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Homology Modeling and *In silico* Docking Studies of DszB Enzyme Protein, Hydroxyphenyl Benzene Sulfinate Desulfinase of *Streptomyces* sp. VUR PPR 101

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Abstract

Biodesulfurization of organosulfur compounds in fossil fuels by employing microbes is advantageous over traditional hydrodesulfurization. Dibenzothiophene (DBT) is the most common model organosulfur compound used in biodesulfurization studies by means of microbes. The microbial desulfurization of DBT via the 4S pathway involves four enzymatic steps. The present study investigated the activity of wild type DszB (Hydroxyphenyl benzene sulfinate desulfinase), the last enzyme in the 4S pathway, and several mutant forms. The 3-D protein model of DszB was developed and mutant proteins of DszB viz., Q65H, Y63F and Y63A were constructed. Docking studies were done between wild DszB and the substrate, hydroxy phenyl benzene sulfinate (HPBS) as well as between mutant DszB proteins and HPBS. Based on the libdock scores obtained from docked complexes, mutant protein Y63A was found to have highest affinity towards the substrate, HPBS likely suggesting highest activity.

Keywords: Biodesulfurization, Dibenzothiophene (DBT), Hydroxyphenyl benzene sulfinate (HPBS), 4S pathway, DszB, Docking.

1. Introduction

Fossil fuels, containing organosulfur compounds, get oxidized during their utilization for various purposes and release various hazardous gases including sulfur dioxide leading to air pollution (Rhee *et al.*, 1998). Sulfur dioxide, during its persistence period of one to seven days, transforms into sulfates under the influence of sunlight and photochemical oxidants and hence, serves as reservoir of toxic sulfates and sulfuric acid in the air (Rall, 1974). Chronic exposure of humans to sulfur dioxide results in respiratory infections, pulmonary impairment, asthma, Emphysema, etc. (Badenhorst, 2007; Mehta, 2010). Sulfur dioxide also causes deleterious effects in plants by decreasing photosynthetic efficiency as well as promoting enhanced opening of stomata, which results in excessive loss of water in plants, and ultimately leads to the reduction of quality and quantity of plant yield (Varshney *et al.*, 1979). Acid rain with sulfurous acid, formed from sulfur dioxide in the air, as one of the major components is hazardous to aquatic life, vegetation and human health. Human beings may suffer from brain damage, kidney problems and Alzheimer's disease, when they consume plant or animal products that absorbed soil toxins that leached due to acid rain (Wondyfraw, 2014).

The hydrodesulfurization process normally employed by oil refineries to eliminate the organosulfur compounds from oil is not so effective particularly in the removal of polycyclic aromatic organosulfur compounds (Rhee *et al.*, 1998). Biodesulfurization by means of microorganisms that selectively attack organosulfur compounds and remove sulfur atoms appears to be a most viable and genuine method over the traditional hydrodesulfurization (Calzada *et al.*, 2009). Organosulfur compounds mainly dibenzothiophene (DBT) and its derivatives are unaffected by traditional hydrodesulfurization of crude oils. Therefore, DBT is treated as a model compound for desulfurization studies (Abo-State *et al.*, 2014). In nature, some microorganisms degrade organosulfur compounds by breaking the ring skeleton of organosulfur compounds leading to a reduction of the calorific value of the fuel. Hence, such microbes are not considered commercially viable. In the contrary, some microorganisms metabolize organosulfur compounds by selectively removing the sulfur atom without breaking the ring structure of the compounds. Such microorganisms are equipped with a specialized enzymatic pathway called the 4S pathway, which specifically removes the sulfur atom from DBT, the

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model organosulfur compound (Campos-Martin *et al*., 2010). Hence, microbes exhibiting DBT desulfurization via the 4S pathway which includes four enzymatic reactions, are obviously commercially important. The prominent DBT desulfurizing bacteria exhibiting the 4S pathway include *Rhodococcus rhodochrous* IGTS8, *Rhodococcus erythropolis* D-1, *Corynebacterium* sp. strain SY1, etc. (Rhee *et al*., 1998). The first enzyme, DBT monooxygenase, catalyzes a two-step oxidation reaction which results in the formation of $DBTO_2$ (Dibenzothiophene oxide) from DBT. The second enzyme, $DBTO_2$ monooxygenase, catalyzes the conversion of $DBTO_2$ to HPBS (Hydroxyphenyl benzene sulfinate). The third enzyme, HPBS desulfinase, catalyzes the hydrolysis of HPBS to form the end products of the pathway, 2-HBP (2-Hydroxy biphenyl) and sulfite (Folsom *et al*., 1999). The 4S pathway enzymes are synthesized by *dsz* operon genes, *dszA*, *dszB* and *dszC*. The *dszA* and *dszC* genes encode flavin dependent $DBTO_2$ monooxygenase (DszA) and DBT monooxygenase (DszC), respectively. The *dszB* gene synthesizes HPBS desulfinase (DszB) (Duarte *et al*., 2001). Using PCR, *dsz* operon genes can be amplified and sequenced (Shavandi *et al*., 2010).

The DBT desulfurization activity is directly proportional to the overall activity of the 4S pathway enzymes. To enhance the DBT desulfurization activity, the activity of the 4S pathway enzymes must be increased. The activity of the 4S pathway enzymatic proteins can be enhanced using computational programs in protein engineering. Protein engineering emphasizes on developing modified proteins by replacing amino acids at specific sites and substrate interaction at the catalytic site (Prokop *et al*., 2000). The current study presents the results of docking studies between wild type DszB protein, translated from the nucleotide sequence of *dszB* gene of *Streptomyces* sp. VUR PPR 101, and the substrate, HPBS, as well as between mutant DszB proteins, constructed via protein engineering by replacing single amino acids at selected sites and HPBS. These results demonstrate the reactivity of wild type and mutant DszB enzyme proteins towards HPBS.

2. Material and Methods

2.1. Translation of dszB Gene Sequence into Protein Sequence

The sequence of *dszB* gene of *Streptomyces* sp. VUR PPR 101 was submitted to NCBI-ORF Finder in FASTA format to generate different reading frames and the frame with highest length was selected for the study (Hung and Lin, 2013).

2.2. Homology Modeling of DszB Protein

The sequence of DszB protein in FASTA format was submitted to SWISS-MODELWORKSPACE automated mode to develop a protein model by homology modeling (Bordoli *et al*., 2008). DszB protein and its sequence were designated as target protein and query sequence, respectively.

2.3. DszB Protein Validation

The modeled DszB protein quality was validated by Ramachandran plot using Rampage (Read *et al*., 2011) and in SPDBV (Deep View – Swiss – Pdb Viewer) version 4.10 based on the RMSD value obtained by superimposing the DszB protein model on its template (Savarino, 2007).

2.4. Energy Minimization and Refinement of Modeled Protein DszB

The modeled DszB protein valency and chemistry were corrected in Discovery Studio (DS) (Accerlys 2.1). To obtain a protein with least energy, energy minimization and refinement were performed by employing CHARMm force field (Nousheen *et al*., 2014; Jin *et al*., 2015).

2.5. Construction and Energy Minimization of DszB Mutant Proteins

Mutant DszB proteins of *Streptomyces* sp. VUR PPR 101 were constructed using "Build Mutant" protocol (Nousheen *et al*., 2014; Raghunathan *et al*., 2012). The substitution of single residues at 63 and 65 positions were made in the modeled DszB protein to generate mutant DszB proteins following the model of Ohshiro *et al.* (2007). Table 1 shows the positions at which amino acid residues in the DszB protein were replaced with different amino acids. Energy of mutant proteins was minimized by applying CHARMm force fields in DS (Hanyog *et al*., 2015).

Table 1. Positions on DszB protein at which amino acids were replaced to generate mutant DszB proteins

ID.	Position of amino acid in DszB protein	Original amino acid	New amino acid
1.	63	Tyrosine	Phenylalanine
2.	63	Tyrosine	Alanine
3.	65	Glutamine	Histidine

2.6. Generation of ~~Substrates~~ Substrate Structures

Chemical structures were drawn in the front end of the chemsketch software (ACDLABS 12.0 version software). The substrate structure, i.e., HPBS, which was used for binding at active sites of wild type and mutant DszB proteins, was drawn in Chemsketch and saved in mol2 format to obtain a three-dimensional structure in DS (Archana *et al*., 2014; Park *et al*., 2009).

2.7. Prediction of Active Site

ERASER algorithm of DS 2.1 (Shanthipriya and Victor, 2013; Naika *et al*., 2015) was used to identify the active site pocket of modeled DszB wild type and mutant proteins. In the pocket site, substrate interacting amino acids were determined.

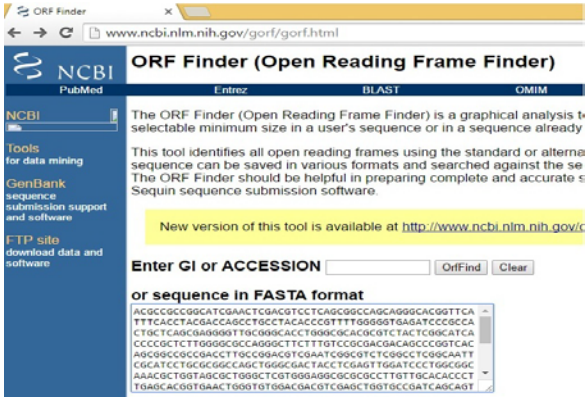
2.8. Docking Studies

The optimized substrate compound HPBS was docked at the catalytic sites of wild type and DszB mutant enzyme proteins using the Libdock algorithm in DS 2.1 utilizing default Libdock parameters. The ligand (substrate) was allowed to be flexible to determine the correct conformation and configuration having minimum energy structures (de Maglhaes *et al*., 2004; Bai *et al*., 2014; Abdel-Hamid and McCluskey, 2014). The parameters used for docking studies are,100 hotspots and docking tolerance of 0.25. User specified docking preferences were employed and the FAST algorithm was used as the endorsement method.

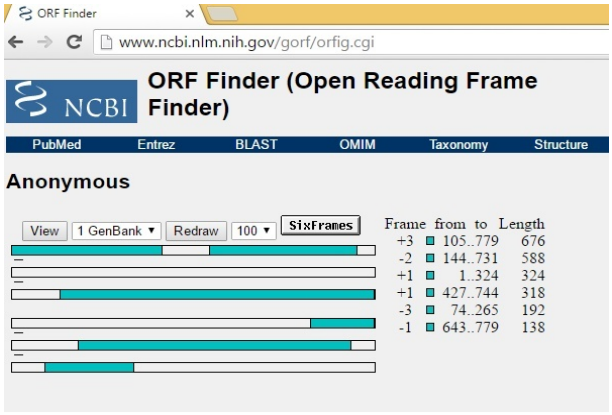
3. Results and Discussion

3.1. Translation of dszB Gene Sequence to Amino Acid Sequence

Submission of *dszB* gene sequence of *Streptomyces* sp. VUR PPR 101 to NCBI-ORF Finder in FASTA format (Figure 1A) resulted in generation of six different reading frames (Figure 1B). The frame showing highest length was selected leading to a protein with a sequence of 259 amino acids. This protein with a linear sequence of amino acids was the DszB protein (primary structure).



(A)



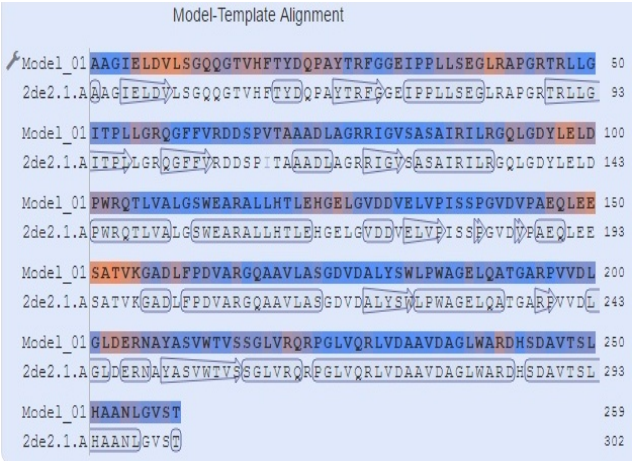
(B)

Figure 1. Translation of *dszB* gene (A) Nucleotide sequence of *dszB* gene of *Streptomyces* sp. VUR PPR 101 in FASTA format submitted to NCBI Open Reading Frame (ORF) finder; (B) Six reading frames generated in NCBI-ORF

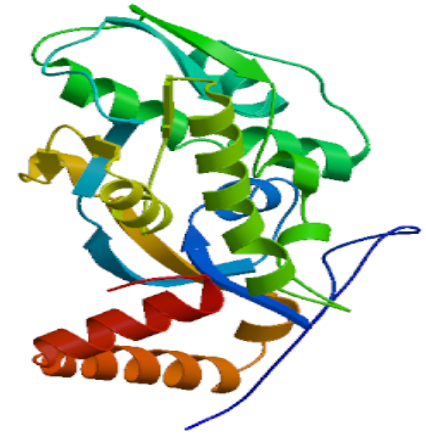
The 259 amino acid sequence of the obtained DszB protein was:
AAGIELDVLSGQQGTVHFTYDQPAYTRFGGEIPPLLS EGLRAPGRTRLLGITPLLGRQGFFVRDDSPVTAAAD LAGRRIGVSASAIRILRGQLGDYLELDPWRQTLVAL GSWEARALLHTLEHGELGVDDVELVPISSPGVDVPA EQLEESATVKGADLFPDVARGQAAVLASGDVDALY SWLPWAGELQATGARPVVDLGLDERNAYASVWTV SSGLVRQRPGLVQRLVDAAVDAGLWARDHSDAVT SLHAANLGVST.

3.2. Sequence Alignment and Homology Modeling of DszB Protein

In SWISS MODEL WORKSPACE automated mode, fifty templates of query sequence (DszB protein sequence) were generated. The template, 2de2.1.A (Figure 2 C) demonstrated highest sequence identity to query sequence (Figure 2 A) and was used to develop the model of DszB protein (Figure 2 B). Tahri *et al*. (2015) also modeled the Thaumetopoein protein using SWISS MODEL WORKSPACE.



(A)



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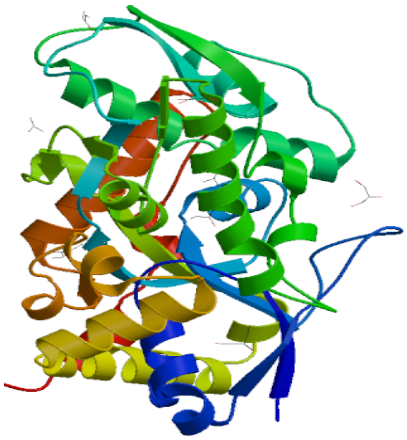


(C)

Figure 2. Homology modeling of DszB protein in SWISS MODEL WORKSPACE automated mode (A) Sequence alignment of DszB protein of *Streptomyces* sp. VUR PPR 101 with template, 2de2.1.A (B) Modeled structure of DszB of *Streptomyces* sp. VUR PPR 101; (C) Structure of template, 2de2.1.A.

3.3. Model Validation of DszB by Rampage

Table 2 depicts the Ramachandran plot values of modeled DszB protein and its template 2de2.1.A. In the Ramachandran plot generated for DszB protein of *Streptomyces* sp. VUR PPR 101, 94.9% residues were found in favoured, 4.7% residues in allowed, and 0.4% in outlier regions (Figure 3A). The Ramachandran plot of template 2de2.1.A showed 97.7% residues in favored, 2.0% residues in allowed, and 0.3% in outlier regions (Figure 3 B). The data of Ramachandran plot clearly indicate the reliability of the DszB protein model. Similarly, Bilal *et al.* (2009) validated the P2RY5 wild and mutant gene proteins by Rampage.

Table 2. Ramachandran plot values showing number of residues in favoured, allowed and outlier regions through RAMPAGE evaluation server

Structure	Number of residues in favoured region (%)	Number of residues in allowed region (%)	Number of residues in outlier region (%)
Modeled DszB	94.9%	4.7%	0.4%
Template (2de2.1.A)	97.7%	2.0%	0.3%

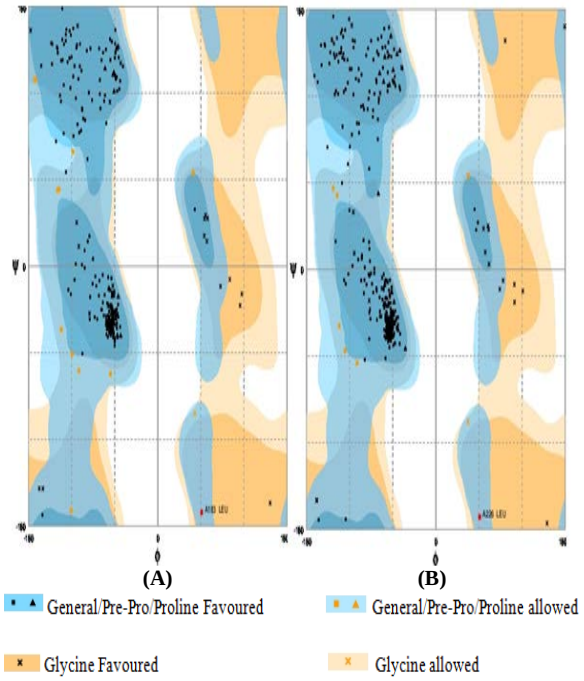


Figure 3. Validation of DszB protein model:(A) Ramachandran Plot of DszB protein of *Streptomyces* sp. VUR PPR 101; (B) Ramachandran Plot of template 2de2.1.A

3.4. Model Validation of DszB of Streptomyces sp. VUR PPR 101 in SPDBV

After superimposing main-chain atoms of modeled DszB protein on template, 2de2.1.A (Figure 4) in Swiss PDB Viewer (SPDBV), the Root-Mean-Square-Deviation (RMSD) was determined at 0.07 A^{o} which indicates close homology and ensures reliability of the model. Devi (2015) also superimposed Thyroid peroxidase (TPO) enzyme protein model on its template, 3BXI in SPDBV to validate the TPO model.

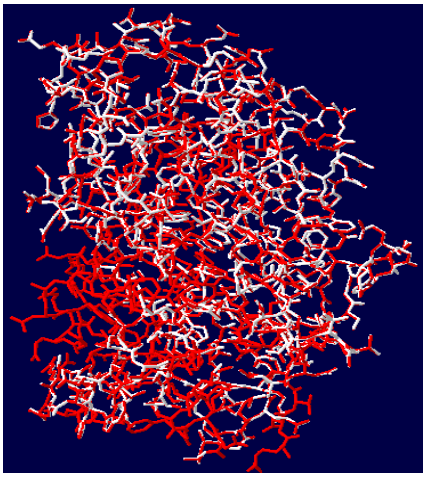


Figure 4. Superimposition of DszB protein of *Streptomyces* sp. VUR PPR 101 on template 2de2.1.A in Swiss PDB Viewer. Red color: Template, White color: DszB protein

3.5. Mutant Protein Construction

Mutant proteins, developed for DszB in DS, were Q65H with replacement of Glutamine by Histidine at 65 position (Figure 5A), Y63A with replacement of Tyrosine by Alanine at 63 position (Figure 5B), and Y63F in which Tyrosine was replaced with Phenylalanine at 63 position (Figure 5C). Ohshiro *et al.* (2007) in their *in vitro* experiment made replacements of same amino acids in same positions in DszB protein via site directed mutagenesis during their work on DszB protein of *Rhodococcus erythropolis* KA 2-5-1 to construct mutant DszB proteins to determine their catalytic efficiency over wild DszB protein and reported an increased catalytic activity in all the mutant proteins over wild DszB protein.

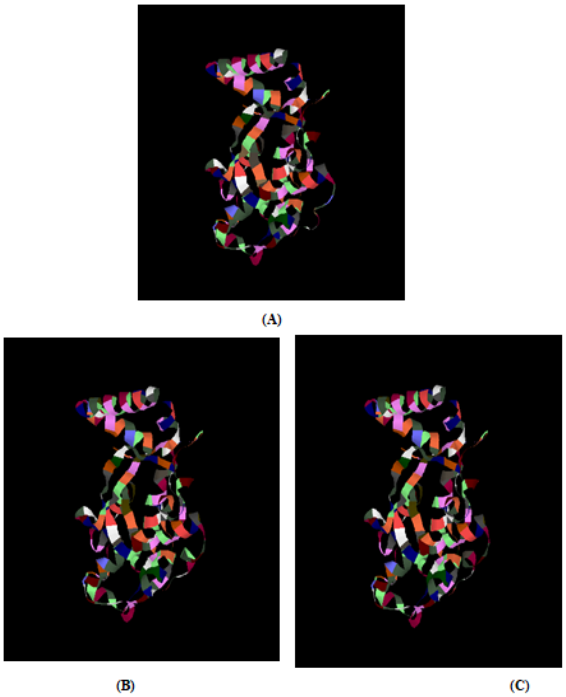


Figure 5. Structures of DszB mutant proteins of *Streptomyces* sp. VUR PPR 101. (A) Q65H mutant (B) Y63A mutant (C) Y63F mutant

3.6. Docking Studies

Molecular docking studies of wild type and mutant DszB proteins were performed with HPBS (mol format) in DS v2.1 using the Libdock algorithm. Binding modes of HPBS in the active sites of modeled wild type and mutant proteins were identified by this algorithm. Libdock makes use of protein site features, known as hotspots, which are of two types: polar and non-polar. The ligand (substrate) poses were fixed into the polar and non-polar receptor interaction sites (Kalani *et al.*, 2013; Alam and Khan, 2014). High Libdock scores were used to measure the ligand (substrate)-binding energies of top ranked conformations. In addition, other input factors, like Van der waal's forces and electrostatic interactions, were also considered for evaluating the docking efficacy of HPBS with modeled wild type and mutant DszB mutant proteins. Docking of HPBS into the active sites of the wild type and mutant models of DszB resulted in the generation of 10 conformations, however, only top ranked docked complex scores were considered for measuring binding affinity analysis (Table 3). Ligand (substrate) – receptor interaction plots for docked complexes were created in DS to determine the organization of key intermolecular interactions that aid in binding of HPBS to receptor sites of wild and mutant DszB proteins. The interaction of HPBS with wild DszB is depicted in Figure (6). The interaction of HPBS with mutant DszB proteins is shown in Figures 7A to 7C.

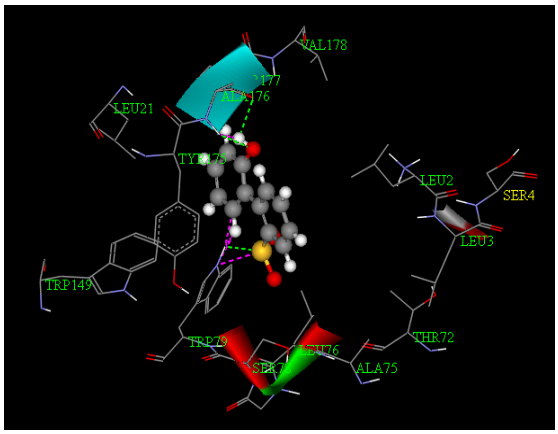
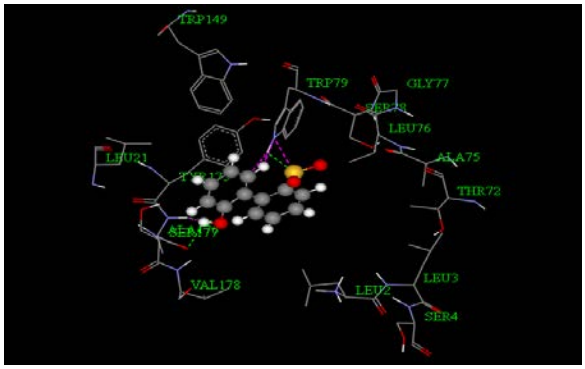
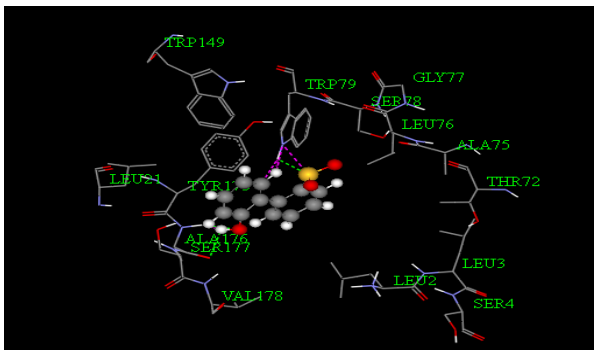


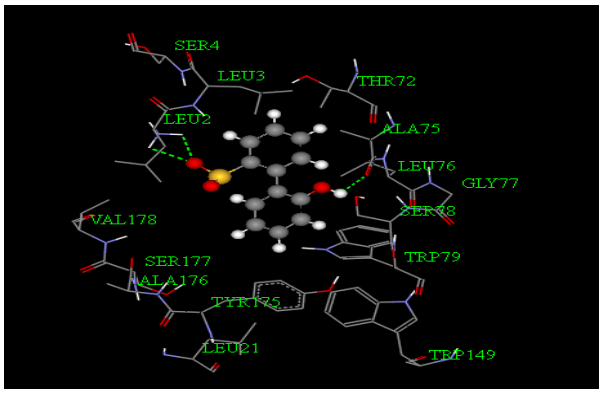
Figure 6. Interaction of HPBS at the active site of wild DszB protein of *Streptomyces* sp. VUR PPR 101



(A)



(B)



(C)

Figure 7. Interaction of HPBS at the active site of mutant DszB proteins. (A) Q65H mutant (B) Y63F mutant (C) Y63A mutant

Table 3. Docking studies between HPBS and DszB wild and mutant proteins of *Streptomyces* sp. VUR PPR 101

Enzyme protein And substrate	Libdock score (Binding energy)	Electrostatic Energy	Vanderwaal Energy	Number of Hydrogen bonds	Interacting aminoacids	Interacting atoms
Wild DszB + HPBS	60.757	10.832	5.115	3	Trp79 Ala176 Leu21 Tyr175 Val178 Trp149 Ser78 Leu2 Leu3 Ser4	A:TRP79:HE1 - Hydroxyphenylbenzosulfinate:S13 A:ALA176:HN - Hydroxyphenylbenzosulfinate:O16 Hydroxyphenylbenzosulfinate:H25 - A:ALA176:O Hydroxyphenylbenzosulfinate:C10 - A:TRP79:HE1 Hydroxyphenylbenzosulfinate:S13 - A:TRP79:NE1 Hydroxyphenylbenzosulfinate:H25 - A:ALA176:HN
Q65H mutant + HPBS	59.185	10.832	5.115	3	Trp79 Ala176 Leu21 Tyr175 Val178 Trp149 Ser78 Leu2 Leu3 Ser4	A:TRP79:HE1 - Hydroxyphenylbenzosulfinate:S13 A:ALA176:HN - Hydroxyphenylbenzosulfinate:O16 Hydroxyphenylbenzosulfinate:H25 - A:ALA176:O A:ALA176:HN - Hydroxyphenylbenzosulfinate:H25 A:TRP79:NE1 - Hydroxyphenylbenzosulfinate:S13
Y63F mutant + HPBS	56.605	10.832	5.115	3	Trp79 Ala176 Leu21 Tyr175 Val178 Trp149 Ser78 Leu2 Leu3 Ser4	A:TRP79:HE1 - Hydroxyphenylbenzosulfinate:S13 A:ALA176:HN - Hydroxyphenylbenzosulfinate:O16 Hydroxyphenylbenzosulfinate:H25 - A:ALA176:O A:ALA176:HN - Hydroxyphenylbenzosulfinate:H25 Hydroxyphenylbenzosulfinate:S13 - A:TRP79:NE1
Y63A mutant+ HPBS	61.497	10.832	5.115	3	Trp79 Ala176 Leu21 Tyr175 Val178 Trp149 Ser78 Leu2 Leu3 Ser4	A:LEU2:HT1 - Hydroxyphenylbenzosulfinate:O15 A:LEU2:HT2 - Hydroxyphenylbenzosulfinate:O15 Hydroxyphenylbenzosulfinate:H25 - A:ALA176:O A:TRP79:HE1 - Hydroxyphenylbenzosulfinate:S13 Hydroxyphenylbenzosulfinate:H19 - A:ALA176:HB2 Hydroxyphenylbenzosulfinate:H23 - A:SER78:HB1

HPBS: hydroxyphenyl benzene sulfinate

3.7. Interaction between DszB (Wild Type Protein) and Hydroxyphenyl Benzene Sulfinate (HPBS)

HPBS interacted with receptor site of wild type DszB (Figure 6) involving three hydrogen bonds. The binding energy (Libdock score) calculated during interaction between HPBS and receptor was 60.757 K.cal/mol. The amino acids interacting with HPBS in the active site were Trp79, Ala176, Leu21, Tyr175, Val178, Trp149, Ser78, Leu2, Leu3 and Ser4. The atoms of Trp79 (A:TRP79 : HE1 - Hydroxyphenylbenzosulfinate : S13), Ala176 (A : ALA176:HN – Hydroxyphenylbenzosulfinate : O16 ; Hydroxyphenylbenzosulfinate : H25 – A : ALA176 : O), Trp79 (Hydroxyphenyl benzosulfinate : C10-A : TRP79 : HE1 ; Hydroxyphenyl benzosulfinate : S13 - A : TRP79 : NE1) and Ala176 (Hydroxy biphenyl benzosulfinate : H25-A:ALA176:HN) were involved in bond formation. The remaining amino acids were involved in non-bonding interactions (Table 3).

3.8. Interaction between DszB Mutant Protein Q65H and HPBS

Three hydrogen bonds were observed between receptor site of mutant protein Q65H and HPBS, with a binding energy of 59.185 K.cal/mol (Figure 7A). The amino acids interacting with HPBS were Trp79, Ala176, Leu21, Tyr175, Val178, Trp149, Ser78, Leu2, Leu3 and Ser4. The atoms of Trp79 (A: TRP78: HE1 –

Hydroxyphenyl benzosulfinate: S13), Ala176 (A : ALA176 : HN – Hydroxyphenyl benzosulfinate : O16), and Trp79 (Hydroxyphenyl benzosulfinate : C10 – A : TRP79 : HE1) were involved in bond formation. The remaining amino acids exhibited non-bonding interactions (Table 3).

3.9. Interaction between DszB Mutant Protein Y63F and HPBS

Three hydrogen bonds were observed during the interaction between HPBS and DszB mutant Y63F (Figure 7B) with a calculated binding energy of 56.605 K.cal/mol. The amino acids interacting with HPBS were Trp79, Ala176, Leu21, Tyr175, Val178, Trp149, Ser78, Leu2, Leu3, and Ser4. The atoms of Trp79 (A : TRP78 : HE1 – Hydroxyphenyl benzosulfinate : S13), Ala176 (A : ALA176 : HN – Hydroxyphenyl benzosulfinate : O16; Hydroxyphenyl benzosulfonate : H25 – A : ALA176 : O; A : ALA176 : HN – Hydroxyphenyl benzosulfinate : H25), and Trp79 (Hydroxyphenyl benzosulfinate : S13 – A:TRP79 : NE1) were involved in bond formation. The remaining amino acids disclosed non-bonding interactions (Table 3).

3.10. Interaction between DszB Mutant Protein Y63A and HPBS

Three hydrogen bonds were formed during the interaction between HPBS and receptor site of mutant DszB Y63A (Figure 7C). The binding energy calculated during the interaction between receptor site and HPBS was 61.497 K.cal/mol. The amino acids interacting with HPBS were Trp79, Ala176, Leu21, Tyr175, Val178, Trp149, Ser78, Leu2, Leu3 and Ser4. The atoms Leu2 (A:LEU2:HT1-Hydroxyphenyl benzosulfonate:O15), Ala176 (Hydroxyphenyl benzosulfinate: H25-A:ALA176:O), Trp79 (A:TRP78:HE1 – Hydroxyphenyl benzosulfinate : S13), Ala176 (Hydroxybiphenyl benzosulfonate:H19-A:ALA176:HB2), and Ser78 (Hydroxybiphenyl benzosulfinate:H23 – A: SER78:HB1) were involved in bond formation. The remaining amino acids showed non-bonding interactions (Table 3).

Libdock score (binding energy) generated during the formation of docking complex was used to measure the affinity and binding strength between the substrate and protein. All docked poses of the complexes were ranked on the basis of Libdock Score (Zhou *et al.*, 2016). Libdock score is a measure of strength of binding affinity between ligand substrate and receptor protein (Rani *et al.*, 2014). That means the enzymatic protein that has maximum binding affinity towards HPBS exhibits highest Libdock score indicating highest activity (Chen *et al.*, 2015; Guo *et al.*, 2015). Highest Libdock score was observed for the docked complex which formed between Y63A mutant protein and HPBS, when compared to wild type DszB and other mutant DszB proteins docked complexes. The order of Libdock scores for the docked complexes was mutant Y63A > wild type DszB > mutant Q65H > mutant Y63F. Binding energy is the basic factor that influences the proximity, alignment effects, etc., during the enzyme-substrate interaction, which affects the catalytic activity of the enzyme (Dmitri *et al.*, 2015). Henceforth, docking studies reveal that mutant Y63A DszB protein of *Streptomyces* sp. VUR PPR 101 has increased affinity towards HPBS, therefore possessing higher DBT desulfurization activity.

4. Conclusion

DszB enzyme protein of *Streptomyces* sp. VUR PPR 101 was modeled in SWISS MODEL WORKSPACE and validated by Rampage and in SPDBV. Three mutant DszB proteins were constructed by replacing single amino acid residue at selected sites. The wild type and mutant DszB enzyme proteins were docked against the substrate HPBS. Highest Lipdock score (binding energy) was found during the interaction between Y63A mutant DszB protein and HPBS. Thus, Y63A mutant DszB protein may exhibit higher catalytic activity when compared to wild and other mutant DszB proteins. Based on the *in silico* and docking studies results of present study, similar mutations at the identified sites of *dszB* gene can be carried out using *in vivo* conditions through site-directed mutations which may pave the way for developing improved strain of *Streptomyces* sp. VUR PPR 101 with a modified *dszB* gene, that exhibit enhanced biodesulfurization activity. Such improved strains could gain ecological and commercial importance in Biodesulfurization of fuels.

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