



Mutation study of DsrM from *Allochromatium vinosum* using the amino acid sequences[☆]

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ABSTRACT

Sulfur metabolism is one of the oldest known environmental processes. The operon involved in this process is called the *dsr* operon. The vital role of the operon is to maintain the environmental sulfur balance. The *dsr* operon of proteobacteria consists of 15 genes, viz. *dsrABEFHCMKIJOPNRS*. The proteins encoded by the *dsr* operon are essential for the transfer of sulfur globules from periplasm to cytosol and oxidation of the stored sulfur. In the present study we tried to analyze the probable molecular details of the DsrM proteins from a diverse set of microbial species using their sequence information. There are certain mutations in the sequences of the DsrM proteins from the different proteobacterial species. The effects of mutations in the sequences of DsrM proteins were predicted from the evolutionary point of view. This is so far the first report of its kind. Our study would therefore enable the researchers to predict the hitherto unknown biochemistry of sulfur oxidation using the amino acid sequences of the DsrM proteins.

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1. Introduction

Sulfur cycle is one of the important biogeochemical cycles in the world. Sulfur has a wide range of oxidation states viz., +6 to −2 and this makes the element capable of taking part in a number of different biological processes. Sulfur based chemo or photolithotrophy is one of such processes involving the transfer of electrons from reduced sulfur compounds. The sulfur metabolism is mediated by a diverse set of microorganisms. These prokaryotes are abundant in nature and are important because of their environmental as well as industrial importance. The different sulfur anions that are abundant in nature and are used by these sets of microorganisms are sulfide, polysulfide, thiosulfate, as well as elemental

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sulfur. Only very little is known about the molecular mechanisms of this ecologically as well as industrially important process. One of them is *Allochromatium vinosum* a dominant member of the purple sulfur bacteria (Weissgerber et al., 2011). Recent studies with *A. vinosum* revealed that a multiple gene cluster comprising of genes *dsrA*, *dsrB*, *dsrE*, *dsrF*, *dsrH*, *dsrC*, *dsrM*, *dsrK*, *dsrL*, *dsrJ*, *dsrO*, *dsrP*, *dsrN*, *dsrS* and *dsrR* is involved in the process [2]. The organism *A. vinosum* has been used in different industrial processes, viz., waste remediation and removal of toxic compounds, e.g. odorous sulfur compounds like sulfide or even explosives (Kobayashi and Kobayashi, 1995; Siefert et al., 1978; Soli, 1973). This organism has also been used in the production of industrially relevant organo-chemicals such as vitamins or bio-polyesters (Liebergessell and Steinbüchel, 1996; Sasikala and Ramana, 1995a, 1995b) and the production of biohydrogen (Sasikala et al., 1993). From the currently available literatures it was revealed that DsrM is one of the key components of the sulfur metabolizing gene cluster (Grein, 2010). DsrM is proposed to be an integral membrane protein with five transmembrane helices. DsrM is a quinone-reactive di-heme cytochrome b. DsrM may work as a quinol oxidase donating electrons to DsrK (Grein et al., 2010). DsrK is proposed to be a cytoplasmic iron sulfur protein, and the catalytic subunit of the DsrMKJOP protein complex (Pott and Dahl, 1998). DsrJ is a periplasmic protein which covalently binds three hemes c, one of which is characterized by an unusual His/Cys ligation (Pires et al., 2006). DsrO is a periplasmic iron–sulfur, ferredoxin-like protein (Grein, 2010). DsrP is an integral membrane protein with ten predicted transmembrane helices and belongs to the NrfD/PsrC protein family (Grein et al., 2010).

The putative function of DsrM is known but the biochemical mechanistic details of the function of the DsrM protein is not yet understood. So we made an attempt to characterize DsrM protein at the sequence level. We analyzed the amino acid sequences of DsrM proteins from 30 different microorganisms. We predicted the putative conserved domains present in the DsrM protein. Sequence analyses of the DsrM proteins revealed the presence of certain mutations in the protein. We also predicted the effects of those mutations present in the conserved domain of DsrM protein and correlated the effects of mutations with the taxonomical distributions of the microorganisms. Till date there are no such reports that deal with the analyses of the mutations in the DsrM proteins using bioinformatics approach. This work is therefore first of its kind. Since there are no previous reports regarding the molecular details of DsrM proteins our work would therefore be important to analyze the biochemical details of the *dsr* operon.

2. Materials and methods

2.1. Sequence homology search and pair wise alignment of sequences

We first download the complete genome of *A. vinosum* strain DSM 180 chromosome (NCBI Reference Sequence: NC_013851.1). The culture collection ID is DSM 180, ATCC 17899 and GOLD stamp ID is Gi02104. The organism was isolated from ditch water. The habitat details and properties of the organism are as follows: oxygen requirement – anaerobe, cell shape – rod-shaped, motility – motile, sporulation – non sporulating, temperature range – mesophile, temperature optimum – 25 °C, gram staining – gram negative, biotic relationship – free living, disease causing – none, habitat – fresh water, and energy source – phototroph. The bacteria were grown anaerobically in the light in RCV medium at a temperature of 30 °C and the DNA was isolated from 50 mg cell pellet. The genome of the bacteria was sequenced at the Joint Genome Institute (JGI) using a combination of Illumina and 454 technologies (Weissgerber et al., 2011). The DsrM protein sequence was extracted from *A. vinosum* having a protein ID ADC62196.1. Initially 42 amino acid sequences of DsrM proteins were extracted from refseq of NCBI of different microorganisms. Then 30 amino acid sequences of DsrM proteins with 50% and more than 50% sequence identities were chosen for our study from BLAST (Altschul et al., 1990) search results. We removed the uncultured bacterial species and redundancies from all the collected sequences. Only those amino acid sequences that were chosen where clear annotations and no ambiguities were present. NCBI refseq was selected for collecting our required sequences because it provides comprehensive, integrated, non-redundant and a well-annotated set of sequences. The accession numbers of the DsrM proteins of the finally selected microorganisms were presented in Table 1.

These sequences were used as inputs to run the program BLAST (Altschul et al., 1990), using the default parameters, in order to find out the conserved domains in the DsrM protein. The BLAST results again produced the same set of sequences as obtained previously. This could be considered as a double check of

Table 1

List of DsrM proteins of the 30 different proteobacteria used in our analysis.

Species	Protein accession number
Seq1: <i>Allochromatium vinosum</i>	[GenBank: YP_003443228.1]
Seq2: <i>Alkalilimnicola ehrlichii</i> MLHE-1	[GenBank: YP_742495.1]
Seq3: <i>Beggiatoa alba</i> B18LD	[GenBank: ZP_10114655.1]
Seq4: <i>Beggiatoa</i> sp. PS	[GenBank: ZP_02001527.1]
Seq5: <i>Beggiatoa</i> sp. SS	[GenBank: ZP_01997347.1]
Seq6: <i>Burkholderiales bacterium</i> JOSHI_001	[GenBank: ZP_09752884.1]
Seq7: <i>Candidatus Ruthia magnifica</i> str	[GenBank: YP_904051.1]
Seq8: <i>Candidatus Vesicomysocius okutanii</i> HA	[GenBank: YP_001219619.1]
Seq9: endosymbiont of <i>Bathymodiolus</i> sp.	[GenBank: ZP_09785184.1]
Seq10: endosymbiont of <i>Tevnia jerichonana</i>	[GenBank: ZP_08817265.1]
Seq11: <i>Halorhodospira halophila</i> SL1	[GenBank: YP_001003523.1]
Seq12: <i>Magnetococcus marinus</i> MC-1	[GenBank: YP_863984.1]
Seq13: <i>Magnetospirillum gryphiswaldense</i> MSR-1	[GenBank: CAM75802.1]
Seq14: <i>Magnetospirillum magneticum</i> AMB-1	[GenBank: YP_422736.1]
Seq15: <i>Magnetospirillum magnetotacticum</i> MS-1	[GenBank: ZP_00052646.1]
Seq16: <i>Magnetospirillum</i> sp. SO-1	[GenBank: EME69579.1]
Seq17: <i>Marichromatium purpuratum</i> 984	[GenBank: ZP_08774771.1]
Seq18: <i>Rhodomicrobium vannielii</i> ATCC 17100	[GenBank: YP_004010972.1]
Seq19: <i>Sideroxydans lithotrophicus</i> ES-1	[GenBank: YP_003524300.1]
Seq20: <i>Sulfuricella denitrificans</i> skB26	[GenBank: ZP_10383243.1]
Seq21: <i>Thioalkalivibrio nitratireducens</i> DSM 14787	[GenBank: YP_007216025.1]
Seq22: <i>Thioalkalivibrio sulfidophilus</i> HL-EbGr7	[GenBank: YP_002514258.1]
Seq23: <i>Thioalkalivibrio thiocyanoxidans</i> ARh 4	[GenBank: ZP_08930267.1]
Seq24: <i>Thiobacillus denitrificans</i> ATCC 25259	[GenBank: YP_316237.1]
Seq25: <i>Thiocapsa marina</i> 5811	[GenBank: ZP_08771170.1]
Seq26: <i>Thiocystis violascens</i> DSM 198	[GenBank: YP_006412734.1]
Seq27: <i>Thioflavicoccus mobilis</i> 8321	[GenBank: YP_007242654.1]
Seq28: <i>Thiorhodococcus drewsii</i> AZ1	[GenBank: ZP_08823216.1]
Seq29: <i>Thiorhodovibrio</i> sp. 970	[GenBank: ZP_09866978.1]
Seq30: <i>Thiothrix nivea</i> DSM 5205	[GenBank: ZP_10105081.1]

our initial results of downloading the sequences. The BLAST search results revealed the presence of a conserved domain of the family of proteins belonging to the Nitrate red gam protein family.

2.2. Prediction of transmembrane helix region

The amino acid sequences of the proteins were further used to find the membrane spanning regions. The DsrM protein has five transmembrane helices (Grein et al., 2010). The transmembrane topology was predicted from the amino acid sequence of DsrM by averaging the results from six different programs: DAS (Lavigne et al., 2000), HMMTOP (Rost et al., 1995), TMHMM (Tusnady and Simon, 1998), TMPRED (Sonnhammer et al., 1998) TOPPED II (Claros and Von Heijne, 1994) and GENEIOUSPRO. We used different software tools in order to have a consensus result (Bagchi, 2013; Bagchi and Ghosh, 2006).

2.3. Multiple sequence alignment (MSA)

In order to study the sequence conservations among the 30 DsrM proteins we generated a sequence profile by MSA, using the default parameters in the software tool ClustalW (Thompson et al., 1994). We used the sequence of the conserved domain from all the 30 DsrM proteins as mentioned in Section 2.1 of the manuscript. From the results of MSA the presence of mutations in the sequences were detected. The result was presented in Fig. 1. The MSA, showed the presence of conserved sites and mutations in the sequences.

2.4. Mutation study within the conserved protein domain

The functions of the different parts of the DsrM protein were predicted from the outputs of Pfam (Bateman et al., 2004). The amino acid sequences of the aforementioned 30 proteins were incorporated one by one to Pfam and the results were analyzed. Pfam analyses of the protein sequences present in the conserved domain of the DsrM proteins enabled us to detect the presence of additional sub domains in the DsrM proteins. These additional sub domains are involved in different metabolic processes as required by the organisms depending on their habitat.

3. Result & discussion

3.1. Predicted transmembrane helix pattern of DsrM

The DsrM proteins were proposed to be integral membrane proteins (Grein et al., 2010). The proteins were found to be rich in hydrophobic helical regions as observed in case of transmembrane proteins (Lodish). The transmembrane topology was predicted from the amino acid sequences of DsrM proteins using six different web servers. All the software tools produced nearly identical results (Table 2). This further established the likelihood of the hydrophobic helical regions present in the DsrM proteins comprised of the five transmembrane helices. The transmembrane topology was predicted from the amino acid sequence of DsrM of *A.vinosum* using Geneious Pro shown in Fig. 2.



Fig. 1. Multiple sequence alignment of DsrM from 30 different proteobacteria using ClustalW default parameters, showing their conserved/variable regions by * and . respectively.

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Seq4      FGKYASFAMFLGFLIGLLIRRMVERIRYISSPSDYLMILLLVIGGSGTMITFVT-HTDI 101
Seq5      FQYQGSFVMLFGLLGLLVRRLFVERIRYISNPSDYLMILLLVIGSGVMLSFVT-RTDI 101
Seq3      LGIYAGFIFIFLGLIGLLRRLCLERIRYISSPSDYLMLLLLLIIAGSGLLMRFFIL-HTDI 119
Seq1      FGIYGGIAMVVGLAGLWARRFLVDRVKYITSTSDHLILGLLVAIGCTGLLMKFFV-HVDV 94
Seq28     YGIYGGFAMIVGLAGLWVRFFVDRVKYITSLSDHLILGLLIIIGCTGLLMKFFV-HVDV 95
Seq26     FGIYGGMAMIVGLAGLWARRFLVDRVRYISSPSDHLILALLIIGCSGLLMKFFV-HTDV 97
Seq25     FGIYGGIAMVVGLAGLWARRFFVDRVRYISSPSDHLILALLIAGCTGLLMKFFV-HTDV 94
Seq17     FGIYGGIAMVIGLIGLWARRFLVDRVRYISSTSDHMLALLVIGTSGLLMKFFV-HTDV 93
Seq27     FGIYGGIAMVVGLAGLWARRVLVDRVRYITSPDHLILLLLLIIGVSGLSMKFAV-HTDI 98
Seq29     FGIYGGMAMIVGLAGLWTRRFVDRVRYISSRSDHLILGLLIIAGTGLMMRFV-HTDI 99
Seq2      FGLYAGFVMVAALIGLWIRRIIGVARIKAITGSPDHLMLALLVAIGLSGLGMKYIQ-PTDI 101
Seq11     LGLYAAFYVLAALVGLWLRRLWIDRVRHISTPSDHAALALLLAIIGTGLGLQHFQ-PTDI 102
Seq21     FGKYAAFAMVIGLAGLWARRVFFVERIRYISSPSDHLMLALLIIGTGLAMTYLW-HVDI 101
Seq23     FGKYAAFAMVIGLAGLWARRLFVERIRYISSPSDHLMLALLIIGTGLAMTYLW-HVDI 102
Seq22     FGIYAGFAMVAALFGLWARRLFVERIRYISSPSDHLMLVLLIIGASGLAMKYIW-HTDI 99
Seq18     LGIYAGFAMVGFVWVRVAISRMRYSIDAFDHLILLLLLIAGTGLGMTFIA-PTDI 101
Seq6      FGIYAGFAMVAGLAGLWARRFLVDRVRYISTPSDHLHLALLIIGTGLGMRFFVA-PTDI 90
Seq20     FGKYAGFAMVAGLGLWARRFLVDRVRYISTPSDHLMLVLLIIGLSGLGMSFVA-HTDI 103
Seq24     FGKYASIMMVIGLGLLARRFLVDRVRYISTPSDYLMVLLLAIIGLSGMMTFTVT-HTDI 103
Seq19     FGMYAGITMLLGLGLLWLRRLFVERIRYISTPSDHLMLLLMGIAASGLSMKFLA-HTDV 92
Seq14     LAQPAGLAMVAGLALLLVRRLVVERVRYVTGSPDI LMLVLLIIGVSGMLKCVF-HTDI 98
Seq16     LAQPAGLAMVAGLTL LLLARRFVIERVRYVTGSPDI LMLALLLAIIGVTGMLKCVI-HTDI 97
Seq15     LAIPAGLAMVAGLALLLARRFLIPRVRYVTGSPDI LMLVLLIIGVSGMAMKCLV-HTDI 99
Seq13     FGIYGGMAMIAGLAALLVRRIVARIAYISGPSDYAMLVLLLAIIGASGMMMKVA-HTDI 101
Seq30     FGRYAGITMVLGLAGLWARRFLVDRVRYISAPSDHLMLALLIAGSGLMMSFVS-HTDV 101
Seq7      PFKYAAPTMTVIGLVGLMLRRIFVERIRYISAPSDYLMIMLLIIGISGAVMTFTSNHTDI 102
Seq8      PFKYAAPTMTVIGLIGLILRRIFVERIRYISAPSDYLMIMLLIIGISGAVMTFTSNHTDI 102
Seq9      PFKYAAPTMTVIGLIGLMGRRIAIERIRYISAPSDYLMILLLVIGSGVVMFTFTSNHTDI 101
Seq10     IGKYAAFAMVAGLVGLLVRRI FVDRVRYISAPSDYLLWLLMLVIGLSGLMFTFV-HTDV 101
Seq12     VGIVAGIAMVGGGLFLFRRLMVDVFRHISSKADYLLI LLLLMAIGSGLLMTFAI-HPDV 92
          .. . * * * * . * . : : * * * : * . : * : * : * :
Seq4      VQFKAFMLSVMYLVNFGQHL LPTDGFLLHLTSVILLMIIFPFSKLLHA----- 151
Seq5      VQFKAFMLSVMYFDFFN-WQPIPTDKMLLVHLSVILLMIIFPFSKLLHA----- 150
Seq3      VQFKIFMRSLFS--FQ-WQAIPTDPVLLLHLGSALLLLLPPFSKLLHATGVFPSPSRY 175
Seq1      VEIKTFFLGLYSYDIEP---LPADPLLLLHLLLVALLMIIFPYSKLLHM----- 140
Seq28     VEIKTFFQGLYSFDIKP---LPADPLLLLHLLVSLMIIFPYSKLLHA----- 141
Seq26     VEIKAFFLGLYSYDIQP---LPADPLLLLHLFLVAVLMIIFPYSKLLHA----- 143
Seq25     VAVKSPFIFGLYSFDQP---LPADPLLLLHLFLVAALMIIFPYSKLLHA----- 140
Seq17     VAIKSPFIFGLYALDPEP---LPADPLLLLHLLLVALLMIIFPYSKLLHA----- 139
Seq27     VAVKSPFIFGLYALDQVNP---LPADPLLLLHLLVAVLMIIFPYSKLLHA----- 144
Seq29     IRVKEFFAGLYQFEVVFV---LPADPVL LLLLHLLLVALLMIIFPYSKLLHA----- 145
Seq2      TVGKAYMLGLMRFDWQP---LPADPL LLLLHLLLVALLMIIFPFSKLLHA----- 147
Seq11     VAVKAFFIGLMQFNWQP---LPSEPL LLLLHLTLVALLMMVFPFSKLLHAA----- 149
Seq21     TAVKLFFTGMRFDNFNP---LPAHPML LLLLHLLLVAGLMIIFPFSKLLHA----- 147
Seq23     TAVKLFFTGMRFDNFNP---LPAHPML LLLLHLLLVAGLMIIFPFSKLLHA----- 148
Seq22     TAVKLFFLGLMRFDIQP---LPTDAM LLIHLTLVAAALMIIFPFSKLLHA----- 145
Seq18     VALKGFPLGLMAFNWQP---LPADPL LLSHLALVALLMVFPFTKLLHA----- 147
Seq6      VALKAFMLGLVRLDIQP---LPTDPL LLLLHLALVALLMAVFPISKLLHA----- 136
Seq20     VAVKEFFLGLMVFDLHP---LPADPVL LVLVHLVALLMVFPISKLLHA----- 149
Seq24     IALKGFPLGLMYLDIQP---LPQDPAL LVHLALVALLMVFPISKLLHA----- 149
Seq19     IAVKEFFLGLMHLNIQA---LPADLL IIVHLSLVVSLVFPFSKLLHA----- 138
Seq14     VAVKAFFTGMLGFELRP---LPADPL LGLHLLLVCLLMIIFPFSKLLHA----- 144
Seq16     VMVKAFFTGMLGFELRP---LPADPL LGLHLLLVCLLMIIFPFSKLLHA----- 143
Seq15     VAVKAFFTGLTSLRLRP---LPDPL LGLHLLLVCLLMIIFPFSKLLHA----- 145
Seq13     IAVKAFFIGLVMFELRP---LPADPL IGLHLLLVAAALMVFPFSKLLHA----- 147
Seq30     TVQKAFPPGMLTTFGFFGEEGGLPADPIVLVHLLLVALLMIIFPISKLLHA----- 151
Seq7      IMVKEFASGLVFNFWAN---LPTEVH FLVHIFLVFVLMVIFPISKLLHV----- 148
Seq8      IMVKEFTSGLVFNWKN---LPTEVY FLIHI FLVFLVLMVIFPISKLLHI----- 148
Seq9      IMVKHFASGLLSFDWGN---LPTEVH FLVHIFLAFSLLAIFPISKLLHV----- 147
Seq10     VMVKQFDGLPTFSGGA---LPMDFAL LVHLLLVAVLMLLPFSKLLHI----- 147
Seq12     VEIKAAMMGVLGMQGFEE---TPSDV VFLHFLVLLVALLMIIFPFSKLMHMVG----- 140
          . * . * . * . * : : * * * : * : * : * :

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Fig. 1 (continued).

Table 2

Result of six different servers used for five transmembrane helix regions.

Server name	Transmembrane helix I	Transmembrane helix II	Transmembrane helix III	Transmembrane helix IV	Transmembrane helix V
Das	8–23	77–88	111–123	143–162	188–206
TMHMM	4–23	71–93	108–130	142–164	184–206
HMMTOP	7–24	71–88	107–124	143–162	189–206
TOPRED II	4–24	69–89	105–125	142–162	187–207
TMPRED	6–24	70–86	104–125	143–161	183–205
Geneious Pro	3–23	74–94	110–130	143–163	184–204

3.2. Functionally conserved domain of DsrM

DsrM proteins play the important roles in the transfer of elemental sulfur from extracellular region to cytosol (Grein et al., 2010). It is also well established from various works that DsrM is a quinone-reactive di-heme cytochrome b protein (Grein et al., 2010). So, to search whether or not there was any functional diversity in DsrM of different proteobacterial species, we ventured into Pfam (Bateman et al., 2004)-based functional studies. Pfam search results revealed that the most conserved region of the DsrM proteins in all of the 30 different organisms had the signature sequence similar to the Nitrate reductase, gamma subunit (Short name: Nitrate_red_gam). In *A. vinosum* this conserved protein domain has been found to be present at amino acid residues 73–212. In other organisms this domain covers nearly the same set of amino acid residues as observed in the case of *A. vinosum*. Nitrate reductase gamma subunit resembles cytochrome b and transfers electrons from quinones to the beta subunit of cytochrome b (Pantel et al., 1998).

3.3. Functional and mutational analysis

The amino acid sequences of the conserved domain i.e. Nitrate red gam family domain in all the 30 organisms obtained from Pfam search were analyzed from the sequence profile that was generated by MSA using the software tool ClustalW (Fig. 2). The MSA results showed the presence of 16 synonymous substitutions in the global alignment of the amino acid sequences from the conserved domains of the DsrM proteins. The remaining positions of the alignment have undergone some kind of significant mutations. We analyzed those significant mutations using Pfam and obtained the presence of some new sub domains in the DsrM proteins. The significant sub domains analyzed from Pfam were described below:

The amino acid sequence of DsrM protein of *A. vinosum* had a significant mutation at amino acid residue position 171. Pfam search results revealed that the mutation created a new sub-domain the Permease FtsX-like (IPR003838) domain spanning the amino acid residues 143–212. The amino acid sequence of DsrM protein from another gammaproteobacteria, *Thioflavicoccus mobilis* 8321 from chromatiaceae family (Imhoff and Pfennig, 2001) had a significant mutation at amino acid residue position 100. Pfam search results revealed that the mutation created a new sub-domain called the Malvel (IPR008253) domain. MALVEL [MAL (myelin and lymphocyte) and related proteins for vesicle trafficking and membrane link] domain comprises of a four transmembrane-helix (Sánchez-Pulido et al., 2002). The amino acid sequences of DsrM proteins of *Candidatus Ruthia magnifica* str., *Candidatus Vesicomysocius okutani* HA, and endosymbiont of *Bathymodiolus* sp. had a significant mutation at amino acid residue positions 163. Pfam search results revealed that the mutation created a new sub-domain called Ni_hydr_CYTB-Cytochrome b561, bacterial/Ni-hydrogenase (IPR011577) (http://microbewik.kenyon.edu/index.php/Candidatus_ruthia_magnifica). Cytochrome b561 is an integral membrane and electron transport protein that binds two haem groups non-covalently and interacts with quinines. In the DsrM proteins from *C. R. magnifica* str. and endosymbiont of *Bathymodiolus* sp. showed the presence of two more protein sub-domains called PepSY-associated TM helix (IPR005625) [amino acid position 146–160] and Sulfate transporter (IPR011547) [amino acid position 145–205] respectively. PepSY-associated TM helix represents a conserved transmembrane (TM) helix. Coil residues are significantly more conserved than

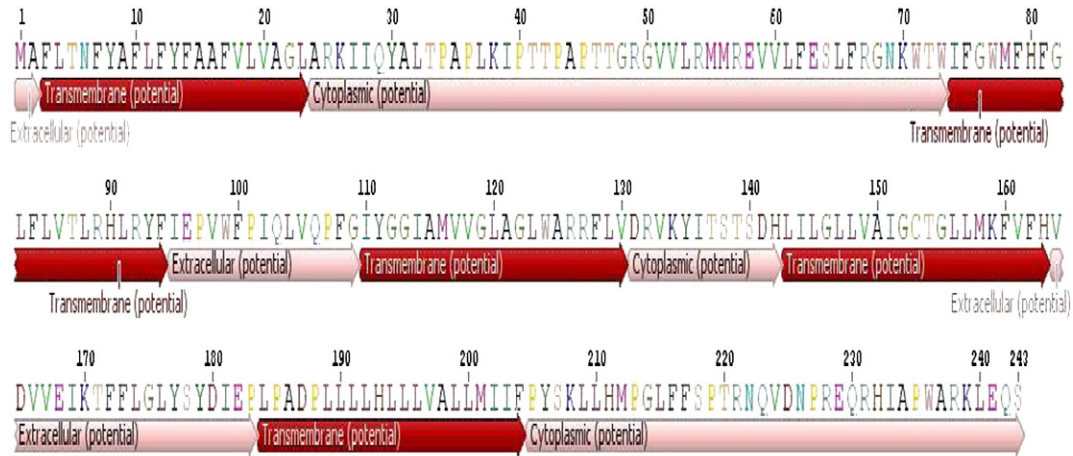


Fig. 2. Integral membrane protein, DsrM of *Allochromatium vinosum* showing presence of extracellular, 5 trans-membrane helices and cytoplasmic domain using Geneious Pro software.

other residues and are frequently found within channels and transporters, where they introduce the flexibility and polarity required for transport across the membrane (Kauko et al., 2008). Sulfate transporter is involved in the transport of sulfate across a membrane (Sandal and Marcker, 1994; Smith et al., 1995). The DsrM protein from *C. V. okutanii* HA showed the presence of a new sub-domain Sodium/calcium exchanger (IPR004837) at amino acid position 144–204. Sodium/calcium exchanger participates in ion transport in melanosomes (Lamason et al., 2005). This organism also shows another synonymous substitution at amino acid position 96. Pfam search result of the amino acid sequence of DsrM protein from *C. V. okutanii* HA showed the presence of a new sub-domain called Ferric reductase transmembrane component-like domain (IPR013130). This protein family may be a family of flavocytochromes capable of moving electrons across the plasma membrane (Roman et al., 1993). The amino acid sequence of DsrM protein of another sulfur-oxidizing endosymbiont of *Tevnia jerichonana* showed a significant mutation at amino acid residue position 168. Pfam search results revealed that the mutation created a new sub-domain called CopD, Copper resistance D (IPR008457) spanning the amino acid residues 140–200. Copper resistance operon (cop) CopD, together with CopC, perform copper uptake into the cytoplasm (Cooksey, 1994). Another significant protein sub-domain namely MerC, Mercury resistance protein (IPR004891) was found in the amino acid sequences of DsrM proteins of *Sulfuricella denitrificans* skB26 (betaproteobacteria) and *Thioalkalivibrio thiocyanoxidans* ARh 4 (gammaproteobacteria) at amino acid residue positions 159 and 158 respectively (Kojima and Fukui, 2010; Sorokin et al., 2002). The mercury resistance protein, MerC, is an inner membrane protein that mediates Hg²⁺ transport into the cytoplasm, thereby conferring mercury resistance (Liebert et al., 2000). The amino acid sequence of DsrM protein of *Halorhodospira halophila* SL1 had a significant mutation at amino acid residue position 133 creating a new sub-domain called the BioY protein (IPR003784) sub-domain spanning the amino acid residues 107–167. BioMNY, a high-affinity biotin transporter, is a member of the ECF (energy-coupling factor) transporters (Finkenwirth et al., 2010).

All the species discussed here have significant mutations in the amino acid sequences in the conserved 'Nitrate red gam protein family' which is the signature protein domain of the DsrM proteins. The presence of these mutations created some significant protein sub-domains that confer some additional functionalities to the DsrM proteins in these organisms. The presence of the mutations in the DsrM proteins correlates with the habitats of the organisms. By and large, these protein sub-domains are found to be present in the integral transmembrane ion transporters. The presence of these additional sub-domains would therefore be safely considered to facilitate the function of DsrM proteins as well as would be required for the survival of the organisms in their respective environments.

4. Conclusions

In this work we tried to analyze the details of DsrM protein of the *dsr* operon. The operon is involved in the balancing and utilization of environmental sulfur compounds. The DsrM protein is one of the central players of the *dsr* operon. The analysis of DsrM protein has revealed the presence of a conserved domain. There are certain mutations present inside the conserved domain of the protein. Those mutations confer some additional functionality to the protein. We also predicted the amino acid residues involved in membrane spanning region in the DsrM protein. There were no previous works that deal with the mutational aspect of DsrM proteins from their amino acid sequences. So, our study is the first of its kind. Our study would therefore pave the pathway to future genetic and mutational studies using DsrM proteins that would lead to illumination of the biochemical mechanism of sulfur metabolism.

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