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ORIGINAL ARTICLE

In Silico Site-Directed Mutagenesis of some Amino Acids in the C-Terminal Domain of Glutathione s-transferase from *Acidovorax* sp. KKS102

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Abstract

A cytosolic glutathione S-tranferase from *Acidovorax* sp. KKS102, a biphenyl/polychlorobiphenyl degrading organism is recently known to have a dehalogenation function on various organochlorine substrates. However, little is known about the specific amino acids involved in this catalytic process. The *in silico* site-directed mutagenesis of a highly conserved region; Ala^{154}, Asp^{155} and Tyr^{157} in the C-terminal domain of the cytosolic glutathione S-transferase from *Acidovorax* sp. was carried out using Deep View/Swiss-Pdb Viewer molecular graphics program for all the proteinogenic amino acids. Sustitutions at position 154 of Ala for Phe, Trp and Tyr showed tendency of greater effect on the protein. At position 155, substitution of Asp for His, Phe, Trp and Tyr have greater effect on the 3D model of the protein. For substitution of Tyr at position 157, only Pro substitution showed greater effect on the protein model. The amino acid substitutions in this region may likely affect the theoretical 3D model of the transferase protein entity through alterations of some stabilization forces which may in turn affect the structural stability and perhaps the activity of the enzyme.

Keywords: *Acidovorax* sp. KKS102, C-terminal domain, Glutathione S-transferase, Site-directed mutagenesis, 3D model

Introduction

The glutathione S-transferases (GSTs) are ubiquitous and multifunctional proteins that play an essential role in detoxification processes on a vast array of hydrophobic as well as electrophilic substrates (Habig et al., 1974; Simarati et al., 2016). GSTs mainly confer solubility to their substrates by employing the sulfhydryl group of glutathione (GSH) to catalyse the nucleophilic attack on the electrophilic centres of the various susceptible compounds (Wilce and Parker, 1994; Edwina et al., 2007). Some bacterial GSTs were reported to have a unique function of catalyzing reductive dehalogenation of some chlorinated substrates (Warner et al., 2005; Fortin et al., 2006; Shehu and Zazali, 2018).

A homologous gene of *Bphk* (biphenyl upper pathway K) from *Acidovorax* sp. KKS102, a biphenyl/polychlorobiphenyl degrading organism was identified and named *Bphk-kks*. This gene product, GST-KKS102 was recently reported to have a dechlorination function on various

organochlorine substrates (Shehu and Zazali, 2018). It was reported to act on 1 -chloro -2,4 dinitrobenzene (CDNB) (Shehu and Zazali, 2018). *Acidovorax* sp. KKS102 was initially isolated from the soil sample near a refinery in Japan (Ohtsubo *et al.,* 2012). Recent searches of the database revealed that *Acidovorax* sp. KKS102 contained at least eleven putative GSTs. These bacterial cytosolic GSTs are shown to be dimeric proteins with average molecular weight of 25,000 Da. They have two domains, the N-terminal and C-terminal domains. Amino acid residues from 80-202 residues constitute the C-terminal domain. The GSH binding site (G site) is located in the N-terminal thioredoxin-like domain and the hydrophobic electrophilic substrate binding site (H site) in the C-terminal α-helix domain (Federici et al., 2007; Allocati et al., 2012).

Several mutagenesis studies have been carried out on bacterial GSTs to unveil the specific amino acids involved in thermal and structural stability as well as catalysis (Inoue et al., 2000; Gilmartin et al., 2005). Many site-directed mutagenesis studies have shown that both a conserved tyrosine and serine residues in N-terminal domain of some bacterial GST classes have been identified in stabilizing the thiolate form of GSH that enhance GST catalysis (Armstrong, 1997; Favaloro et al., 1998; Wilce and Parker, 1994). However, the majority of the mutagenesis studies conducted on GST were focused on the N-terminal domain, which is involved in glutathione binding and is more strongly conserved than the C-terminal domain, which has a sequence similarity below 20% in automated searches of sequence databases (Gilmartin et al., 2005).

In-silico side-directed mutagenesis is an essential tool for predicting the effect of mutation on the theoretical 3D model and function of protein prior to the actual *in-vitro* side-directed mutagenesis (Murtala et al., 2019). In line with this, Murtala et al. (2018) showed that *in silico* site-directed mutagenesis on the evolutionarily conserved Ser^{11} and Lys^{107} may affect the structural stability of a GST from *Acidovorax* sp. KKS102. Recently, some structural stability effects were observed on the *in silico* substitutions of conserved Trp^{164} and Ser^{165} in the C-terminal domain of GST from *Acidovorax* sp. KKS102 (Murtala et al., 2019). This study is aimed to investigate the *in silico* effect on substitution of the conserved amino acids, Ala^{154}, Asp^{155} and Tyr^{157} in the C-terminal domain on the predicted 3D model of GST-KKS102 in an attempt to learn more about the functional role of the amino acids in the stability of the protein model.

Materials and Methods

Protein Homology Studies

The protein sequence homology analysis of cytosolic GST-KKS102 was carried out using the European Molecular Biology Laboratory and European Bioinformatics Institute (EMBL-EBI) Clustal Omega Online Resources (https://www.ebi.ac.uk/Tools/services/web). Clustal Omega is a new multiple sequence alignment program that uses seeded guide trees and HMM profile-profile techniques to generate alignments between three or more sequences.

Four protein sequences, *Bphk* LB400 (PDB file ABE37052.1), *Pseudomonas pseudoalcaligenes* (PDB file Q52037), *Pseudomonas* sp. B4 (PDB file Q9RBS6) and *Paraburkholderia xenovoras* LB400 (PDB file Q59721) were used as templates for the sequence homology studies.

3D Structure Prediction

The theoretical 3D model of GST-KKS102, with 202 amino acid residues was predicted using the SWISS-MODEL homology modelling alignment interface approach mode (Guex and Peitsch, 1997; Schwede et al., 2003). Homology modelling determines structure based on the target sequence, GST-KKS102, possessing homology with template sequence in the structural database. The ABE37052.1, Q52037, Q9RBS6 and Q59721 were used as template sequences. The predicted theoretical 3D model was viewed and manipulated using DeepView/Swiss-PdbViewer version 3.7 (SP5) (Guex and Peitsch, 1997).

In Silico Site-Directed Mutagenesis

The effect of *in silico* site-directed mutagenesis of the evolutionarily conserved Ala^{154}, Aps^{155} and Tyr^{157} on the predicted 3D model of GST-KKS102 was carried out using the DeepView/Swiss-PdbViewer version 4.1 (SP5) molecular graphics program. New amino acid side chains were selected by opting for the mutate tool in accordance with DeepView/Swiss-PdbViewer user guide protocols.

Results and Discussion

Sequence Similarity Studies

The protein sequence alignment studies of cytosolic GST-KKS102 showed at least more than 40% sequence similarity with all the biphenyl/polychlorobiphenyl degrading templates, ABE37052.1, Q52037, Q9RBS6 and Q59721 in the structural database (Table 1).

In both prokaryotic and eukaryotic GSTs, the most important criterion for classifying cytosolic GSTs is the use of percentage sequence similarity, which is generally accepted that proteins with at least 40% and above of the sequence similarity belongs to the same class, while those sharing less than 20% are classified into a different class (Brennan et al., 2009). All the templates used in this study were beta class GSTs. Thus, going by the above criterion, cytosolic GST-KKS102 belongs to the same class with all the templates used.

Table 1: Percentage Sequence Similarity between various Bacterial GST Templates and Cytosolic GST-KKS102

Template	% Sequence similarity with GST-KKS102
ABE3705.1	47.26
Q52037	46.77
Q9RBS6	46.67
Q59721	47.26

The templates are in form of Protein Data Bank (PDB) designation files

The three evolutionarily conserved residues Ala^{154}, Asp^{155} and Tyr^{157} (Figure 1) selected in this study were part of α helices in the C-terminal domain and located in the core region of the GSTs protein entity as shown in Figure 2. This region contains some conserved motif (Ser/Thr-Val-Ala-Asp-Xaa-Tyr) which was shown to be implicated in substrate specificity in the insect, *Anopheles dirus* GST and in protein folding and stability in human GST (Dragani et al., 1997; Wongtrakul et al., 2003).

In silico Site-Directed Mutagenesis

The *in silico* substitutions of Ala^{154}, Asp^{155} and Tyr^{157} of cytosolic GST-KKS102 were carried out using all the proteinogenic amino acids. Rotamer and score values were allocated for each substitution depending on how much disruption the new amino acid was predicted to affect the theoretical 3D model of cytosolic GST-KKS102 (Table 2). DeepView/Swiss-PdbViewer selects the best rotamer or conformation of the new amino acid side chain from libraries of rotamers within the program. The best rotamer is defined as one that produces the lowest score indicating the least amount of effect on the structure of the protein and is based on minimization of energy. However, any higher score, indicated by a zero to positive integer, have a tendency to affect the energy level which creates great effect on the stability of the protein’s 3D structure.

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kks102                     MKLYYAPGACSLAVHIALREVGVAFDLVKVDLVRHTTETGANYLDISPRGYVPLLELADQ    60
sp|Q52037|Q52037_PSEPS     MKLYYSPGACSLSPHIALREAGLNFELVQVDLASKKTASGQDYLEINPAGYVPCLQLDDG    60
ABE37052.1                 MKLYYSPGACSLSPHIALREAGLNFELVQVDLASKKTASGQDYLEINPAGYVPCLQLDDG    60
sp|Q9RBS6|Q9RBS6_9PSED     MKLYYSPGACSLSPHIALREAGLNFELVQVDLASKKTASGQDYLEINPAGYVPCLQLDDG    60
sp|Q59721|Q59721_BURXL     MKLYYSPGACSLSPHIALREAGLNFELVQVDLASKKTASGQDYLEINPAGYVPCLQLDDG    60
                           *****:******: ******.*: *:**:***. :.* :* :**:*.* **** *:* *

kks102                     SRHTEAAALLQYVADLDPARALIGQPGSSERLAVLEWLTFVSTELHKGFSPWLWHKETAD    120
sp|Q52037|Q52037_PSEPS     RTLTEGPAIVQYVADQVPGKQLAPANGSFERYHLQQWLNFISSELHKSFSPLFNP-ASSD    119
ABE37052.1                 RTLTEGPAIVQYVADQVPGKQLAPANGSFERYHLQQWLNFISSELHKSFSPLFNP-ASSD    119
sp|Q9RBS6|Q9RBS6_9PSED     RTLTEGPAIVQYVADQVPGKQLAPANGSFERYHLQQWLNFISSELHKSFSPLFNP-ASSD    119
sp|Q59721|Q59721_BURXL     RTLTEGPAIVQYVADQVPGKQLAPANGSFERYHLQQWLNFISSELHKSFSPLFNP-ASSD    119
                              **. *::*****  *.: *    ** **  : :**.*:*:****.*** :    ::*

kks102                     STRQAVKAKLAVRFAEMEAVLSRSDFL-AGGYSVADAYGFTIVNWSHLLGIPLTAYPHLQ    179
sp|Q52037|Q52037_PSEPS     EWKNAVRQSLNTRLGQVARQLEHAPYLLGDQLSVADIYLFVVLGWSAYVNIDLSPWPSLQ    179
ABE37052.1                 EWKNAVRQSLNTRLGQVARQLEHAPYLLGDQLSVADIYLFVVLGWSAYVNIDLSPWPSLQ    179
sp|Q9RBS6|Q9RBS6_9PSED     EWKNAVRQSLNTRLGQVARQLEHAPYLLGDQLSVADIYLFVVLGWSAYVNIDLSPWPSLQ    179
sp|Q59721|Q59721_BURXL     EWKNAVRQSLNTRLGQVARQLEHAPYLLGDQLSVADIYLFVVLGWSAYVNIDLSPWPSLQ    179
                           . ::**: .* .*:.::   *.:: :* ..  **** * *.::.**  :.* *: :* **

kks102                     AWMARVAARPQVQAALRAEGLLS-     202
sp|Q52037|Q52037_PSEPS     AFQGRVGGREAVQSALRAEVLIKE     203
ABE37052.1                 AFQGRVGGREAVQSALRAEGLIKE     203
sp|Q9RBS6|Q9RBS6_9PSED     AFQGRVGGREAVQSALR-------     196
sp|Q59721|Q59721_BURXL     AFQGRVGGREAVQSALRAEGLIKE     203
                           *: .**..*  **:***
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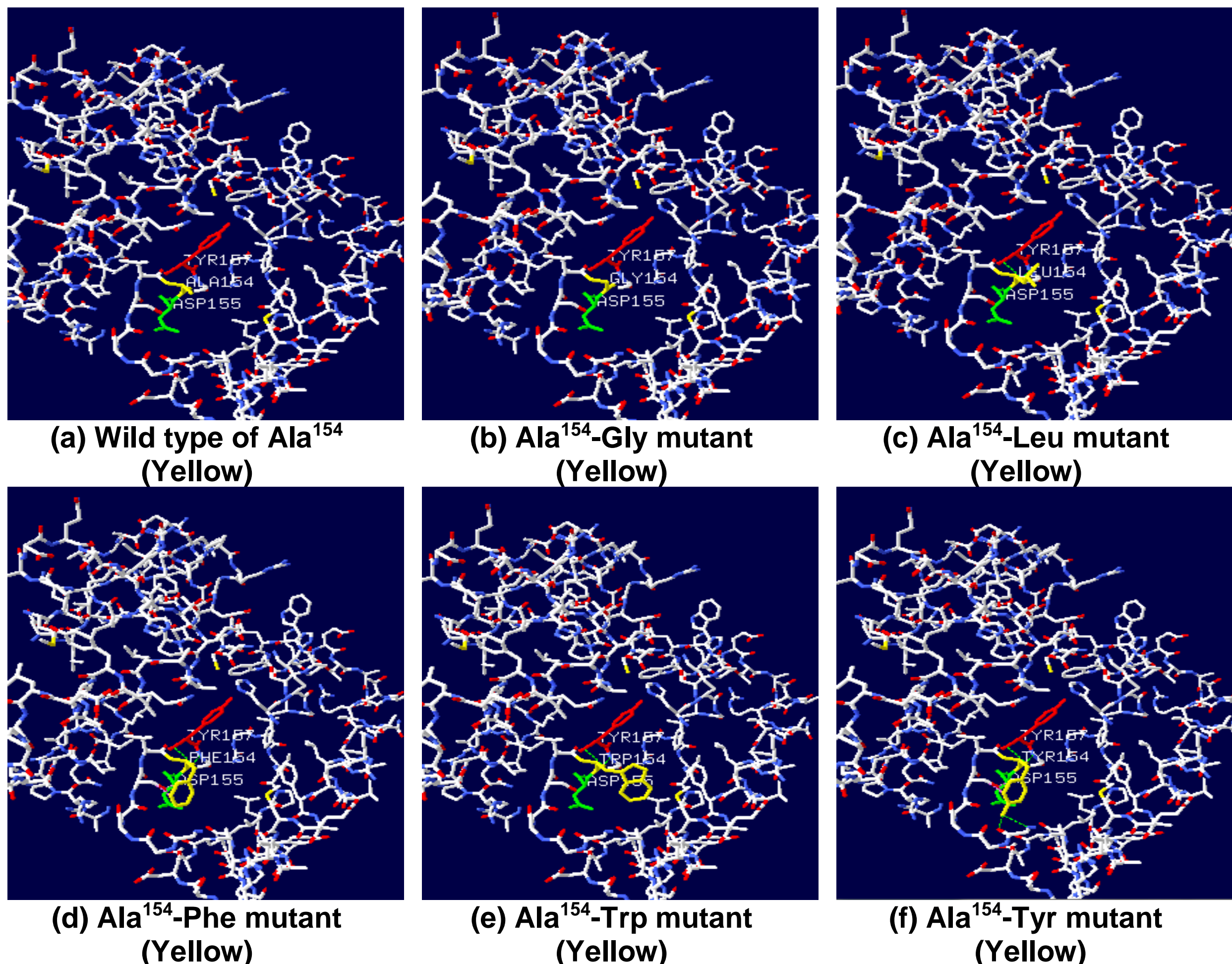
Figure 1: Sequence Homology of Cytosolic GST-KKS102 and various GST Templates, Indicating Evolutionarily Conserved Ala^{154} (A), Asp^{155} (D) and Tyr^{157} (Y) Residues in the Yellow Boxes



(a) Wild type of Ala^{154} (Yellow) **(b) Ala^{154}-Gly mutant (Yellow)** **(c) Ala^{154}-Leu mutant (Yellow)**

(d) Ala^{154}-Phe mutant (Yellow) **(e) Ala^{154}-Trp mutant (Yellow)** **(f) Ala^{154}-Tyr mutant (Yellow)**

Figure 2: The 3D models of wild type (a) and Ala^{154} substituted mutants of GSTKKS102. Position of the mutation is shown in yellow

Table 2: Rotomer and Score Values of *In-Silico* Site-Directed Mutagenesis of Ala^{154}, Asp^{155} and Tyr^{157}

Mutation	Ala^{154} Rotamer	Score	Asp^{155} Rotamer	Score	Tyr^{157} Rotamer	Score
Ala	-	-	1/1	-3	1/1	-4
Arg	2/26	-2	18/28	-4	15/26	-6
Asn	2/5	-1	2/5	-5	1/5	-2
Asp	3/4	-1	-	-	1/4	-4
Cys	1/3	-3	2/3	-4	1/3	-4
Gln	11/14	-2	12/14	-4	7/14	-4
Glu	12/14	-2	12/14	-4	7/14	-4
Gly	1/1	0	1/1	0	1/1	0
His	4/6	-1	1/6	+2	4/6	-4
Ile	1/5	-2	1/5	-3	1/5	-4
Leu	1/4	0	2/4	-3	1/2	-4
Lys	4/16	-2	12/16	-3	6/16	-5
Met	2/12	-3	4/12	-3	1/12	-4
Phe	5/5	+6	1/5	+9	1/5	-2
Pro	1/2	-2	1/2	-3	1/2	+4
Ser	1/3	-3	2/5	-4	1/3	-5
Thr	1/2	-3	2/2	-4	1/2	-5
Trp	1/6	+18	5/6	+5	2/6	-4
Tyr	5/5	+4	1/5	+9	-	-
Val	1/3	-2	1/3	-3	1/3	-4

Effects of In Silico Site-Directed Mutagenesis on the 3D Model of GST-KKS102

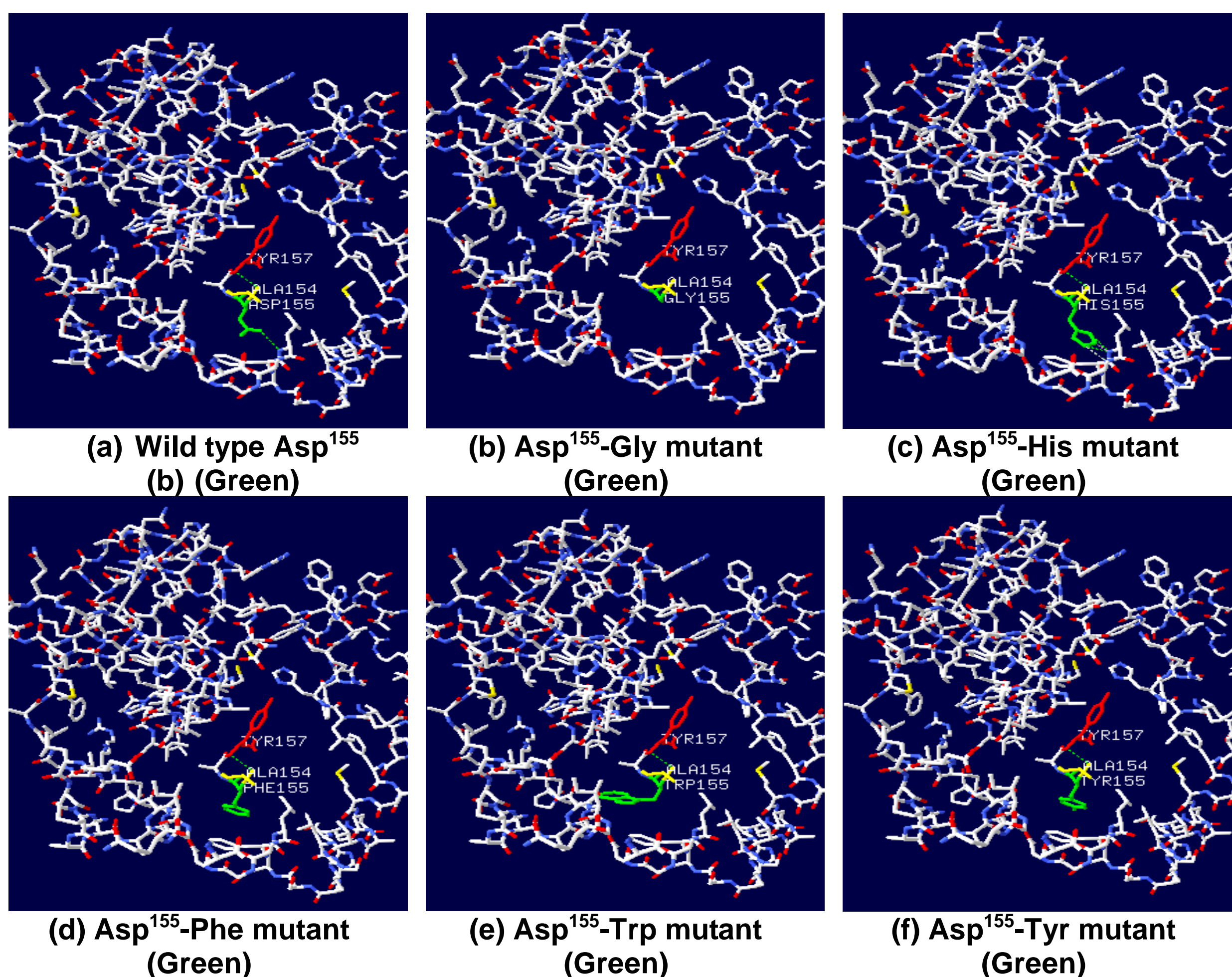
In all the three positions targeted, the *in silico* substitution with glycine were likely to affect the stability of the 3D model of GST-KKS102. This may be due to the ability of glycine to introduce kinks into the chain, possibly affecting the overall structure of the C-terminal domain. Additionally, in Asp^{155}-Gly and Tyr^{157}Gly mutants, there is possibility of disruption of hydrogen bonds due to the absence of carboxyl group (COOH) and hydroxyl group (OH) in glycine. Similar scenario was reported by Gilmartin et al. (2005) that Ser^{152}-Gly mutant showed decreased structural stability and drop in the activity of protein due to the absence of hydrogen bond interactions.

The all proteinogenic amino acids substitutions using Swiss PBD viewer program of alanine at position 154, the Ala^{154}-Tyr, Ala^{154}-Phe and Ala^{154}-Trp mutants (Figure 2), showed greater effect on the theoretical 3D model of GST-KKS102 with higher scores accordingly (Table 2). Phenylalanine, tyrosine and tryptophan are large aromatic residues that are essential for protein stability (Federici et al., 2007; Brennan et al., 2009). Thus, substitution by these amino acids could affect the stability, structure and perhaps the activity of GST-KKS102 as indicated by the higher scores as shown in Table 2. However, with exception of leucine, substitutions using Swiss PBD viewer program by hydrophobic and other charged amino acids in the same position

(Table 2) showed no significant effect on the 3D model. This is due to the insignificant changes in the hydrogen bond interactions (Gilmartin et al., 2005).

In all the *in silico* substitutions of aspartate for all the proteinogenic amino acids at position 155; the Asp^{155}-His, Asp^{155}-Phe, Asp^{155}-Trp and Asp^{155}-Tyr mutants showed great structural effects as indicated by the higher scores as shown in Table 2. In the 3D model of Asp^{155}-His mutant as in Figure 3c, histidine as a positively charged amino acid replaces aspartic acid, a negatively charged amino acid in the wild type (Figure 3a). This likely cause a disruption of hydrogen bonds between Asp^{155} and Ser^{152} in the histidine mutant, as similar scenario was observed in GST from *Bphk* as reported by Gilmartin et al., (2005).

Asp^{152} is known to be involved in the thermostability of human GST (Dragani et al., 1997), which corresponds to the Asp^{155} in *Bphk* GST (Gilmartin et al., 2005) and possibly in the GST-KKS102 protein. Since both *Bphk* GST and GSTKKS102 are beta class GSTs. Similarly, the other aromatic amino acid mutants, Asp^{155}-Phe, Asp^{155}-Trp and Asp^{155}-Tyr may cause disruption and/or formation of hydrogen bond interactions in these GST-KKS102 mutants which may affect the overall 3D model of the proteins. It was reported that substitution of Asp^{155} with Tyr resulted in loss of two H-bonds in GST from *Bphk* (Gilmartin et al., 2005).

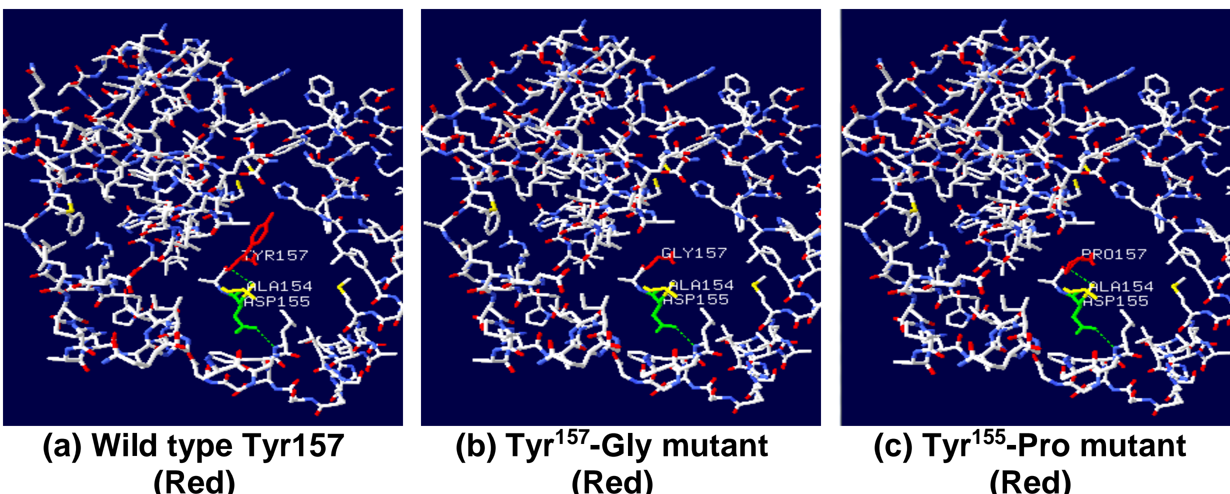


(a) Wild type Asp^{155} (b) (Green)
(b) Asp^{155}-Gly mutant (Green)
(c) Asp^{155}-His mutant (Green)
(d) Asp^{155}-Phe mutant (Green)
(e) Asp^{155}-Trp mutant (Green)
(f) Asp^{155}-Tyr mutant (Green)

Figure 3: The 3D models of wild type (a) and Asp^{155} substituted mutants of GSTKKS102. Position of the mutation is shown in green

On the other hand, the *in silico* substitution of tyrosine for all the proteinogenic amino acids at position 157 on the 3D model of GST-KKS102, proline was the only mutant with greatest effect as

indicated by the higher score of +6 (Table 2). Proline is a non-polar amino acid with unique structural properties such as turning points in β-sheets and structural disruption of α-helices (Edwina et al., 2007). Tyr^{157}Pro mutant (Figure 4) might thus, affect the stability of predicted 3D model and function of GSTKKS102. In E. coli GST, Tyr^{157} is hydrogen bonded with a water molecule, which in turn is bonded to His^{106}. This His^{106} is known to have a catalytic role and it is suggested to be involved in deprotonation of the GSH thiol. It has been suggested that Tyr^{157} may assist $histidine^{106}$ in its catalytic function (Nishida et al., 1994).



(a) Wild type Tyr157 (Red) **(b) Tyr^{157}-Gly mutant (Red)** **(c) Tyr^{155}-Pro mutant (Red)**

Figure 4: The 3D models of wild type (a) and Tyr^{157} substituted mutants of GSTKKS102. Position of the mutation is shown in green

Conclusion

The *in silico* site-directed mutagenesis of a highly conserved region, Ala^{154}, Asp^{155} and Tyr^{157} in the C-terminal domain of cytosolic glutathione S-transferase from *Acidovorax* sp., a biphenyl/polychlorobiphenyl degrading organism was carried out using Swiss PBD viewer program for all the proteinogenic amino acids. Sustitutions at position 154 of Ala for Phe, Trp and Tyr showed tendency of greater effect on the protein. At position 155, substitution of Asp for His, Phe, Trp and Tyr have greater effect on the 3D model of the protein. For substitution of Tyr at position 157, only Pro substitution showed greater effect on the protein model. The amino acid substitutions in this region may likely affect the structural stability of the GSTKKS102 3D model by possibly altering some stabilization forces. However, laboratory work is required to validate these findings.

References

Allocati, N., Federici, L., Masulli, M. & Di Ilio, C. (2012). Distribution of glutathione transferases in Gram-positive bacteria and Archaea. *Biochimie* 94(3), 588-596. doi. 10.1016/j.biochi.2011.09.008.

Armstrong, R. N. (1997). Structure, catalytic mechanism, and evolution of the glutathione transferases. *Chem Res Toxicol,* 10(1), 2-18. doi.10.1021/tx960072x

Brennan, E., Mcguinness, M. & Dowling, D.N. (2009). Bioinformatic analysis and in vitro site-directed mutagenesis of conserved amino acids in BphK LB400, a specific bacterial glutathione transferase. *Int Biodeter Biodegrad* 63, 928-932. doi.10.1016/j.ibiod.2009.03.008

Dragani, B., Stenberg, G., Melino, S., Petruzzelli, R., Mannervik, B. & Aceto, A. (1997). The conserved N-capping box in the hydrophobic core of glutathione S-transferase P1-1 is essential for refolding: identification of a buried and conserved hydrogen bond important for protein stability. *J Biol Chem* 272, 25518–25523. PMID: 9325266.

Edwina, B., McGuinness, M. & Dowling, D. (2007). *In silico* site-directed mutagenesis and the predicted 3d structure of *BphK*LB400. Proceedings of ESAI Environ, 2007. Ireland. pp. 42-44,

Favaloro, B., Tamburro, A., Angelucci, S., Luca, A.D., Melino, S., Di Ilio, C. & Rotilio, D. (1998) Molecular cloning, expression and site-directed mutagenesis of glutathione S-transferase from Ochrobactrum anthropi. *J Biochem.* 335 (Pt 3), 573–579.

Federici, L., Masulli, M., Bonivento, D., Di Matteo, A., Gianni, S., Favaloro, B., Di Ilio, C. & Allocati, N. (2007). Role of Ser (11) in the stabilization of the structure of Ochrobactrum anthropi glutathione transferase. *J Biochem* 403(2), 267-74. doi. 10.1042/BJ20061707

Fortin, P.D., Horsman, G.P., Yang, H.M. & Eltis, L.D. (2006). A glutathione S-transferase catalyzes the dehalogenation of inhibitory metabolites of polychlorinated biphenyls. *J Bacteriol,* 188, 4424-4430. doi. 10.1128/JB.01849-05

Gilmartin, N., Ryan, D. & Dowling, D. (2005). Analysis of the C-terminal domain of *Burkholderia sp.* strain LB400 BphK reveals a conserved motif that affects catalytic activity. *FEMS Microbiol letters* 249, 23-30. doi.10.1016/j.femsle.2005.05.056

Guex, N. & Peitsch, M.C. (1997). SWISSMODEL and Swiss-PdbViewer: An environment for comparative protein modeling. *Electrophoresis* 18, 2714-2723. doi.10.1002/elps.1150181505

Habig, W.H., Pabst, M.J. & Jakoby, W.B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem,* 249, 7130–7139. PMID: 4436300

Inoue, H., Nishida, M. & Takahashi, K. (2000). Effects of Cys10 mutation to Ala in glutathione transferase from *Escherichia coli. J Organomet Chem* 611, 593-595. doi.10.1016/S0022-328X(00)00395-8

Murtala, Y., Nwanguma, B. C. & Ezeanyika, L. U. S. (2018). *In Silico* Site-Directed Mutagenesis of Ser11 and Lys107 on the Predicted 3D Structure of glutathione s-transferase from *Acidovoras sp.* KKS102. *Chemsearch J,* 9(2), 33-39

Murtala, Y., Nwanguma, B. C. and Ezeanyika, L. U. S. (2019). Effect of *In Silico* Site-Directed Mutagenesis of Trp164 and Ser165 on the Predicted 3D structure of the C-Terminal Domain of Glutathione s-transferase from *Acidovoras sp.* KKS102. *Nig Res J Chem Sci,* 6, 10-20

Nishida, M., Kong, K.H., Inoue, H. & Takahashi, K. (1994) Molecular cloning and site-directed mutagenesis of glutathione S-transferase from Escherichia coli. The conserved tyrosyl residue near the N terminus is not essential for catalysis. *J Biol Chem* 269, 32536–32541.

Ohtsubo, Y., Maruyama, F., Mitsui, H., Nagata, Y. & Tsuda, M. (2012). Complete genome sequence of *Acidovorax* sp. strain KKS102, a polychlorinated-biphenyl degrader. *J Bacteriol* 194, 6970–6971. doi.10.1128/JB.01848-12.

Schwede, T. Kopp, J., Guex, N. & Peitsch, M.C. (2003). SWISS-MODEL: An automated protein homology modelling server. *Nucleic Acids Res* 31(13), 3381-3385. doi. 10.1093/nar/gkg520

Shehu, D. & Zazali, A. (2018). Functional Role of Tyr12 in the Catalytic Activity of Novel Zeta-like Glutathione *S*-transferase from *Acidovorax* sp. KKS102. *J Protein* 37(3), 261-269. doi.10.1007/s10930-018-9774-x

Simarani, K., Yusoff, W.H.A.C. & Alias, Z. (2016). Purification of Glutathione Transferases (GSTs) from Identified Rhizospheric Bacteria. *Sains Malays* 45(3), 1057-1062

Warner, J.R., Lawson, S.L. & Copley, S.D.A. (2005). Mechanistic Investigation of the Thiol-Disulfide Exchange Step in the Reductive Dehalogenation Catalyzed by Tetrachlorohydroquinone Dehalogenase. *Biochem-US*. 44(30), 10360-10368

Wilce, M.C.J. & Parker, M.W. (1994). Structure and function of glutathione S-transferases. *Biochimica et Biophysica Acta* 1205, 1-18.

Wongtrakul, J., Udomsinprasert, R. & Ketterman, A.J. (2003). Non-active site residues Cys69 and Asp150 affected the enzymatic properties of glutathione S-transferase AdGSTD3-3. *Insect Biochem Mol Biol* 33, 971–979.

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