

Bioinformatics analysis of few Zinc metalloproteases from anoxygenic phototrophic bacteria

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ABSTRACT

Metalloproteases from nine photosynthetic bacterial species were analyzed and presented in this communication. The composition of alanine, glycine and leucine, valine was high while low concentrations of asparagines, histidine, tyrosine, tryptophan, cysteine and lysine residues were seen. The composition of other aminoacids was intermediate. The numbers of negative charged residues are more than positively charged residues. The relative volume of protein occupied by aliphatic side chains was found to span within a range of 99 to 113. Secondary structural analysis of the metalloproteases showed the dominance of α -helices followed by random coils. Less number of beta strands was found in all metalloproteases. The sequences were aligned and PIM was identified with Clustal analysis. Swiss modeling was performed to predict the structure of the proteases.

Keywords: Photosynthetic bacteria, metalloproteases, *in silico* analysis, Swiss modeling.

1. INTRODUCTION

Hydrogen production by phototrophic bacteria is significant in the total biohydrogen production which is taking place. Nitrogenase and hydrogenase enzymes are the major enzymes which play an important role in hydrogen production from photosynthetic bacteria. Photo hydrogen production by photosynthetic bacteria has been widely investigated [1-7]. Presence of two component metalloproteins in nitrogenase system was proposed by Burris [8]. *nifHDK* and *anfHDGK* genes play a role in the expression of nitrogenases [9]. Glutamate limitation has been reported to stimulate nitrogenase expression [10]. Nitrogenase requires ATP and electrons in order to produce hydrogen. Under anaerobic conditions photosynthetic bacterial nitrogenase is involved in hydrogen production under anaerobic conditions [11]. Nitrogenase is inhibited in the presence of ammonium [12] while induction takes place in presence of light [13]. Limitation of

Molybdenum leads to reduced expression of *nifK* in *R. sphaeroides* [14]. Metalloproteases (*metallo*, metal) are members of a clan of proteases that contain metal ion acting as a catalyst in the hydrolysis of peptide binds [15]. The most common metal ion metalloproteases utilize is a zinc ion (Zn^{2+}) [16] or Co^{2+} and Mn^{2+} when Zn^{2+} core is absent [17]. A tetrahedral conformation of amino acids is commonly seen at the active site which associate with the metal ion for hydrolysis [18]. There are two kinds of metalloproteases namely metalloendopeptidases and metalloexopeptidases based on which the reaction takes place [19,20]. In the present work, metalloproteases which play an important role in photobiological hydrogen production have been analyzed for various proteomic parameters *in silico* and the results are discussed.

2. MATERIALS AND METHODS

Retrieval of several Metalloprotease protein sequences was done from UniProtKB/Swiss-Prot [21]. These sequences were used for further analysis by ExPASy's ProtParam tool for the computation of various physical and chemical parameters of protein like the Number of Amino Acids, amino acid composition, molecular weight, -ve charged residues, +ve charged residues, theoretical pI, instability index, aliphatic index and grand average of hydropathicity (GRAVY). [22]. SOPMA tool (Self-Optimized Prediction Method with Alignment) server was used to obtain several parameters like Alpha helix, 310 helix, Pi helix, Beta

bridge, Extended strand, Beta turn, Bend region, Random coil, Ambiguous state, Other states for prediction of the secondary structural features of the proteins [23]. Further by using by Clustal Omega tool the protein sequences were virtually aligned accurately and quickly compared the homologous sequences and obtained Percent Identity Matrix to find the best match for the selected sequences, and to line them up so that the identities, similarities and differences can be seen. Swiss Model software was used to generate reliable three-dimensional protein structure models.

Table 1: Different Zinc Metalloproteases from Phototrophic bacteria along with the sequences.

Zinc Metalloproteases	Species /strain	Accession no.
RsZnmp1	[Rhodospirillum photometricum DSM 122] MREGSPARGLLFLSACSGAPPGGAEETRAMPEIFHTVLAFLLVLTVVVVFHVELGHFLVARWNGVRVEVFSI GMGPELLGYTDRHGTRWRLLSLLPVGGYVRFVFGDADESSGSAEVDLEAMTPEKAVCFHHKRVQRAAIVA AGPLANFAFVAILVFAFLFMHGHQTRSPVVIDAVLAGSAAEAAGLKVGDRIVALNGKTVERFQDVQRVAL SDGKVMQVGLVRDQPLRVEVQPRFVNDNDGLGNTVKMAQLGIQIKLSPGDVHRLGPVQALEQVQAQWT LSADTLTYVQGMISGFRSASELGGPIRIALFSGQAERGVADLVFIALLSANLGLINLFPIMLDGGHL MFYTIELRGRPLTERIQEMGLKVGLALVVALMLFATWNDLGLKGLWG	CCG06778.1
RsZnmp2	[Rhodospirillum rubrum] MLDLLHTVLSFLVLTAVVVFVHEFGHFLVARLNGVRVEVFSIGFRELFGFNDRYGTRWRLLPLGGYV RFFGDADETSGETAETTRPLSKAEAEAVSFHHKRVQRFVAVLAGPMANFLSIVVFAAGLYMTIGQPHSAPV VGEVIAGSAAEAAGLLAGDRIVADGTPIDRFQDVRRVPLSNGAPLHIDILRDNAPLAVIALPRMVETD DGLGNKVQVAVLQGVKVSLSQADVQLRGLDALGQAVGQVWLSADTLTYLQVVRGNRSAAELGGPVRIA QFSGKAAERGVLDLVTFIALLSVNLGLINLFPIMLDGGHLMFYTIELRGRPLGARAQEYGLRFLGLALV LAMMVFATWNDLSLINW	WP_01138 9468.1
RsZnmp3	[Rhodobacter sp. SW2] MDIIGLVPSFGGMAWTHIAFIVAILVIVAVHEYGHYIVGRWSGIHAEVFSIGFPGVIVSRIDKRGTRWQL AALPFGGYVRFVLDSSAASGKDGALISQLSPEERRHMHGAPLWARAATVAAGPAFNFIIVFAAGFFLAYG VATDRPVVVGALKPMEATQSLQPGDLILAVDGGQATPDLETYVAVGKELPHQASFDYRIERAGVETTL TGPHFPPIADAVQPRSAAMEAGIKVGDVVTVDGTPVAVFQQLRDMVGESGGKTLHLQWRDGTTEADLT PRRDLPLEAGGFETRWLIGLSGGGMFTPEIRTPGPWETTLTAVDRVWYIVKVSLSIWSMITGAIS SCNMAGPIGMAEAMGDAARGGLEMFVQTLAMFSLGIGLMNLFPIVLDGGHLVVFHVEAVTGKPPSDGAM RILMTTGLVLLLLLMVFAVTNDLFCP	WP_04067 1922.1
RsZnmp4	[Rhodobacter sphaeroides KD131] MDIVSLVPQFGGVIWTLIAFVVALSIVVAVHEYGHYIVGRWSGIHAEVFSLGMGPVIASRVDRRGTRWQLAAF PVGGYVRFVLDGDAASSRASVSVHKLNEQERGRMHGAPLWARAATVAAGPLFNALSILVFCFFM VKGVATELPPVGEVKALPEASQSLVEGDRIADGQETPTLSDFVRVANELPPAPTAAYRIERDGAEMDVTA YPPFPVDAVQAPSGAHEAGIEAGDVLEVNGAPIASFRELRAVGLSNGDPLTMTVWRAGETYEAS LTPRRMDIPLPTGGFETRWLIGLSGGLLFEPETRTPGLEAIWLGIIQQTITITITSLGLWHMVTGAISSCNLQ PIGIAEISGAAASQAGNFIWFIAMLSTAVGLMNLFPVPILDGGHLVHFAYEAVAGKPPSDRVLRLVMTGGLA VLLSLMVFAVTNDLFC	ACM00886. 1
RsZnmp5	[Rhodopseudomonas palustris CGA009] MADFLNSFHLSHGLVGYVVPFLVLTIVVFFHELGHFLVARWNGVKVLTFSIGFPEIVGFNDRYGTRWKL SAVPLGGYVVFVGGDSEASTPSGDALSQMSASERAVSFHHKPVGPRAAIVVAGPLANFILAVLFTF LFSVFGVPNTSARVDGVPQGSAAEAAGFKPGDVVTSINGSAINFLEMQRVFGAEAGNQLKFTVKRGDSTVDL VATPQLKEIKDRFGNVQRLGILGISRSTAAGEVTTEQVNPVAVAFWMGIKETWVVDRTFSYIGGIFT GREAADQLGGPLRIAQVSGQVATIGFTPLHLAAVLSISIGLLNLPVPLLDGGHLLFYGIEAARGRPLSERAQE LGRIGLALVLMMLMFATYNDILHLASS	CAE28356. 1
RsZnmp6	[Rhodobacter sp. CACIA14H1] MEIVSLIPSGGLAYTVLAFVAALTVIVVVFVHEYGHYIVARWSGIHADVFSIGFPGVLSRVDRRGTRWQVAAL PFGGYVVFVGGDSNAASGGHADDVSALSAEQRRATMHGAPLWARSATVAAGPVFNFILSIIVFAGIFL VNGVATDRPTVGEVRPLPGVEAELRAGDVLALLEGTEPTDFGFSIDAAGAHPLERTLQYRVDRGGEQVTLRD AHPLPPVETVQPDASAARAAGIEAGDVIVSVNGQPVATFPQLREVMVSSDGKVLTLGLMRGEQALEMV LEPKRMDLPLSEGGFETRWLIGLTGALYFEPETRRPAVLESVSLGAQQSWDIAVTSLSGLWHMITGAISSCNL RGPPIAETSAAAASAGLSFIWFIAMLSTAVGLMNLFPVPLDGGHLLVFFAWEAVTGKPTARALR VLMVGLTAILTMLVFALSNDLFCP	ESW62223. 1
RsZnmp7	[Rhodobacter sp. SW2] MAWTHIAFIVAILVIVAVHEYGHYIVGRWSGIHAEVFSIGFPGVIVSRIDKRGTRWQLAALPFGGYVRFVLDGSS	EEW25109. 1

	AASGKDGALISQLSPEERRHTMHGAPLWARAATVAAGPAFNFIFAILVFAGFFLAYGVATDRPVVG ALKPMPEATQSLQPGDLILAVDGGQATPDLETYVAVGKLPHQASFDYRIERAGVETTLTGPHFPPIADAVQP RSAAMEAGIKVGDVVTTVDGTPVAVFQQLRDMVGESGGKTLHLQIWRDGTTHIADLTPRRADLPLEA GGFETRVLIGLSGGGMFTPEIRTPGPWETLTLAVDRVWYIVKVSLSIWSMITGAISSCNMAGPIGMAEAM GDAARGGLEMFVQTLAMFSLGIGLMNLFPIPVLDGGHLVFHVWEAVTGKPPSDGAMRILMTTGLVLLL LLMVFVAVTNDLFCP	
RsZnmp8	[Rhodobacter sphaeroides 2.4.1] MDIVSLVPQFGGVIWTLAFVVALSIVVAVHEYGHYIVGRWSGIHAEVFSLGMPVIASRVDRRGRTRWQLAAF PVGGVYRFLGDADAASSRASVSVHKLNEQERGRMHGAPLWARAATVAAGPLNFALSILVFCAFFM VKGVATELPPVVEVKSLPEASQSLSEEGDRILADGQETPTLSDFVQVANELPPAPTAAYRIERDGAEMDVTAP YFPFPVVDVAVQAPSGAHEAGIEAGDVLAVNGAPIASFRELRDAVGLSNGDPLTMTVWRAGETYEAS LTPRRMDIPLPTGGFETRWLIGLSGGLLFEPETRTPGPLEAIWLGIIQTTITITSLSGLWHMVTGAISSCNLQG PLGIAEISGAAASQGAGNFIWFIAMLSTAVGLMNLFPVPILDGGHLVFHAYEA VAGKPPSDRVLRL VLMTGGLAVLLSLMVFVAVTNDLFC	YP_352765.1
RsZnmp9	[Rhodospirillum centenum SW] MDAVGYVWNYGVVFLVVLTVLVFVHELGHYVWARRNGVRVEVFSIGFGPELFGFNDRAGRTRWKFSAVPLGG YVKMFGDADAASRPDFRLDDLPEERARSFYHQSLGSRAAIVAAGPAANFAFAIVALALLFTVYGGQFPTAPVI EEVSPDGAEEAGLLPGDRVLSIDGQTIERFEDITQLVVQYPRPLALVVQRDGLVPPVTPRTVEVEDRFG NTHITIGRIGVLRGADEFKRRDPLSAVWYAGKETLSLTLGTLKAVGQMISGTRGTDELGGPLRI AQMSGEVAQTGFVALVWVFAILSINLGLINLFPIMLDGGHLLFYGIEAVRGRPLGERAQEYGFRIGLALVLT MVFATWNDLVHLRVVQFFVDVLS	ACI98615.1

Table 2: Amino acid composition of different Zinc Metalloproteases from *Rhodobacter*

Zn Metalloprotease	Rs Znmp1	Rs Znmp2	Rs Znmp3	Rs Znmp4	Rs Znmp5	Rs Znmp6	Rs Znmp7	Rs Znmp8	Rs Znmp9
Ala	42	38	49	53	37	54	49	53	36
Arg	26	25	21	23	18	25	21	22	26
Asn	8	11	4	9	12	7	4	9	8
Asp	17	18	21	17	14	18	20	17	19
Cys	2	0	2	3	0	2	2	3	0
Gln	15	13	11	10	11	10	11	11	11
Glu	20	15	19	24	15	23	19	24	20
Gly	47	39	52	46	42	47	49	46	41
His	9	8	10	9	9	9	10	9	6
Ile	18	17	31	27	20	23	29	26	17
Ileu	51	51	43	44	43	48	42	45	43
Lys	9	5	8	4	10	4	8	4	6
Met	12	8	17	12	7	11	16	12	6
Phe	21	22	23	21	32	24	22	21	25
Pro	17	15	29	29	17	26	28	29	19
Ser	18	17	21	27	27	29	20	28	14
Thr	16	16	29	25	20	25	29	25	19
Trp	5	4	11	9	4	9	11	9	6
Tyr	4	6	7	7	6	6	7	7	10
Val	41	39	38	45	39	45	37	44	45
Pyl	0	0	0	0	0	0	0	0	0
sec	0	0	0	0	0	0	0	0	0

Table 3: Physico chemical characteristics of different Zinc Metalloproteases from *Rhodobacter*

Zinc metalloproteases	No of amino acids	Molecular weight	PI	-ve charged residues	+ve charged residues	Instability index	Aliphatic index	gravy
Rsmmp1	398	42670.6	6.52	37	35	28.04	108.04	0.344
Rsmmp2	367	39643.0	6.25	33	30	22.67	113.43	0.399
Rsmmp3	446	47610.3	5.37	40	29	35.76	100.40	0.401
Rsmmp4	444	47095.3	5.08	41	27	36.06	103.69	0.413
Rsmmp5	383	41069.4	6.76	29	28	23.04	103.34	0.431
Rsmmp6	445	47220.4	5.23	41	29	38.54	103.69	0.423
Rsmmp7	434	46422.9	5.47	39	29	35.30	99.82	0.379
Rsmmp8	444	47055.2	5.01	41	26	36.62	103.04	0.402
Rsmmp9	377	41111.4	5.47	39	32	31.78	106.23	0.326

3. RESULTS AND DISCUSSION

The most extensively studied extracellular zinc-containing metalloproteases are widely distributed in the bacteria. Smaller family units of bacterial zinc-

containing metalloendopeptidases such as elastase like, thermolysin-like and Serratia protease-like are present in different bacteria [24]. They are associated with bacteria having industrial significance. They are found

in both gram-negative and gram-positive microorganisms and are known to contribute for bacterial pathogenicity. The protein sequences retrieved from UniProtKB/Swiss-Prot were analyzed by ExPASy's ProtParam tool revealed that, the composition of alanine, glycine and leucine, valine were high while concentrations of asparagines, histidine, tyrosine, tryptophan, cysteine and lysine residues were low. The composition of other aminoacids was intermediate (Table 1 and 2). The numbers of negative

charged residues are more compared to positively charged residues. Molecular weight of Rsmmp3 was the highest while Rsmmp2 Metalloprotease had the lowest molecular weight. pI value of Rsmmp5 was the highest with 6.76 while the lowest pI 5.01 was seen in Rsmmp8. The instability index of all the metalloproteases was between 22 to 38 showing that most of them were stable. The relative volume of protein occupied by aliphatic side chains was found to span within a range of 99 to 113 (Table 3).

Table 4: Secondary structure analysis of different Zinc Metalloproteases from *Rhodobacter*

Zinc Matrix metalloproteases	Alpha helix	310 helix	Pi helix	Beta bridge	Extended strand	Beta turn	Bend region	Random coil	Ambiguous state	Other states
RsZnmp1	34.17	0.00	0.00	0.00	27.39	13.32	0.00	25.13	0.00	0.00
RsZnmp2	37.87	0.00	0.00	0.00	24.25	11.72	0.00	26.16	0.00	0.00
RsZnmp3	31.17	0.00	0.00	0.00	22.42	12.78	0.00	33.63	0.00	0.00
RsZnmp4	35.81	0.00	0.00	0.00	20.50	12.61	0.00	31.08	0.00	0.00
RsZnmp5	32.64	0.00	0.00	0.00	28.46	10.70	0.00	28.20	0.00	0.00
RsZnmp6	34.61	0.00	0.00	0.00	22.25	12.36	0.00	30.79	0.00	0.00
RsZnmp7	32.03	0.00	0.00	0.00	21.89	12.67	0.00	33.41	0.00	0.00
RsZnmp8	35.36	0.00	0.00	0.00	20.27	12.39	0.00	31.98	0.00	0.00
RsZnmp9	35.01	0.00	0.00	0.00	25.73	13.53	0.00	25.73	0.00	0.00

Table 5: CLUSTAL O (1.2.1) multiple sequence alignment results for Zinc Metalloproteases from *Rhodobacter*

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RsZnmp3      -----MDI-----IG-----LVPSFGGMAWTIIAFIVAILVIVAVHEYGHYIVGRW
RsZnmp7      -----MAWTIIAFIVAILVIVAVHEYGHYIVGRW
RsZnmp6      -----MEI-----VS-----LIPSPGGGLAYTVLAFVAALTIVIVFVHEYGHYIVARW
RsZnmp4      -----MDI-----VS-----LVPQFGGVIWTILAFVVALSIVVAVHEYGHYIVGRW
RsZnmp8      -----MDI-----VS-----LVPQFGGVIWTILAFVVALSIVVAVHEYGHYIVGRW
RsZnmp5      -----MADLFLNSFH-----TLSHGLVGVVVPFLVFLTVVFFHELGHFLVARW
RsZnmp9      -----MDAVGVVWNYGVVFLVVLTVLVFVHELGHYVVAR
RsZnmp1      MREGSPARGLLFLSACSGAPPGGAEETRAMPEIFHTVLAFLVLTAVVVFHELGHFLVARW
RsZnmp2      -----MLDLLHTVLSFLVVLTAVVVFHEFGHFLVARL
                :      : * : . : : * . * * * : * . *

RsZnmp3      SGIHAEVFSIGFGPVIWSRIDKRGTRWQLAALPFGGYVRFGLDSSAASGKDGALISQLSP
RsZnmp7      SGIHAEVFSIGFGPVIWSRIDKRGTRWQLAALPFGGYVRFGLDSSAASGKDGALISQLSP
RsZnmp6      SGIHADVFSIGFGPVLWSRVDKRGTRWQVAALPFGGYVRFGLDSSAASGKDGALISQLSP
RsZnmp4      SGIHAEVFSIGFGPVIWSRVDKRGTRWQVAALPFGGYVRFGLDSSAASGKDGALISQLSP
RsZnmp8      SGIHAEVFSIGFGPVIWSRVDKRGTRWQVAALPFGGYVRFGLDSSAASGKDGALISQLSP
RsZnmp5      NGVKVLTFSIGFGPEIVGFNDRYGRWKLAVPLGGYVRFGLDSSAASGKDGALISQLSP
RsZnmp9      NGVRVEVFSIGFGPEIVGFNDRYGRWKLAVPLGGYVRFGLDSSAASGKDGALISQLSP
RsZnmp1      NGVRVEVFSIGFGPEIVGFNDRYGRWKLAVPLGGYVRFGLDSSAASGKDGALISQLSP
RsZnmp2      NGVRVEVFSIGFGPEIVGFNDRYGRWKLAVPLGGYVRFGLDSSAASGKDGALISQLSP
                . * : . * * * * : . * : * * * * : . * * * * : * * : *

RsZnmp3      EERRHTMHGAPLWARAATVAAGPAFNFI F A I L V F A G F F L A Y G V A T D R P V V G A L K P M P E A T
RsZnmp7      EERRHTMHGAPLWARAATVAAGPAFNFI F A I L V F A G F F L A Y G V A T D R P V V G A L K P M P E A T
RsZnmp6      EQRRATMHGAPLWARAATVAAGPAFNFI F I L S I I V F A G I F L V N G V A T D R P T V G E V R P L P G V E
RsZnmp4      QERGRTMHGAPLWARAATVAAGPLFNFALS I L V F C A F F M V K G V A T E L P V V G E V K L P E A S
RsZnmp8      QERGRTMHGAPLWARAATVAAGPLFNFALS I L V F C A F F M V K G V A T E L P V V G E V K L P E A S
RsZnmp5      SERAVSFHHKPVGPRAAIVVAGPLANFILA V V L F T F L F S V F G V P N T S -----
RsZnmp9      EERARSFYHQSLGSRAAIVVAGPAANFAFA I V A L A L L F T V Y G Q P F T A -----
RsZnmp1      EEKAVCFHHKRVGQRAAIVVAGPLANFILA V L V F A G L F M I H G Q T R S V -----
RsZnmp2      AEEAVSFHHKRVGQRF A I V L A G P M A N F L F S I V V F A G L Y M T I G Q P H S A -----
                : . : : : * * * * * * * * : : : : : : *

RsZnmp3      QSLQPGDLILAVDQATPDLETYVAVGEKLPHQASFYRIERAGVETTLTGPHFPPIAD
RsZnmp7      QSLQPGDLILAVDQATPDLETYVAVGEKLPHQASFYRIERAGVETTLTGPHFPPIAD
RsZnmp6      AELRAGDVLILALEGTETPDFGSFIDAAGAHPLERLTQYRVDRGGQVTLRDAHPLPPVVE
RsZnmp4      QSLVEGDRILAI D G Q E T P T L S D F V R V A N E L P P A T A A Y R I E R D G A E M D V T A P Y P F P P V V D
RsZnmp8      QSLVEGDRILAI D G Q E T P T L S D F V R V A N E L P P A T A A Y R I E R D G A E M D V T A P Y P F P P V V D
RsZnmp5      -----ARVD
RsZnmp9      -----PVIE
RsZnmp1      -----PVID
RsZnmp2      -----PVVG

RsZnmp3      AVQPRSAAMEAGIKVGDVVTTVDGTPVVAFFQQLRDMVGS GGKTLHLQIWRDGTTEADL
RsZnmp7      AVQPRSAAMEAGIKVGDVVTTVDGTPVVAFFQQLRDMVGS GGKTLHLQIWRDGTTEADL
RsZnmp6      TVQPDSAARAAGIEAGDVI V S V N G Q P V A T F P Q L R E M V G S S D G K V L T L G L M R G E Q A L E M V L
RsZnmp4      AVQAPSGAHEAGIEAGDVL V E N G A P I A S F R E L R D A V G L S N G D P L T M T V V R A G E T Y E A S L
RsZnmp8      AVQAPSGAHEAGIEAGDVL V A N G A P I A S F R E L R D A V G L S N G D P L T M T V V R A G E T Y E A S L
    
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RsZnmp5      GVQPGSAAEAAGFKPGDVVTSINGSAISNFLEMQRFFVGAEEAGNQLKFTVKRGDSTVDLVA
RsZnmp9      EVSPDGAEEAGLLPGDRVLSIDGQTIERFEDITQLVVQYPGRPLALVVQRDGLVPPVTV
RsZnmp1      AVLAGSAAEAAGLKVGDRIVALNGKTVERFQDVQRRVALSDGKVMQVGLVRDGGQPLRVEV
RsZnmp2      EVIAGSAAEAAGLLAGDRIVAIDGTPIDRFQDVRVVPVPLSNGAPLHIDILRDNAPLAVIA
*      . . *   ** :   ** :   : : *   :   *   :   *   *   :   .   :   *

RsZnmp3      TPRRADLPLEAGGFETRWLIGLSGGGGMFTPEIRTPGPWETLTLAVDRVWYIVKVSLSASI
RsZnmp7      TPRRADLPLEAGGFETRWLIGLSGGGGMFTPEIRTPGPWETLTLAVDRVWYIVKVSLSASI
RsZnmp6      EPKRMDLPLESEGGFETRWLIGLGTG--ALYFEPETRRPAVLESVSLGAQQSWDIAVTSLSGL
RsZnmp4      TPRRMDIPLPTGGFETRWLIGLSG--GLLFEPEPTRTPGPLEAIWLGIQQTITITITTSLSGL
RsZnmp8      TPRRMDIPLPTGGFETRWLIGLSG--GLLFEPEPTRTPGPLEAIWLGIQQTITITITTSLSGL
RsZnmp5      TPQLKEIKDRFGNVQRLGILGISRSTAAGEVTTQVNPVAVAFWFMGIKETWVVDRTFSYI
RsZnmp9      TPRTVVEVDRFGNTHITIGRIGVLRG--ADEFK--KRDPLSAVWYAGKETLSLTLGLTKAV
RsZnmp1      QPRFVDNDDGLGNVTKMAQLGIQIKLSPGDVH--RLGPVQALEQVAVQTWTLSDADTLTYV
RsZnmp2      LPRMVEITDDGLGNKVQVAQLGVKVSLSQADVQ--RLGPLDALGQAVGQWQLSADTLTYL
* :   :   *   *   : : *   :   .   .   .   :   : :   :

RsZnmp3      WSMITGAISSCNMAGPIGMAEAMGDAARGGLEMFVQTLAMFSLGIGLMNLFPIPVLDGGH
RsZnmp7      WSMITGAISSCNMAGPIGMAEAMGDAARGGLEMFVQTLAMFSLGIGLMNLFPIPVLDGGH
RsZnmp6      WHMITGAISSCNLRGPIGIAETSAAAASAGLLSFIWFIAMLSLSTAVGLMNLFPVPLDGGH
RsZnmp4      WHMVTGAISSCNLQGPPIGIAEISGAAAASQAGNFIWFIAMLSLSTAVGLMNLFPVPLDGGH
RsZnmp8      WHMVTGAISSCNLQGPPIGIAEISGAAAASQAGNFIWFIAMLSLSTAVGLMNLFPVPLDGGH
RsZnmp5      GGIFTGREAADQLGGPLRIAQVSGQVATIGFTPLHLHAAVLSISIGLLNLFPPVPLDGGH
RsZnmp9      GQMISGTRGTDELGGPLRIAQMSGEVAQTGFVALVWFVALLSINLGLINLFPIMPDLGGH
RsZnmp1      GQMSIGFRSASELGGPIRIALFSGQAERGVADLIVFIALLSANLGLINLFPIMPDLGGH
RsZnmp2      GQVVRGNRSAAEELGGPVRIAQFSKAAERGVLDLVTFIALLSVNLGLINLFPIMPDLGGH
. :   *   . :   : : ** : *   *   . *   *   : :   * : *   : ** : ** : * : ** : ** :

RsZnmp3      LVFHVWEAVTGKPPSDGAMRILMTTGLVLLLLLMLVFAVTNDFLFCP-----
RsZnmp7      LVFHVWEAVTGKPPSDGAMRILMTTGLVLLLLLMLVFAVTNDFLFCP-----
RsZnmp6      LVFFAWWEAVTGKPPPTARALRVLMVAVGLTALITLMLVFALSNDLFCP-----
RsZnmp4      LVFHAYEAVAGKPPSDRVLRLVMTGGLAVLLSMLVFAVTNDFLFC-----
RsZnmp8      LVFHAYEAVAGKPPSDRVLRLVMTGGLAVLLSMLVFAVTNDFLFC-----
RsZnmp5      LLFYGIEAARGRPLSERAQELGFRIGLALVLMMLMFATYNDILHLASS-----
RsZnmp9      LLFYGIEAVRGRPLGERAQEYGFRIGLALVLTMLVFAVTNDFLHRLRVVQFFVDVLS
RsZnmp1      LMFYTI EALRGRPLTERIQEMGLKVGLALVVALMLFATWNDLGLKGLWG-----
RsZnmp2      LMFYTI EALRGRPLGARAQYGLRFGALVLMMLVFAVTNDFLHRLRVVQFFVDVLS
* : *   *   * : *   .   :   * : *   : : * : *   * : *
    
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Table 6: Percent Identity Matrix as calculated by Clustal 2.1 for Zinc Metalloproteases from *Rhodobacter*

100.00	100.00	59.33	62.84	62.61	35.73	38.25	37.87	37.26
100.00	100.00	59.12	62.50	62.27	36.16	38.61	39.12	37.57
59.33	59.12	100.00	62.61	62.39	37.97	39.18	41.44	39.56
62.84	62.50	62.61	100.00	98.87	38.61	39.56	40.48	39.67
62.61	62.27	62.39	98.87	100.00	38.87	39.84	40.48	39.94
35.73	36.16	37.97	38.61	38.87	100.00	52.72	46.72	47.68
38.25	38.61	39.18	39.56	39.84	52.72	100.00	51.89	54.95
37.87	39.12	41.44	40.48	40.48	46.72	51.89	100.00	68.12
37.26	37.57	39.56	39.67	39.94	47.68	54.95	68.12	100.00

Secondary structural analysis by SOPMA tool (Self-Optimized Prediction Method with Alignment) of the Metalloproteases showed the predominance of α -helices followed by random coils. Less number of beta strands was found in all Metalloproteases (Table 4). The sequences were aligned by Clustal Omega and Percent identity matrix (PIM) was used for secondary

structure prediction (Table 5). Swiss modeling was performed to predict the 3-D structure of the proteases (Figures 1 to 9). Further studies on metalloproteases from phototrophic bacteria will provide important additional information into the regulation of Nitrogenase enzyme involved in hydrogen production.

RsZnmp1

RsZnmp2

RsZnmp3

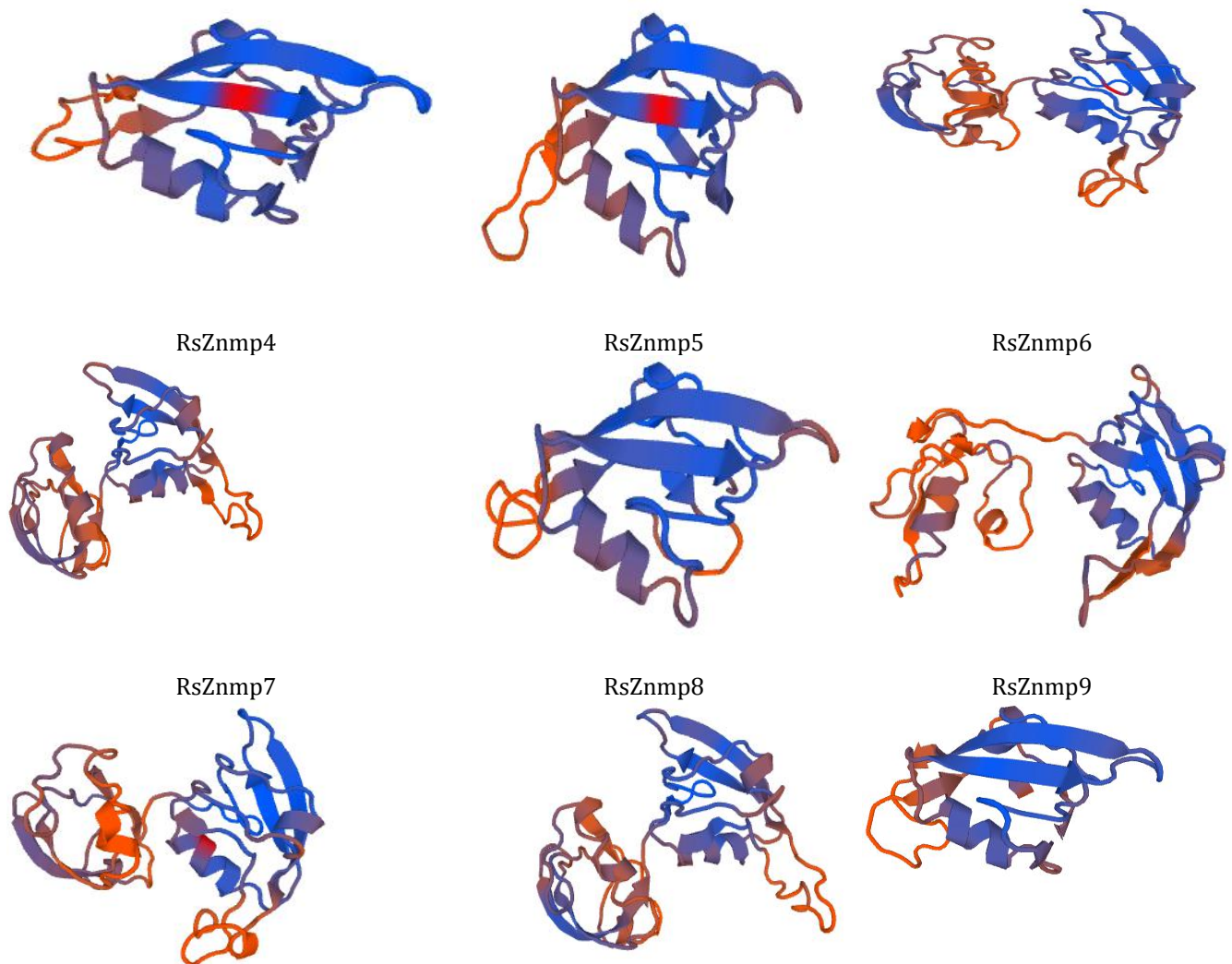


Figure 1 to 9: Three dimensional structure prediction using Swiss modeling for Zinc Metalloproteases from *Rhodobacter*

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