1.4	1.4	1.1	2.8	3.6
Ile	2.3	2.3	4.2	6.9	7.5	7.6	6.5	6.3
leu	14.6	14.6	14.2	7.9	8.1	5.8	10.0	6.1
Lys	0.7	0.7	1.1	3.4	6.4	6.9	6.7	6.8
Met	1.4	1.4	0.7	0.7	4.4	3.6	3.3	3.0
Phe	2.1	2.1	2.2	3.4	1.7	5.1	4.3	4.0
Pro	7.5	7.5	7.8	4.8	2.7	3.3	5.0	4.8
Ser	3.4	3.4	4.4	5.2	3.4	2.5	4.3	4.8
Thr	3.4	3.4	4.4	4.8	4.4	5.5	5.7	4.2
Trp	0.9	0.9	0.7	1.0	0.0	0.0	0.9	1.5
Tyr	0.2	0.2	0.4	2.8	3.4	2.5	2.2	4.6
Val	8.0	8.0	6.9	7.9	7.1	8.0	8.5	6.5
Pyl	0	0	0	0	0	0	0	0
sec	0	0	0	0	0	0	0	0

Table 3: Physicochemical characteristics of nitrogenases from *Rhodobacter* species

Name of species	No of amino acids	Molecular weight	pI	-ve charged residues	+ve charged residues	Extinction coefficient	Instability index	Aliphatic index	gravy
N 1	439	43927.8	6.02	40	35	23740/23490	33.63	110.25	0.431
N 2	439	43954.8	6.16	40	36	23740/23490	32.87	109.59	0.421
N 3	451	45426.3	5.74	43	37	19730/19480	40.35	109.45	0.331
NIP 1	290	30784.9	5.65	36	32	28670/28420	28.36	94.28	0.002
NIP 2	295	32112.8	4.98	44	32	15275/14900	36.42	91.29	-0.163
NIP 3	275	30177.6	5.06	41	32	10805/10430	31.86	84.40	-0.154
AN 1	460	50702.5	5.25	64	47	37275/36900	20.92	96.85	-0.016
AN2	526	59061.1	5.91	73	61	80385/79760	36.36	75.49	-0.016

Table 4: Secondary structural analysis of nitrogenases from *Rhodobacter* species

Species	Alpha helix (%)	310 helix	Pi helix	Beta bridge	Extended strand (%)	Beta turn	Bend region	Random coil (%)	Ambiguous state	Other states
N 1	43.28	0	0	0	15.26	9.11	0	32.35	0	0
N 2	44.19	0	0	0	14.12	8.20	0	33.49	0	0
N 3	46.12	0	0	0	13.53	8.20	0	32.15	0	0
NIP 1	40.34	0	0	0	18.97	7.59	0	33.10	0	0
NIP 2	42.03	0	0	0	16.61	9.49	0	31.86	0	0
NIP 3	37.82	0	0	0	18.91	9.45	0	33.82	0	0
AN 1	45.65	0	0	0	13.48	3.70	0	37.17	0	0
AN2	39.35	0	0	0	15.21	4.75	0	40.68	0	0

Table 5: SOSUI server analysis of nitrogenases from *Rhodobacter* species

Species	Soluble/ Transmembrane	Transmembrane regions
N(RC)	Soluble	
N(RC)	Soluble	
N(RS)	Transmembrane	QASCALHGAILTAAAIPGVIPLV, IGGSLAAPGLARFLTTVLGWKTV, GRAGAARLLAALVTGLEAADAPA
NIP(RC)	Soluble	
NIP(RC)	Soluble	
NIP(RC)	Soluble	
AN(RC)	Soluble	
AN(RC)	Soluble	

RESULTS AND DISCUSSION

Nitrogen is a constituent of many biomolecules. In the biosphere nitrogen is present as dinitrogen which is of no use to most living forms [19]. Industrial nitrogen reduction is accomplished by Haber-Bosch process with a metal, hydrogen under high pressures and temperature. Biological nitrogen fixation is catalyzed by microorganisms, called diazotrophs which accounts for majority of N_2 reduction. N_2 reduction is catalyzed biologically by the enzyme nitrogenase which functions at room temperature and pressure and energy in the form of ATP [20]. There are four known types of nitrogenases which have a different combination of metals at the active site [21, 22]. The nitrogenase system composes of dinitrogenase reductase and dinitrogenase. Molybdenum dependent enzyme, having an active site of FeMo-cofactor is the most widely studied nitrogenase [23]. FeMo-cofactor contains Fe, S, homocitrate and X(unknown atom) [24] . In continuation of earlier studies on phototrophic bacteria [25-31], a study has been done on the nitrogenases which are known to play a crucial role in hydrogen production by the bacteria. Nitrogenases of different *Rhodobacter* species obtained from database are presented in Table 1. Table 2 shows that the amino acid composition of eight different nitrogenases of *Rhodobacter* species obtained from biological databases. The composition of alanine, glycine and leucine was high while low concentrations of asparagines, tyrosine, tryptophan, cysteine and lysine residues were seen when compared to other aminoacids. The number of negative charged residues is more compared to positively charged residues (Table 3). Molecular weight of AN2 was the highest while NIP3 nitrogenase had the lowest molecular weight. pI value of N2 was the highest with 6.1 while the lowest pI 4.98 was seen in NIP2 . The instability index of all the nitrogenases varied while for most of them it was less than 40 except N3 showing that most of them are stable. Aliphatic index shows the relative volume of protein occupied by aliphatic side chains was found to be within a range of 75 to 110. From Table 4, Secondary structural analysis of the nitrogenases showed the dominance of α-helices and random coils equally for all the nitrogenases. Beta turns were less in number for all the nitrogenases. SOSUI server analysis (Table 5) has shown that all the nitrogenases so far characterized from *Rhodobacter genus* are soluble proteins except N3 which is a membrane protein.

CONCLUSION

These *In silico* findings can be used for working on proteomic properties of nitrogenases in solution and for comparisons with related nitrogenases from other groups.

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